various sites including the lumbar spine and femoral neck, adjusting for covariates [14]. Higher serum uric acid levels were also associated with a lower prevalence of osteoporosis, vertebral fracture ascertained by lateral spine scans, and history of nonvertebral fracture [14]. Another large cross-sectional study replicated the association of uric acid positively with BMD and negatively with lower prevalence of vertebral fracture in postmenopausal women [15]. This study also demonstrated that uric acid suppressed osteoclastogenesis and reduced the production of reactive oxygen species in osteoclast precursors, providing important evidence that the positive association between uric acid and bone mineral density may be related to the antioxidant effect of uric acid. Moreover, in a longitudinal study on peri- and postmenopausal female twins, higher uric acid levels at baseline were associated with higher BMD at baseline and a slower rate of decline in BMD thereafter, independent of covariates [16].

However, there is also strong evidence linking hyperuricemia with increased risk of cardiovascular disease [17, 18] in which oxidative stress plays an important pathophysiological role [19, 20]. One of the proposed hypotheses explaining this paradox is related to a shift in the prooxidant/antioxidant properties of uric acid depending on its concentration. Experimental studies suggested that uric acid may become prooxidant under certain conditions [21, 22], particularly when it is supersaturated in blood. Therefore, it is conceivable that uric acid may confer protective antioxidant effects or detrimental prooxidant effects when, respectively, present at normal levels or at supersaturated concentrations [23]. One cross-sectional study on young men and women actually demonstrated that *higher* levels of serum uric acid were associated with *lower* BMD at the femoral neck in women after controlling for age, weight, and serum creatinine [24]. Interestingly, uric acid levels in most female participants were within the normal range. Estrogen has an antioxidant property [1] and also reduces serum uric acid by enhancing renal clearance [25]. Therefore, the finding of an inverse association between estrogen and uric acid may be attributable to the confounding effects of estrogen, considering that the women in this study were predominantly premenopausal. However, the effects of age and menopause on the association between uric acid and osteoporosis have not been empirically examined, and further research is needed.

In the present study, we examined the association between uric acid and BMD in peri- and postmenopausal Japanese women. We hypothesized that BMD and uric acid are linearly and positively associated independent of covariates including the menopausal status in the normal range of serum uric acid, but the association becomes inverse in the hyperuricemic range.

Methods

Subjects

This was a retrospective analysis of medical records obtained from Kanto Central Hospital which is a 470-bed urban teaching hospital in Tokyo funded and run by the Mutual Aid Association of Public School Teachers. Teachers who work at public schools and belong to the Association have health checkup annually at the Center for Health Check-up and Preventive Medicine of the Hospital since workers are required by law to have annual health checkup regardless of their age in Japan. Health checkup is performed in a standardized manner, consisting of consultation with a doctor, height and weight measurement, laboratory tests, and several studies including chest X-ray. Lumbar spine BMD measurement by dual-energy X-ray absorptiometry (DXA) is offered optionally for teachers with financial subsidy from the association.

We drew data from the medical records of 3,814 women aged between 45 and 75 years who received a health checkup at the Center from August 2011 to July 2012. Of the women, 638 (16.7 %) out of 3,814 had lumbar spine BMD measurement. Women with chronic kidney disease (estimated glomerular filtration rate (GFR) lower than 60 mL/min/1.73 m^2) ($n=10$) or who had received treatment for osteoporosis ($n=8$) were excluded from the analysis. Those who had received treatment for either hypothyroidism ($n=4$) or hyperthyroidism ($n=1$) were also excluded because of the effect of thyroid hormones on bone [26]. No women received oral steroids, loop diuretics, high-dose thiazide diuretics, hormone replacement therapy, or treatment for hyperuricemia or chronic liver disease. After exclusion, 615 women were included in the analysis. This study was approved by the Ethics Committee of Kanto Central Hospital.

Measurements

Standardized interviews and self-reported questionnaires were used to obtain the following information: age (years), smoking habit (current smoker, past smoker, or never smoked), drinking habit [abstainer, infrequent (non-abstainer but one or less drink per week), and light (more than one drink per week but one or less per day), or moderate to heavy (more than one drink per day)], physical activity (any regular exercise or none), age at menopause, medical history, and use of prescription medication. Height and weight were measured using a fixed stadiometer and a digital scale, with the participant wearing light clothing. Body mass index (BMI) was calculated from weight and height.

Fasting blood samples were collected from each participant, and serum uric acid, creatinine, calcium, and alkaline phosphatase were measured using a standard technique with a medical autoanalyzer (BioMajesty JCA-BM2250). The assay

range for serum uric acid was 0.2–200 mg/dL. Plasma C-reactive protein (CRP) was measured using a latex immunoassay with the assay range of 0.2–4,000 mg/L. Estimated GFR was calculated from age, sex, and serum creatinine [27].

Subjects with a reported history of diabetes mellitus, fasting glucose of 126 mg/dL or higher, or glycosylated hemoglobin levels at 6.5 % or higher were classified as diabetic. Those with a reported history of hypertension, systolic blood pressure of 140 mmHg or higher, or diastolic blood pressure of 90 mmHg or higher were classified hypertensive.

Bone mineral density measurements

BMD of the lumbar spine was measured by DXA using a GE Lunar Prodigy. A standard quality control program included daily calibrations with machine-specific phantoms to ensure machine accuracy of greater than 98 %.

Statistical analysis

Uric acid becomes insoluble and supersaturated in bodily fluids above a concentration of about 7 mg/dL. The nonparametric locally weighted scatterplot smoothing (LOESS) method was used to determine whether the saturation point affects the functional form of the association between uric acid and BMD. The LOESS method generated a smooth curve of BMD as a function of uric acid. Visual inspection of the LOESS plot indicated that the relationship between BMD and uric acid was piecewise linear with an inflection (change of slope) at the uric acid value of 4.8, above which the slope appeared steeper (Fig. 1). We then fitted piecewise linear spline models to BMD as a function of uric acid with a fixed knot at 4.8. We also employed generalized additive models to examine the shape of the association between uric acid and BMD accounting for other covariates. The generalized additive model is an extension of the generalized linear model in which one or more independent variables can be modeled with nonparametric smooth functions [28].

The model was initially adjusted for age and BMI (model 1). Covariates for lifestyle risk factors for osteoporosis including physical activity; smoking and drinking habit; years after menopause (coded as 0 if subject had not experienced menopause) (model 2); comorbidity including diabetes mellitus and hypertension (model 3); and serum calcium, alkaline phosphatase (ALP), estimated GFR, and log (CRP) (model 4) were successively added to regression models. The selection of covariates was based on the literature review on factors affecting BMD [29–35].

There were missing values for physical activity in 180 women (29.3 %), years after menopause in 140 women (22.8 %), and drinking habit in 1 woman (0.2 %). These were imputed using the expectation–maximization (EM) algorithm [36].

Statistical analyses were performed using SAS, version 9.2 (SAS Institute, Inc., Cary, NC, USA) and R statistical software

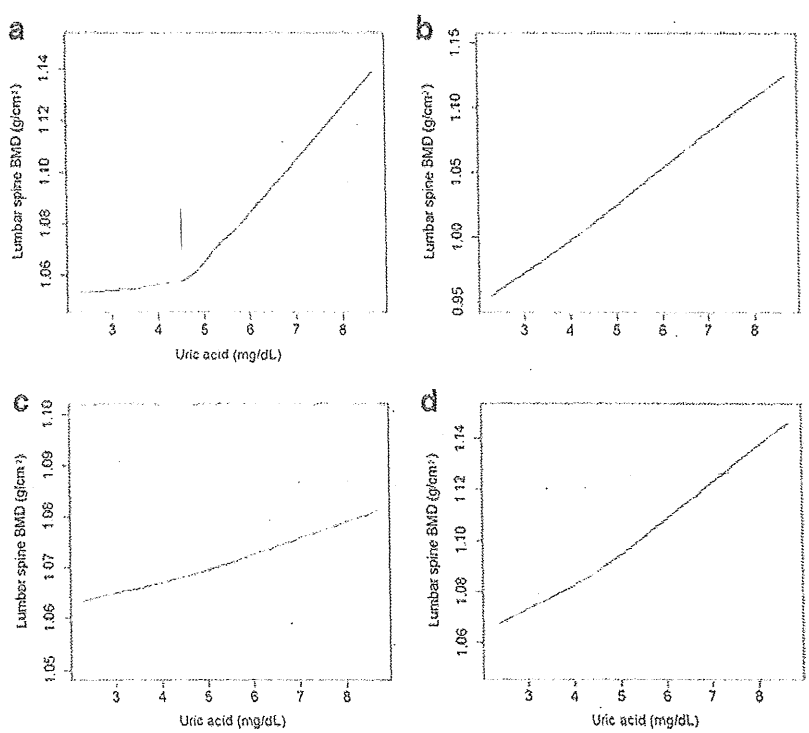


Fig. 1 Plots of lumbar spine bone mineral density against uric acid level. **a** The LOESS plot. **b–d** The plots generated using generalized additive models accounting for age (**b**), body mass index (**c**), or estimated glomerular filtration ratio (**d**). The values of the covariates were fixed at their mean when the association between lumbar spine BMD and uric acid were plotted. *BMD* bone mineral density

version 2.15.2 (R Foundation, Vienna, Austria). All statistical tests were two-sided, and a p value less than 0.05 was considered statistically significant.

Results

Characteristics of study participants are shown in Table 1. Women included in the analysis were similar to those excluded from the analysis with respect to major characteristics. Of the 615 women included in the analysis, serum uric acid had a mean value of 4.7 mg/dL with standard deviation of 1.0 mg/dL. Only 12 women (2.0 %) had hyperuricemia (i.e., uric acid level higher than 7.0 mg/dL), and 19 (3.1 %) women were obese (i.e., BMI equal to or higher than 30 kg/m^2).

Table 1 Characteristics of participants

	Participants (n=615)
Uric acid (mg/dL)	4.7±1.0
Lumbar spine bone mineral density (g/cm^2)	1.06±0.18
Age (years)	57.6±6.4
Log (CRP in mg/L)[a]	0.12±0.17
BMI (kg/m^2)	22.2±3.5
Smoking	
Current	19 (3.1)
Ex	53 (8.6)
Never	543 (88.3)
Drinking[b]	
Abstainer	219 (35.7)
Infrequent	188 (30.6)
Light	171 (27.9)
Moderate to heavy	36 (5.9)
Activity[b]	
Sedentary	283 (65.1)
Active	152 (34.9)
Postmenopausal[b]	373 (78.5)
Age at menopause in postmenopausal women (years)	50.9±3.8
Diabetes	44 (7.2)
Hypertension	114 (18.5)
Serum calcium (mg/dL)	9.3±0.3
Estimated GFR (mL/min/1.73 m^2)	97.8±21.6
ALP (IU/L)	223.5±66.3

For continuous variables, the mean is shown with standard deviation. For categorical variables, the number (percentage) is shown. Percentages may not add up to 100 because of rounding errors

BMD bone mineral density, *CRP* C-reactive protein, *BMI* body mass index, *GFR* glomerular filtration rate, *ALP* alkaline phosphatase, *IU* international unit

[a] The natural log (base e) was taken for CRP due to skewed distribution

[b] There were missing values for physical activity in 180 women (29.3 %), years after menopause in 140 women (22.8 %), and drinking habit in 1 woman (0.2 %)

Association between BMD and uric acid

In piecewise linear regression of BMD as a function of uric acid with a fixed knot at uric acid level of 4.8 mg/dL, the change in slope at the knot was not statistically significant in univariate analysis and all four models of multivariate analyses (p values=0.31–0.79). The generalized additive models also demonstrated that uric acid was approximately linearly associated with BMD when accounting for each of age, BMI, or estimated GFR (Fig. 1). Therefore, the knot was subsequently dropped. The resulting multiple linear regression models fitted simple linear relationship between uric acid and BMD. Serum uric acid levels were significantly and positively associated with lumbar spine BMD adjusting for age and BMI (model 1, Table 2). The association between uric acid and BMD remained significant after successively adjusting for lifestyle risk factors and years after menopause (model 2); comorbidity (model 3); and serum calcium, estimated GFR, log (CRP), and ALP (model 4). Serum uric acid levels explained 0.48–0.63 % of variance in BMD (R^2=0.187–0.258).

Effect modification

One of the presumed mechanisms of the association between BMD and uric acid is the antioxidant property of uric acid. Considering the complicated and interrelated relationship between oxidative stress and inflammation, we postulated that the degree of inflammation modifies the association between BMD and uric acid. To test this hypothesis, we examined the interaction between log (CRP) and uric acid, but it was not significant (p=0.22).

Table 2 Adjusted associations of serum uric acid with lumbar spine bone mineral density (n=615)

	Beta[a]	%V[b]	p	R^2
Model 1	0.084	0.63	0.03	0.187
Model 2	0.081	0.57	0.04	0.199
Model 3	0.084	0.61	0.03	0.206
Model 4	0.078	0.48	0.049	0.258

Model 1—adjusted for age, BMI; model 2—adjusted for age, BMI, smoking, drinking, physical activity, and years after menopause; model 3—adjusted for age, BMI, smoking, drinking, physical activity, years after menopause, diabetes, and hypertension; model 4—adjusted for age, BMI, smoking, drinking, physical activity, years after menopause, diabetes, hypertension, serum calcium, estimated GFR, log (CRP), and ALP. Abbreviations are as in Table 1

[a] Standardized beta coefficient

[b] Variance of lumbar spine bone mineral density explained by uric acid

Sensitivity analysis

Previous studies have demonstrated that menopause is associated with changes in both BMD and uric acid. Women have a minimal decline in BMD until 1–2 years prior to the final menstrual period when they begin to experience a rapid decline in BMD. The decline in BMD decelerates 1–2 years after the final menstrual period, but continues [37]. On the other hand, postmenopausal status was associated with higher levels of uric acid [38, 39]. Therefore, the associations of age with BMD and uric acid in this study sample of peri- and postmenopausal women may not be linear. The LOESS plots of BMD and uric acid as a function of age demonstrated that both of the relationships were piecewise linear, with an inflection at around the age of 60 (Fig. 2a, b). Uric acid rapidly increased with increasing age until age 60 years, then decelerated but continued to increase. Similarly, lumbar spine BMD declined rapidly with increasing age, but the rate of decline slowed down at the age of 60 years but continued to decline. As a sensitivity analysis, we examined the association between uric acid and BMD after excluding 177 women older than 60. The analysis demonstrated significant and positive associations between BMD and uric acid in all models, with effect sizes slightly larger than those observed in the main analyses, supporting the robustness of our scientific conclusion (Table 3).

We also conducted another sensitivity analysis after excluding 281 women with any missing values in covariates. This sensitivity analysis revealed slightly larger effect sizes of the association between UA and BMD than those in the main analyses, but the associations failed to reach statistical significance (data not shown).

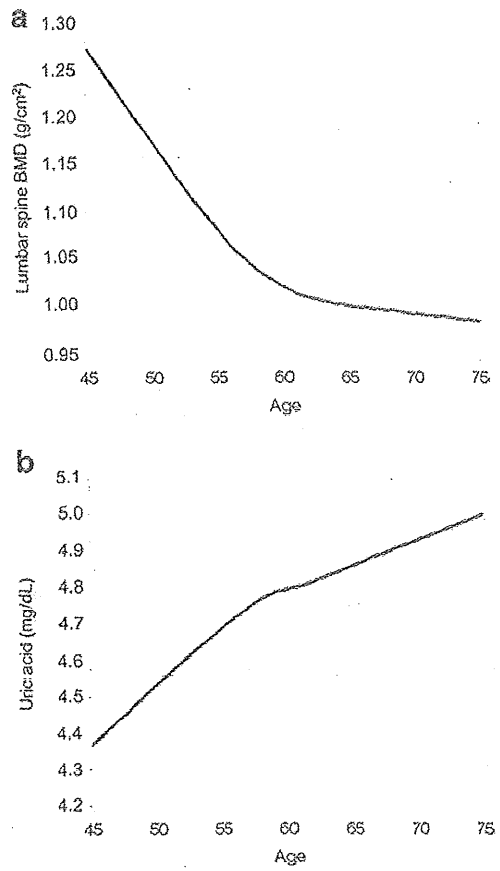


Fig. 2 LOESS plots of bone mineral density and uric acid against age. *BMD* bone mineral density

Discussion

In this cross-sectional analysis of 615 peri- and postmenopausal women aged between 45 and 75 years, higher serum levels of uric acid were significantly associated with higher values of BMD in the lumbar spine, independent of covariates including years after menopause. One standard deviation (1.0 mg/dL in this study population) increment in uric acid was associated with an approximately 0.08 standard deviation increase in lumbar spine BMD. We also demonstrated rapid changes in uric acid and BMD with increasing age until the age of 60, and the rate of changes slowed down thereafter. The positive association between BMD and uric acid remained significant after excluding women older than 60 years.

Our study confirms and extends a previous study that has demonstrated a positive association between BMD and uric acid in peri- and postmenopausal women [15, 16]. We showed that uric acid was positively and linearly associated with lumbar spine BMD, and therefore not only the presence of hyperuricemia but also the magnitude of uric acid elevation plays an important role. Addition of years after menopause did not significantly affect the uric acid–BMD association. We did not observe any sharp inflection point (i.e., change of slope) in the association between uric acid and BMD, incongruent with our hypothesis that the association between uric acid and

Table 3 Adjusted associations of serum uric acid with lumbar spine bone mineral density after excluding 177 women older than 60 years (n=438)

	Beta[a]	%V[b]	p	R^2
Model 1	0.103	0.96	0.02	0.284
Model 2	0.091	0.73	0.04	0.294
Model 3	0.101	0.89	0.02	0.304
Model 4	0.107	0.91	0.02	0.359

Model 1—adjusted for age and BMI; model 2—adjusted for age, BMI, smoking, drinking, physical activity, and years after menopause; model 3—adjusted for age, BMI, smoking, drinking, physical activity, years after menopause, diabetes, and hypertension; model 4—adjusted for age, BMI, smoking, drinking, physical activity, years after menopause, diabetes, hypertension, serum calcium, estimated GFR, log (CRP), and ALP. Abbreviations are as in Table 1

[a] Standardized beta coefficient

[b] Variance of lumbar spine bone mineral density explained by uric acid

BMD becomes inverse in the hyperuricemic range. However, it should be noted that only a small portion of women in this study had hyperuricemia, and further study is needed to determine if the association between BMD and uric acid in the hyperuricemic range may differ from that in the physiologic range.

We also demonstrated that there was a period of rapid increase in uric acid until the age of 60 years when the rate of increase slowed. The observed trajectory of uric acid is consistent with menopause-related changes, rather than changes secondary to chronological aging. This is congruent with previous studies showing that uric acid levels were higher in postmenopausal women compared with pre- or perimenopausal women [38, 39]. We observed a similar menopause-related change in BMD, consistent with previous studies [37]. However, the inflection (i.e., change of slope) was observed at around the age of 60 for both uric acid and BMD in the present study, which appears too far apart from the mean age at menopause of approximately 51 years. The possible explanations for the discrepancy include reporting error and the nature of cross-sectional data, which are predisposed to recall bias and are unable to separate the effects of aging from secular trend. Hence, a longitudinal study is warranted to determine the precise trajectory of uric acid during the menopause transition.

This study has several limitations. First, the study design was cross-sectional and did not allow us to infer a cause–effect relationship between uric acid level and BMD. However, one previous longitudinal study demonstrated that higher serum uric acid levels were associated with slower annual decline in BMD in peri- and postmenopausal women [16]. Second, we employed an EM algorithm to impute missing values in covariates. Missing values occurred mostly in two variables—physical activity and age at menopause. Sensitivity analysis excluding women with any missing values in covariates yielded similar, albeit not significant, effect sizes of the association between BMD and uric acid, indirectly supporting the robustness of the approach. The association failed to reach statistical significance due to the reduced number of women included in the sensitivity analysis. Third, the data were obtained from the medical records of female teachers who had received health checkup annually and were therefore expected to be generally in good health and health conscious. In fact, the prevalence of comorbidity such as hypertension and diabetes, and the smoking rate were lower than those in the general population [40–42]. In addition, the women in this study had lower weight compared with peri- and postmenopausal Australian women in a previous study on uric acid and BMD [16]. Thus, the observed associations of uric acid with menopause and BMD were less likely to be confounded by obesity and other comorbidity, but the generalizability of the findings to other populations may be limited. In addition, BMD measurement was performed voluntarily, which could introduce selection bias. However, women in the analysis were comparable to those excluded from the analysis, most of whom had not had BMD measurement and excluded. Fourth, the observed association was marginally significant. We speculate that it is mostly likely due to relatively small sample size because the finding was consistent throughout various models. Lastly, any observational studies like this one cannot be free of possible confounding due to uncontrolled or unmeasured variables. Several important variables such as bone turnover markers, PTH, and serum 25-hydroxyvitamin D were not measured or available for the analysis.

Despite these limitations, the study has several strengths. Even though this was a retrospective analysis, the data were drawn from medical records for health checkup, which were in general free of missing values except for a few measurements. These measurements were performed voluntarily or as a part of optional examinations. The main finding was robust to the inclusion of a variety of covariates including years after menopause and exclusion of older women.

In conclusion, the present study showed that higher uric acid levels in the physiologic range of uric acid are linearly associated with higher lumbar spine bone mineral density in peri- and postmenopausal Japanese women. Further research is needed to elucidate the precise underlying mechanism of the association between uric acid and bone mineral density and to determine if the positive association between BMD and uric acid is still observed in the hyperuricemic range.

Acknowledgments The authors wish to thank the staff members and patients of the Center for Health Check-up and Preventive Medicine in Kanto Central Hospital.

Conflicts of interest None.

References

1. Manolagas SC (2010) From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis. Endocr Rev 31:266–300
2. Manolagas SC, Almeida M (2007) Gone with the Wnts: beta-catenin, T-cell factor, forkhead box O, and oxidative stress in age-dependent diseases of bone, lipid, and glucose metabolism. Mol Endocrinol 21: 2605–2614, Baltimore, Md
3. Kuyumcu ME, Yesil Y, Ozturk ZA, Cinar E, Kizilarslanoglu C, Halil M, Ulger Z, Yesil NK, Cankurtaran M, Ariogul S (2012) The association between homocysteine (hcy) and serum natural antioxidants in elderly bone mineral densitometry (BMD). Arch Gerontol Geriatr 55:739–743
4. Maggio D, Barabani M, Pierandrei M, Polidori MC, Catani M, Mecocci P, Senin U, Pacifici R, Cherubini A (2003) Marked decrease in plasma antioxidants in aged osteoporotic women: results of a cross-sectional study. J Clin Endocrinol Metab 88:1523–1527
5. Sugiura M, Nakamura M, Ogawa K, Ikoma Y, Ando F, Yano M (2008) Bone mineral density in post-menopausal female subjects is associated with serum antioxidant carotenoids. Osteoporos Int 19: 211–219
6. Sendur OF, Turan Y, Tastaban E, Serter M (2009) Antioxidant status in patients with osteoporosis: a controlled study. Joint, bone, spine 76:514–518

7. Sahni S, Hannan MT, Blumberg J, Cupples LA, Kiel DP, Tucker KL (2009) Protective effect of total carotenoid and lycopene intake on the risk of hip fracture: a 17-year follow-up from the Framingham Osteoporosis Study. J Bone Miner Res 24:1086–1094
8. Sanchez-Rodriguez MA, Ruiz-Ramos M, Correa-Munoz E, Mendoza-Nunez VM (2007) Oxidative stress as a risk factor for osteoporosis in elderly Mexicans as characterized by antioxidant enzymes. BMC Musculoskelet Disord 8:124
9. Sahni S, Hannan MT, Gagnon D, Blumberg J, Cupples LA, Kiel DP, Tucker KL (2009) Protective effect of total and supplemental vitamin C intake on the risk of hip fracture—a 17-year follow-up from the Framingham Osteoporosis Study. Osteoporos Int 20:1853–1861
10. Sahni S, Hannan MT, Gagnon D, Blumberg J, Cupples LA, Kiel DP, Tucker KL (2008) High vitamin C intake is associated with lower 4-year bone loss in elderly men. J Nutr 138:1931–1938
11. Ostman B, Michaelsson K, Helmersson J, Byberg L, Gedeborg R, Melhus H, Basu S (2009) Oxidative stress and bone mineral density in elderly men: antioxidant activity of alpha-tocopherol. Free Radical Biol Med 47:668–673
12. Basu S, Michaelsson K, Olofsson H, Johansson S, Melhus H (2001) Association between oxidative stress and bone mineral density. Biochem Biophys Res Commun 288:275–279
13. Ames BN, Cathcart R, Schwiers E, Hochstein P (1981) Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. Proc Natl Acad Sci U S A 78:6858–6862
14. Nabipour I, Sambrook PN, Blyth FM, Janu MR, Waite LM, Naganathan V, Handelsman DJ, Le Couteur DG, Cumming RG, Seibel MJ (2011) Serum uric acid is associated with bone health in older men: a cross-sectional population-based study. J Bone Miner Res 26:955–964
15. Ahn SH, Lee SH, Kim BJ, Lim KH, Bae SJ, Kim EH, Kim HK, Choe JW, Koh JM, Kim GS (2013) Higher serum uric acid is associated with higher bone mass, lower bone turnover, and lower prevalence of vertebral fracture in healthy postmenopausal women. Osteoporos Int 24(12):2961–2970
16. Makovey J, Macara M, Chen JS, Hayward CS, March L, Seibel MJ, Sambrook PN (2013) Serum uric acid plays a protective role for bone loss in peri- and postmenopausal women: a longitudinal study. Bone 52:400–406
17. Chen JH, Chuang SY, Chen HJ, Yeh WT, Pan WH (2009) Serum uric acid level as an independent risk factor for all-cause, cardiovascular, and ischemic stroke mortality: a Chinese cohort study. Arthritis Rheum 61:225–232
18. Kim SY, Guevara JP, Kim KM, Choi HK, Heitjan DF, Albert DA (2010) Hyperuricemia and coronary heart disease: a systematic review and meta-analysis. Arthritis Care Res 62:170–180
19. Aviram M (2000) Review of human studies on oxidative damage and antioxidant protection related to cardiovascular diseases. Free Radical Res 33(Suppl):S85–S97
20. Rocha M, Apostolova N, Hernandez-Mijares A, Herance R, Victor VM (2010) Oxidative stress and endothelial dysfunction in cardiovascular disease: mitochondria-targeted therapeutics. Curr Med Chem 17:3827–3841
21. Bagnati M, Perugini C, Cau C, Bordone R, Albano E, Bellomo G (1999) When and why a water-soluble antioxidant becomes pro-oxidant during copper-induced low-density lipoprotein oxidation: a study using uric acid. Biochem J 340(Pt 1):143–152
22. Patterson RA, Horsley ET, Leake DS (2003) Prooxidant and antioxidant properties of human serum ultrafiltrates toward LDL: important role of uric acid. J Lipid Res 44:512–521
23. Lippi G, Montagnana M, Franchini M, Favaloro EJ, Targher G (2008) The paradoxical relationship between serum uric acid and cardiovascular disease. Clin Chim Acta 392:1–7
24. Sritara C, Ongphiphadhanakul B, Chailurkit L, Yamwong S, Ratanachaiwong W, Sritara P (2012) Serum uric acid levels in relation to bone-related phenotypes in men and women. J Clin Densitom 16(3):336–340
25. Yahyaoui R, Esteva I, Haro-Mora JJ et al (2008) Effect of long-term administration of cross-sex hormone therapy on serum and urinary uric acid in transsexual persons. J Clin Endocrinol Metab 93:2230–2233
26. Galliford TM, Murphy E, Williams AJ, Bassett JH, Williams GR (2005) Effects of thyroid status on bone metabolism: a primary role for thyroid stimulating hormone or thyroid hormone? Minerva Endocrinol 30:237–246
27. Ando Y, Ito S, Uemura O, Kato T, Kimura G, Nakao T, Hattori M, Fukagawa M, Horio M, Mitarai T (2009) CKD clinical practice guidebook. The essence of treatment for CKD patients. Clin Exp Nephrol 13:191–248
28. Hastie T, Tibshirani R (1995) Generalized additive models for medical research. Stat Methods Med Res 4:187–196
29. Siris ES, Miller PD, Barrett-Connor E, Faulkner KG, Wehren LE, Abbott TA, Berger ML, Santora AC, Sherwood LM (2001) Identification and fracture outcomes of undiagnosed low bone mineral density in postmenopausal women: results from the National Osteoporosis Risk Assessment. Jama 286:2815–2822
30. Waugh EJ, Lam MA, Hawker GA, McGowan J, Papaioannou A, Cheung AM, Hodsman AB, Leslie WD, Siminoski K, Jamal SA (2009) Risk factors for low bone mass in healthy 40–60 year old women: a systematic review of the literature. Osteoporos Int 20:1–21
31. Schwartz AV, Vittinghoff E, Bauer DC et al (2011) Association of BMD and FRAX score with risk of fracture in older adults with type 2 diabetes. Jama 305:2184–2192
32. Ishii S, Cauley JA, Greendale GA, Crandall CJ, Danielson ME, Ouchi Y, Karlamangla AS (2013) C-reactive protein, bone strength, and 9-year fracture risk: data from the Study of Women's Health Across the Nation (SWAN). J Bone Miner Res 28(7):1688–1698
33. Varenna M, Manara M, Galli L, Binelli L, Zucchi F, Sinigaglia L (2013) The association between osteoporosis and hypertension: the role of a low dairy intake. Calcif Tissue Int 93(1):86–92
34. Hulth AG, Nilsson BE, Westlin NE, Wiklund PE (1979) Alkaline phosphatase in women with osteoporosis. Acta Med Scand 206:201–203
35. Miller PD (2009) Diagnosis and treatment of osteoporosis in chronic renal disease. Semin Nephrol 29:144–155
36. Graham JW (2009) Missing data analysis: making it work in the real world. Annu Rev Psychol 60:549–576
37. Greendale GA, Sowers M, Han W, Huang MH, Finkelstein JS, Crandall CJ, Lee JS, Karlamangla AS (2012) Bone mineral density loss in relation to the final menstrual period in a multiethnic cohort: results from the Study of Women's Health Across the Nation (SWAN). J Bone Miner Res 27:111–118
38. Stockl D, Doring A, Thorand B, Heier M, Belcredi P, Meisinger C (2012) Reproductive factors and serum uric acid levels in females from the general population: the KORA F4 study. PloS one 7:e32668
39. Hak AE, Choi HK (2008) Menopause, postmenopausal hormone use and serum uric acid levels in US women—the Third National Health and Nutrition Examination Survey. Arthritis Res Ther 10:R116
40. Marugame T, Kamo K, Sobue T, Akiba S, Mizuno S, Satoh H, Suzuki T, Tajima K, Tamakoshi A, Tsugane S (2006) Trends in smoking by birth cohorts born between 1900 and 1977 in Japan. Prev Med 42:120–127
41. Neville SE, Boye KS, Montgomery WS, Iwamoto K, Okamura M, Hayes RP (2009) Diabetes in Japan: a review of disease burden and approaches to treatment. Diabetes Metab Res Rev 25:705–716
42. Sekikawa A, Hayakawa T (2004) Prevalence of hypertension, its awareness and control in adult population in Japan. J Hum Hypertens 18:911–912

Geriatr Gerontol Int 2014; 14: 159–166

ORIGINAL ARTICLE: EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

Association of decreased sympathetic nervous activity with mortality of older adults in long-term care

Koji Shibasaki,[1] Sumito Ogawa,[1] Shizuru Yamada,[2] Katsuya Iijima,[1] Masato Eto,[1] Koichi Kozaki,[2] Kenji Toba,[3] Masahiro Akishita[1] and Yasuyoshi Ouchi[1]

[1]*Department of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo,* [2]*Department of Geriatric Medicine, Kyorin University School of Medicine, Tokyo, and* [3]*National Center for Geriatrics and Gerontology, Obu, Japan*

Aim: To investigate the relationship between physical function, mortality and autonomic nervous activity measured by heart rate variability of elderly in long-term care.

Methods: Cross-sectional and longitudinal studies were carried out at hospitals and health service facilities for the elderly in Nagano prefecture, Japan, from July 2007 to March 2011. A total of 105 long-term care older adults and 17 control older adults with independent physical function were included. The Functional Independence Measure (FIM) and Barthel Index were determined as indices of physical function. Twenty-four-hour Holter monitoring was carried out. From RR intervals in electrocardiograms, heart rate and standard deviations of all NN intervals in all 5-min segments of the entire recording, power spectral density, low frequency, high frequency and low frequency/high frequency (LF/HF) were calculated.

Results: FIM score and Barthel Index were 46 ± 26 and 30 ± 31, respectively, in long-term care elderly. FIM and Barthel index were significantly correlated with heart rate and the standard deviations of all NN intervals after adjustment for age, sex, cardiovascular risk factors and FIM. Furthermore, LF/HF was significantly decreased in long-term care elderly compared with control elderly after adjustment for covariates. In addition, decrease in LF/HF was an independent risk factor for mortality.

Conclusion: Low LF/HF activity was observed in long-term care elderly and was related to an increase of overall mortality. **Geriatr Gerontol Int 2014; 14: 159–166.**

Keywords: heart rate variability, long-term care, mortality, motor activity, sympathetic nervous system.

Introduction

The number of older adults who require long-term care (LTC) has been increasing in Japan, and it was reported that there were 4.67 million older adults in LTC in 2008.[1] One of the characteristics of older adults in long-term care is physical and cognitive dysfunction. Physical dysfunction, including slow gait, low handgrip strength, low physical activity, weight loss and exhaustion, are reported to be associated with increased overall mortality.[2] In Japan, LTC elderly is defined as those who require assistance with walking, moving, and washing their face, body and mouth, representing functional disability and high mortality.[3] Thus, it is important to maintain or increase physical function in LTC elderly.

The underlying causes of physical dysfunction in Japanese LTC elderly include cerebrovascular disease, dementia, fractures, falls, weakness as a result of aging, and arthritis.[3] Recent studies have shown that these diseases with physical dysfunction are associated with low sympathetic nervous system activity.[4–7]

Skin sympathetic reactivity (SSR) reflects sympathetic nervous system activity. Muslumanoglu *et al.* showed that low SSR was associated with greater severity of paralysis, and depression of sympathetic reflex activity was associated with moderate or severely limited motor function in the chronic phase of ischemic cerebrovascular disease in elderly patients.[5] In addition, low plasma norepinephrine and low iodine-131-meta-iodobenzylguanidine (^{123}I-MIBG) uptake were observed in patients with Lewy body dementia compared with normal healthy subjects.[6,7] RR intervals in the electrocardiogram are utilized to evaluate heart rate variability

Accepted for publication 10 March 2013.

Correspondence: Dr Sumito Ogawa MD PhD, Department of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Email: suogawa-tky@umin.ac.jp

© 2013 Japan Geriatrics Society

(HRV), which reflects autonomic nervous system activity.[8] In practice, low frequency/high frequency (LF/HF), a marker of sympathovagal balance or sympathetic modulation, showed a positive correlation with respiratory and skeletal muscle strength in chronic obstructive pulmonary disease.[4] Furthermore, decreased LF/HF was related to overall mortality in frail older adults.[9]

In addition to measurement of SSR, norepinephrine spillover and ^{123}I-MIBG scintigraphy uptake, HRV has recently been used as a marker of autonomic nervous function.[8] HF was reported to reflect parasympathetic nervous system activity and LF/HF to represent sympathovagal balance or sympathetic modulation. In addition, decreased HRV was associated with cardiovascular disease (CVD),[10] cardiac death[11] and all-cause mortality.[9] Whereas HRV is known to decrease with the aging process,[12,13] little is known about the relationship between sympathetic nervous activity and mortality in LTC elderly.

In the Framingham heart study, a cohort study in American community-dwelling people, mortality and HRV were investigated in older adults, and it was not shown that low LF/HF correlated with mortality,[14] whereas in a cohort study of frail older adults, low LF/HF was significantly correlated with both frailty and mortality in the Women's Health and Aging Study-I (WHAS-I).[9]

Aging attenuates sympathetic nervous modulation,[12,13] and previous studies suggested that low sympathetic nervous activity might be associated with physical and cognitive dysfunction. However, only some of the subjects were frail or LTC elderly,[9,14] and there is little evidence describing the relationship between physical function, mortality and sympathetic nervous activity in LTC elderly. In particular, few studies have focused on the specific characteristics of sympathetic nervous activity in LTC elderly. Therefore, we investigated the relationship between sympathetic nervous activity, measured by HRV, and physical function and mortality in older adults in LTC.

Methods

Study design and participants

The present observational study analyzed 105 consecutive older adults in LTC aged 75 years or older who were admitted to a rehabilitation unit or a health service facility for older adults that provided rehabilitation. All hospitals and health service facilities were located in Nagano prefecture, Japan. Inclusion criteria were older adults in LTC aged 75 years or older receiving rehabilitation. Exclusion criteria were treatment of acute phase diseases within the past 2 weeks, arrhythmia, administration of anti-arrhythmia drugs or β-blockers, malignancy and neurodegenerative diseases other than dementia. As a control for the present study, we recruited 17 elderly outpatients with intact activities of daily living (ADL) who were matched for age, sex and CVD risk factors. The same inclusion and exclusion criteria were used for these control subjects. Medical records were reviewed to obtain information about the medical history of CVD, such as hypertension, diabetes mellitus, hyperlipidemia, chronic heart failure and ischemic heart disease, which was confirmed by the patients or their family. The present study protocol was approved by the institutional review board of the facility. Written informed consent was obtained from all participants or their families.

Heart rate variability

Ambulatory Holter recording was carried out for 24 h using QR2100 (Fukuda ME Kogyo, Tokyo, Japan) and processed with HS1000VL (Fukuda ME Kogyo). For time domain analysis, the standard deviations of all NN intervals in all 5-min segments of the entire recording (SDANN) were calculated, and frequent domain analysis was carried out with fast Fourier transform. From the power spectral density, low frequency (LF; 0.04~0.15 Hz), high frequency (HF; 0.15~0.40 Hz) and low frequency/high frequency (LF/HF) were determined.

Anthropometric, physical function and hematological measures

Height, weight and body mass index (BMI) were measured before Holter monitoring. The Functional Independence Measure (FIM)[15] and Barthel Index[16] were determined in order to assess physical function. Venous blood samples were obtained from participants in the morning after an overnight fast. Blood cell counts and serum levels of chemical parameters were determined by a commercial laboratory (Health Science Research Institute, Yokohama, Japan).

Statistical analysis

Data were analyzed using SPSS software version 11.0.1J (SPSS Japan, Tokyo, Japan). Mann–Whitney *U*-test for continuous variables and χ^2-test for categorical variables were used to compare controls and LTC elderly. Pearson's correlation coefficient was calculated, and standardized multiple regression analysis of HRV indices was carried out with age, sex, FIM, Barthel Index and blood nutritional data as covariates. Multiple regression analysis was used to calculate Cox hazard ratio, with adjustment for age, sex, clinical risk factors and FIM. Kaplan–Meier survival rate was computed for HRV indices.

© 2013 Japan Geriatrics Society

Table 1 Characteristics of long-term care elderly and healthy elderly controls

	LTC elderly	Controls	*P*
No. participants	105	17	
Age (years)	86.5 ± 6.0 (75–100)	86.3 ± 9.1 (75–103)	0.311
Sex, male (%)	29 (27.6)	6 (35.3)	0.999
Body mass index	19.5 ± 3.3	22.0 ± 3.5	0.009
Cardiovascular risk factors, *n* (%)			
Hypertension	57 (54.3)	11 (64.7)	0.590
Diabetes mellitus	13 (12.4)	2 (11.8)	0.999
Hyperlipidemia	14 (13.3)	3 (17.6)	0.921
Chronic heart failure	12 (11.4)	1 (5.9)	0.792
Ischemic heart disease	15 (14.3)	1 (5.9)	0.572
Physical function			
FIM	46 ± 26	116 ± 24	<0.001
Barthel Index	30 ± 31	92 ± 16	<0.001
Blood nutritional data			
Albumin (g/dL)	3.5 ± 0.5	3.9 ± 0.3	<0.001
Hemoglobin (g/dL)	12.0 ± 1.8	12.4 ± 2.2	0.188
Total cholesterol (mg/dL)	177 ± 40	175 ± 34	0.892
Heart rate variability indices			
SDANN	85.0 ± 34.3	112.1 ± 27.2	0.001
Heart rate (b.p.m.)	73.1 ± 12.1	71.5 ± 7.4	0.878
LF (ms^2)	36.1 ± 25.3	42.4 ± 37.5	0.274
HF (ms^2)	65.9 ± 56.3	60.7 ± 52.3	0.813
LF/HF	0.69 ± 0.27†	0.87 ± 0.31	0.023

Values are mean ± standard deviation. †After adjusted for age, sex, cardiovascular risk factors and Function Independent Measure (FIM), low frequency/high frequency (LF/HF) were significantly lower in long-term care elderly than healthy controls ($P = 0.049$). HF, high frequency; LF, low frequency; SDANN, standard deviations of the all NN intervals in all 5-min segments of the entire recording.

Results

We registered 105 elderly in LTC, and assessed HRV from 24-h Holter monitoring. The underlying diseases of older adults in LTC for rehabilitation were cerebrovascular disease ($n = 59$, 56.2%), disuse syndrome ($n = 26$, 24.8%), fracture ($n = 19$, 18.1%) and dementia ($n = 1$, 1.0%). The proportions of underlying diseases were similar to those reported in Japanese older adults in LTC.[3]

The background data of the present study are shown in Table 1. In LTC elderly, mean age was 86.5 ± 6.0 years, blood nutritional data including albumin, hemoglobin and total cholesterol were at the lower limit of the normal range, and physical function represented by FIM and Barthel Index was significantly lower (46 ± 26 and 30 ± 31, respectively) than that in elderly controls (116 ± 24 and 92 ± 16, respectively). Scores for each FIM item were as follow: eating 3.7 ± 2.2, grooming 2.6 ± 1.8, bathing 1.5 ± 1.1, upper body dressing 2.5 ± 1.7, lower body dressing 2.2 ± 1.6, toileting 2.7 ± 2.0, bladder management 2.6 ± 2.1, bowel management 2.4 ± 2.0, bed to chair transfer 3.0 ± 1.9, toilet transfer 2.4 ± 1.7, shower transfer 1.5 ± 1.4, locomotion (ambulatory or wheelchair level) 2.0 ± 1.8, stairs 1.2 ± 0.8, cognitive comprehension 3.6 ± 2.2, expression 3.6 ± 2.2, social interaction 3.2 ± 2.2, problem solving 2.8 ± 1.9 and memory 2.8 ± 1.9. These score showed that the overall participants required moderate care supporting physical and cognitive function. In addition, BMI, albumin, SDANN and LF/HF were significantly decreased in LTC elderly compared with elderly controls. After adjustment for covariance, of all HRV indices, only LF/HF was significantly lower in LTC elderly (Table 1). Data of HRV indices were obtained every 5 min, and averaged every 3 h to examine the circadian rhythm in both LTC elderly and healthy controls. A significant decrease of LF/HF was observed in the night-time in healthy controls, whereas there was a loss of circadian rhythm in LTC elderly (Fig. 1).

Multiple regression analysis showed that the associations between heart rate, SDANN and physical function (Barthel Index and FIM) were independent of age, sex, and CVD. Furthermore, albumin and hemoglobin were also correlated with heart rate and SDANN. In contrast, LF, HF and LF/HF were not significantly correlated with physical function and blood nutritional data (Table 2).

© 2013 Japan Geriatrics Society

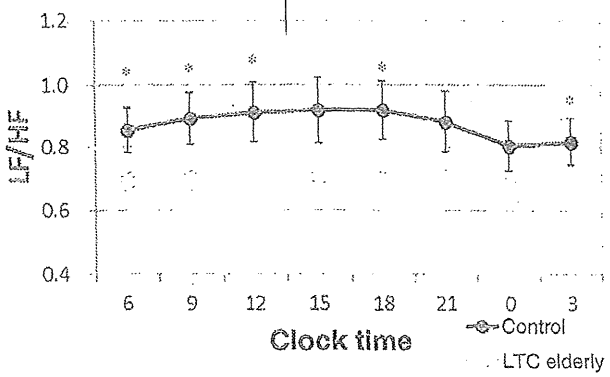


Figure 1 The activity of low frequency/high frequency (LF/HF) in long-term care (LTC) elderly and controls. The RR interval data were measured every 5 min, and averaged every 3 h. $^{*}P < 0.05$, mean ± SEM.

Next, we followed the survival of LTC elderly, and 23 people died among 105 LTC elderly during a mean follow-up period of 8.9 months. The major cause of death was pneumonia ($n = 12$). There was no sign of stroke among the study participants, and one participant with acute myocardial infarction was observed during the follow-up period. Mortality according to HRV indices divided by the average is shown in Table 3. After adjustment for covariates, of all HRV indices, only LF/HF was associated with mortality. Kaplan–Meier survival curves also showed an association between decreased LF/HF and high mortality (Fig. 2). In addition to adjusted covariates, BMI, Barthel Index, and blood nutritional data were not different between the high LF/HF group and low LF/HF group (data not shown).

Discussion

In the present study, we investigated the relationship between physical function, mortality and sympathetic nervous activity measured by HRV in Japanese LTC elderly, and it was shown that LF/HF was significantly decreased in LTC elderly after adjustment for age, sex, CVD risk factors and FIM compared with elderly controls. In addition, the circadian rhythm of LF/HF was lost in LTC elderly, and low LF/HF was associated with overall mortality.

In a previous study, low LF/HF was associated with both frailty and mortality in community-dwelling people of whom one-third were frail elderly,[9] and these associations were consistent with the present data. Additionally, low LF/HF was also shown in LTC elderly, and was independent of physical function.

Elevated heart rate or low SDANN leads to cardiovascular disease and low physical function,[17,18] and the same relationship was also observed in LTC elderly. Furthermore, low albumin and low hemoglobin were observed in the high heart rate group, and limited physical function was observed in LTC elderly. These results are supported by a previous report.[19] So it might be possible to improve the physical function of LTC elderly by maintaining their nutritional state. The high LF/HF group has been reported to show high physical function and muscle mass,[4,20] whereas the present data did not show this association. One of the reasons for this discrepancy is thought to be the effect of aging. Aging generally attenuates LF/HF, and the patients in the present study were older than those in other studies.[9,14] Another reason might be autonomic nervous system disturbance. In particular, the circadian rhythm of LF/HF was impaired in LTC elderly.

Circadian imbalance of LF/HF has been shown in some disorders, such as Parkinson's disease, type 2 diabetes mellitus (T2DM) and ischemic stroke;[21–23] and furthermore, physical activity also influences HRV indices.[24,25] In the present study, LTC elderly with Parkinson's disease were excluded, and CVD risk factors including T2DM were matched between LTC elderly and healthy controls, as stroke and physical activity might affect LF/HF. However, the influence of both conditions on LF/HF is controversial. High physical activity and good posture led to high LF/HF activity,[26] whereas it was also suggested that LF/HF was not affected by physical activity.[13] The effect of LF/HF on stroke is also controversial.[23,27,28] In ischemic stroke patients, LF/HF was higher than healthy controls in some studies,[27,28] whereas another study suggested that LF/HF was lower in patients.[23] So the mechanism of LF/HF circadian rhythm disturbance is not clear, though its recovery might be important to increase physical function in LTC elderly. Other reasons why LF/HF and physical function did not show a correlation in LTC elderly might to be the effects of stroke, insufficient exposure to daylight and posture at daytime. All participants were aged over 75 years in the present study, and there is a possibility that asymptomatic lacunar infarction might be observed. It has also been suggested that lacunar infarction disturbs the autonomic nervous system, leading to a decrease in LF/HF and the related value of the autonomic nervous system, resulting in a disappearance of the correlation between physical activity and LF/HF. In addition, exposure to daylight was known to be one of the most powerful rhythmic regulators in the environment.[29] All participants in the present study spent their time indoors for rehabilitation and care. Furthermore, it is known that the supine position increases HF and decreases LF/HF,[30] and LTC elderly participants who were at rehabilitation units or health service facilities might spend more time in bed compared with outpatient controls, leading to low LF/HF and disappearance of the correlation between LF/HF and physical activity in the present study.

© 2013 Japan Geriatrics Society

Table 2 Multiple regression analysis of heart rate variability indices with physical function and blood nutritional data after adjusted for age, sex and cardiovascular risk factors

	HR	SDANN	LF	HF	LF/HF
FIM	−0.25*	0.28*	0.19	0.15	−0.08
Barthel Index	−0.27*	0.29*	0.08	0.04	0.00
Body mass index	−0.05	0.05	0.00	−0.08	0.19
Albumin	−0.21*	0.25*	0.05	−0.02	0.11
Hemoglobin	−0.20*	0.27*	0.12	0.12	0.05
Total cholesterol	−0.01	−0.05	−0.13	−0.17	0.03

*$P < 0.05$, analyzed in 105 long-term care elderly. FIM, function independent measure; HF, high frequency; HR, heart rate; LF, low frequency; SDANN, standard deviations of the all NN intervals in all 5-min segments of the entire recording.

Table 3 Proportional hazards regression analysis of the impact of heart rate variability measure on overall mortality

	Hazard ratio†	95% Confidence interval	*P*
Unadjusted			
SDANN (ms)	1.84	0.77–4.38	0.171
LF (ms^2)	1.61	0.59–4.38	0.353
HF (ms^2)	2.14	0.72–6.34	0.169
LF/HF	4.73	1.59–14.06	0.005
Age, sex and cardiovascular risk factors adjusted for association with mortality			
SDANN (ms)	1.53	0.60–3.86	0.372
LF (ms^2)	1.65	0.57–4.78	0.357
HF (ms^2)	2.60	0.82–8.22	0.105
LF/HF	3.37	1.02–11.07	0.046
Age, sex, FIM and cardiovascular risk factors adjusted for association with mortality			
SDANN (ms)	1.19	0.44–3.17	0.736
LF (ms^2)	1.49	0.50–4.41	0.475
HF (ms^2)	2.85	0.83–9.83	0.097
LF/HF	3.61	1.08–12.10	0.038

Based on 23 deaths among 105 participants. Mean values of heart rate variability measure are in Table 1. †Hazard ratio of death rates of participants whose heart rate variability were less than average. FIM, function independent measure; HF, high frequency; HR, heart rate; LF, low frequency; SDANN, standard deviations of the all NN intervals in all 5-min segments of the entire recording.

Recent studies showed that decreased HRV indices including LF, HF and LF/HF were associated with CVD risk factors, and decreased LF was an independent predictor of death in elderly people.[31,32] However, the present findings showed that, of all HRV indices, only LF/HF was associated with mortality. This result is supported by a previous study in which, of HRV indices, LF/HF was associated with both frailty and mortality.[9] The major difference between the present study and other studies is whether or not the participants included frail LTC elderly. All participants were LTC elderly in the present study and WHAS-I, which was reported by Varadhan *et al.* and consisted of one-third frail elderly, whereas in other studies the participants were community-dwelling older adults with intact ADL, and they did not consider physical function.[14,32,33] These results suggest that the significance of LF/HF might differ between LTC elderly and elderly with intact ADL and physical function.

There is a discrepancy in the results derived from studies of LTC elderly and studies of elderly with intact physical function regarding sympathetic nervous activity. Exercise activates the sympathetic nervous system, leading to an increase in blood pressure, muscle blood

© 2013 Japan Geriatrics Society

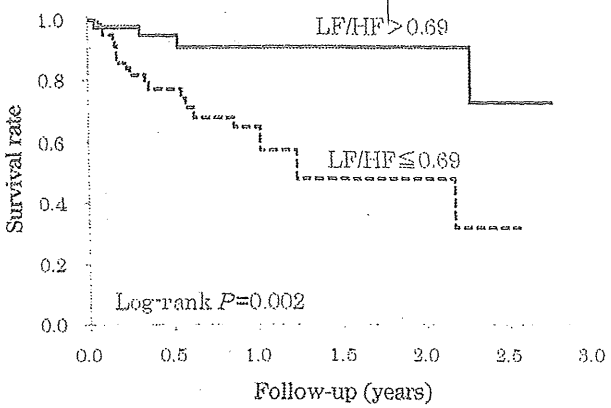


Figure 2 Kaplan–Meyer survival curves for death according to low frequency/high frequency (LF/HF). Mortality was significantly higher for patients with low LF/HF than for patients with high LF/HF. The mean follow-up period was 8.9 months.

flow and muscle strength by inducing muscle protein synthesis,[34–37] suggesting that low sympathetic nervous activity is related to not only physical dysfunction, but also the inability to maintain muscle strength, leading to a worse outcome in LTC elderly. Appropriate activation of the sympathetic nervous system might prevent muscle wasting and improve overall mortality in LTC elderly.

Activation of the sympathetic nervous system has been applied to aging or sarcopenic model rats. The β2-adrenergic agonists, clenbuterol and formoterol, improved muscle mass and muscle strength, and prevented muscle aging in aging, disuse and sarcopenia[38–44] model rats. In contrast, inhibition of sympathetic nervous activity with β-blockers was associated with a worse outcome in older adults.[45] These findings also suggest the importance of preventing a sympathetic nervous activity decline in LTC elderly.

There were several study limitations. First, this was an observational study, and could not provide direct evidence of causality. So it will be necessary to carry out randomized controlled trials to show whether high sympathetic nervous activity leads to a good outcome or not. Second, excessive sympathetic nervous activity is associated with cardiovascular risk factors, such as hypertension, left ventricular myocardial hypertrophy and old cerebrovascular disease.[46,47] In addition, the number of control subjects was relatively small in the present study. Based on these results, it might be hard to apply the findings in the present study to the oldest old population in general. However, some studies, particularly in the elderly, showed that decreased sympathetic nervous activity was associated with a worse outcome.[9] In addition to low physical activity, poor handgrip strength and frailty are known to be important risk factors predicting death older adults,[2,48–50] and few reports have focused on LTC elderly. Therefore, the present study has the possibility of providing evidence to improve physical function and mortality in LTC elderly by means of maintaining or increasing LF/HF.

In summary, the present study showed that LF/HF is a factor that distinguishes LTC elderly from elderly controls independent of physical function. In addition, the circadian rhythm of LF/HF was lost in LTC elderly. Furthermore, low LF/HF was associated with high mortality. For LTC elderly aged 75 years or over, LF/HF might be a predictive biomarker of physical function and mortality.

Disclosure statement

There is no financial support or relationship that might pose conflicts of interest.

References

1 Japanese Ministry of Health, Labor and Welfare. Changes in the number of people certified for long-term care/support need. 2008. [Cited 23 Aug 2011.] Available from URL: http://www.mhlw.go.jp/english/wp/wp-hw3/dL/10-06.pdf
2 Fried LP, Tangen CM, Walston J *et al.* Frailty in older adults: evidence for a phenotype. *J Gerontol* 2001; 56: M146–M156.
3 Japanese Ministry of Health, Labor and Welfare. Welfare policy for the elderly with a focus on long-term care insurance system. 2007. [Cited 23 Aug 2011.] Available from URL: http://www.mhlw.go.jp/english/wp/policy/dL/04.pdf
4 Camillo CA, Pitta F, Possani HV *et al.* Heart rate variability and disease characteristics in patients with COPD. *Lung* 2008; 186: 393–401.
5 Muslumanoglu L, Aki S, Turkdogan D, Us O, Akyuz G. Involvement of sympathetic reflex activity in patients with acute and chronic stroke: a comparison with functional motor capacity. *Arch Phys Med Rehabil* 2004; 85: 470–473.
6 Oka H, Morita M, Onouchi K, Yoshioka M, Mochio S, Inoue K. Cardiovascular autonomic dysfunction in dementia with Lewy bodies and Parkinson's disease. *J Neurol Sci* 2007; 254: 72–77.
7 Oka H, Yoshioka M, Morita M *et al.* Reduced cardiac 123I-MIBG uptake reflects cardiac sympathetic dysfunction in Lewy body disease. *Neurology* 2007; 69: 1460–1465.
8 Heart rate variability. Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology. *Circulation* 1996; 93: 1043–1065.
9 Varadhan R, Chaves PHM, Lipsitz LA *et al.* Frailty and impaired cardiac autonomic control: new insights from principal components aggregation of traditional heart rate variability indices. *J Gerontol A Biol Sci Med Sci* 2009; 64: 682–687.
10 Alter P, Grimm W, Vollrath A, Czerny F, Maisch B. Heart rate variability in patients with cardiac hypertrophy – relation to left ventricular mass and etiology. *Am Heart J* 2006; 151: 829–836.
11 Smilde TDJ, van Veldhusen DJ, van den Berg MP. Prognostic value of heart rate variability and ventricular arrhythmias during 13-year follow-up in patients with mild to moderate heart failure. *Clin Res Cardiol* 2009; 98: 233–239.

© 2013 Japan Geriatrics Society

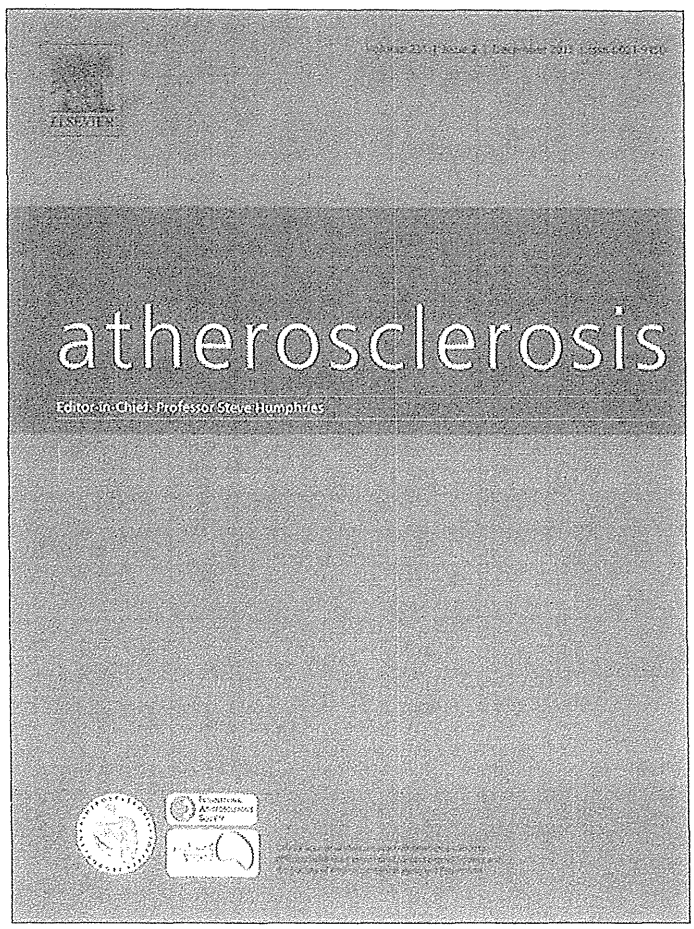
12 Umetani K, Singer DH, McCraty R, Atkinson M. Twenty-four hour time domain heart rate variability and heart rate: relations to age and gender over nine decades. *J Am Coll Cardiol* 1998; **31**: 593–601.
13 Greiser KH, Kluttig A, Schumann B *et al.* Cardiovascular diseases, risk factors and short-term heart rate variability in an elderly general population: the CARLA study 2002–2006. *Eur J Epidemiol* 2009; **24**: 123–142.
14 Tsuji H, Venditti FJ Jr, Manders ES *et al.* Reduced heart rate variability and mortality risk in an elderly cohort. The Framingham Heart Study. *Circulation* 1994; **90**: 878–883.
15 Keith RA, Granger CV, Hamilton BB, Sherwin FS. The functional independence measure: a tool for rehabilitation. *Adv Clin Rehabil* 1987; **1**: 6–18.
16 Mahoney FI, Barthel DW. Functional evaluation: the Barthel index. *Md State Med J* 1965; **14**: 61–65.
17 Graham I, Atar D, Borch-Johnsen K *et al.* European guidelines on cardiovascular disease prevention in clinical practice: executive summary: fourth joint task force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (Constituted by representatives of nine societies and by invited experts). *Eur Heart J* 2007; **28**: 2375–2414.
18 Roach D, Wilson W, Ritchie D, Sheldon R. Dissection of long-range heart rate variability: controlled induction of prognostic measures by activity in the laboratory. *J Am Coll Cardiol* 2004; **43**: 2271–2277.
19 Spurr GB, Barac-Nieto M, Maksud MG. Functional assessment of nutritional status: heart rate response to submaximal work. *Am J Clin Nutr* 1979; **32**: 767–778.
20 David MDR, Martha ERR, Ernesto GR, David MDR, Martha EMDR. Sympathovagal imbalance assessed by heart rate variability correlates with percent body fat and skeletal muscle, independent of body mass index. *Cleve Clin J Med* 2011; **78**: S91a.
21 Niwa F, Kuriyama N, Nakagawa M, Imanishi J. Circadian rhythm of rest activity and autonomic nervous system activity at different stages in Parkinson's disease. *Auton Neurosci* 2011; **165**: 195–200.
22 Boer-Martins L, Figueiredo VN, Demacq C *et al.* Relationship of autonomic imbalance and circadian disruption with obesity and type 2 diabetes in resistant hypertensive patients. *Cardiovasc Diabetol* 2011; **22**: 10–24.
23 Juha T, Korpelainen KA, Sotaniemi HV *et al.* Circadian rhythm of heart rate variability is reversibly abolished in ischemic stroke. *Stroke* 1997; **28**: 2150–2154.
24 Ino-Oka E, Sekino H, Ohtaki Y, Inooka H. Effects of daily physical activity level on the degree of sympathetic tone. *Intern Med* 2009; **48**: 19–24.
25 Fortrat JO, de Germain V, Custaud MA. Holter heart rate variability: are we measuring physical activity? *Am J Cardiol* 2010; **106**: 448–449.
26 Miyamoto S, Fujita M, Sekiguchi H *et al.* Effects of posture on cardiac autonomic nervous activity in patients with congestive heart failure. *J Am Coll Cardiol* 2001; **37**: 1788–1793.
27 Dütsch M, Burger M, Dörfler C, Schwab S, Hilz MJ. Cardiovascular autonomic function in poststroke patients. *Neurology* 2007; **69**: 2249–2255.
28 Colivicchi F, Bassi A, Santini M, Caltagirone C. Cardiac autonomic derangement and arrhythmias in right-sided stroke with insular involvement. *Stroke* 2004; **35**: 2094–2098.
29 Appenzeller O, Oribe E. *The Autonomic Nervous System. An Introduction to Basic and Clinical Concepts*, 5th edn. Oxford: Elsevier Science Publishers, 1997.
30 Huikuri HV, Niemelä MJ, Ojala S, Rantala A, Ikäheimo MJ, Airaksinen KE. Circadian rhythms of frequency domain measures of heart rate variability in healthy subjects and patients with coronary artery disease. Effects of arousal and upright posture. *Circulation* 1994; **90**: 121–126.
31 Stein PK, Brazilay JI, Chaves PH, Domitrovich PP, Gottdiener JS. Heart rate variability and its changes over 5 years in older adults. *Age Ageing* 2009; **38**: 212–218.
32 La Rovere MT, Pinna GD, Maestri R *et al.* Short-term heart rate variability strongly predicts sudden cardiac death in chronic heart failure patients. *Circulation* 2003; **107**: 565–570.
33 Kleiger RE, Miller JP, Bigger JT Jr, Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am J Cardiol* 1987; **59**: 256–262.
34 Nakamura T, Mizushima T, Yamamoto M, Kawazu T, Umezu Y, Tajima F. Muscle sympathetic nerve activity during isometric exercise in patients with cerebrovascular accidents. *Arch Phys Med Rehabil* 2005; **86**: 436–441.
35 Astrand PO, Cuddy TE, Saltin B, Stenberg J. Cardiac output during submaximal and maximal work. *J Appl Physiol* 1964; **19**: 268–274.
36 Koopman R, Ryall JG, Church JE, Lynch S. The role of β-adrenoceptor signaling in skeletal muscle: therapeutic implications for muscle wasting disorders. *Curr Opin Clin Nutr Metab Care* 2009; **12**: 601–606.
37 Lynch GS, Ryall JG. Role of beta-adrenoceptor signaling in skeletal muscle: implications for muscle wasting and disease. *Physiol Rev* 2008; **88**: 729–767.
38 Carter WJ, Dang AQ, Faas FH, Lynch ME. Effects of clenbuterol on skeletal muscle mass, body composition, and recovery from surgical stress in senescent rats. *Metabolism* 1991; **40**: 855–860.
39 Carter WJ, Lynch ME. Effect of clenbuterol on recovery of muscle mass and carcass protein content following dietary protein depletion in young and old rats. *J Gerontol* 1994; **49**: B162–B168.
40 Chen KD, Alway SE. Clenbuterol reduces soleus muscle fatigue during disuse in aged rats. *Muscle Nerve* 2001; **24**: 211–222.
41 Ryall JG, Plant DR, Gregorevic P, Sillence MN, Lynch GS. Beta 2-agonist administration reverses muscle wasting and improves muscle function in aged rats. *J Physiol* 2004; **555**: 175–188.
42 Ricart-Firinga C, Stevens L, Canu MH, Nemirovskaya TL, Mounier Y. Effects of beta(2)-agonist clenbuterol on biochemical and contractile properties of unloaded soleus fibers of rat. *Am J Physiol Cell Physiol* 2000; **278**: C582–C588.
43 Stevens L, Firinga C, Gohlsch B, Bastide B, Mounier Y, Pette D. Effects of unweighting and clenbuterol on myosin light and heavy chains in fast and slow muscles of rat. *Am J Physiol Cell Physiol* 2000; **279**: C1558–C1563.
44 Ryall JG, Scherzer JD, Lynch GS *et al.* Attenuation of age-related muscle wasting and weakness in rats after formoterol treatment: therapeutic implications for sarcopenia. *J Gerontol* 2007; **62A**: 813–823.
45 Peters R, Beckett N, Burch L *et al.* The effect of treatment based on a diuretic (indapamide) +/- ACE inhibitor (perindopril) on fractures in the Hypertension in the Very Elderly Trial (HYVET). *Age Ageing* 2010; **39**: 609–616.
46 Lucini D, Mela GS, Malliani A, Pagani M. Impairment in cardiac autonomic regulation preceding arterial hypertension in humans: insights from spectral analysis of beat-by-beat cardiovascular variability. *Circulation* 2002; **106**: 2673–2679.

© 2013 Japan Geriatrics Society

47 Burns J, Sivananthan MU, Ball SG, Mackintosh AF, Mary DA, Greenwood JP. Relationship between central sympathetic drive and magnetic resonance imaging-determined left ventricular mass in essential hypertension. *Circulation* 2007; **115**: 1999–2005.
48 Stessman J, Rozenberg RH, Cohen A, Mor EE, Jacob JM. Physical activity, function, and longevity among the very old. *Arch Intern Med* 2009; **169**: 1476–1483.
49 Rantanen T, Voipato S, Ferrucci L, Heikkinen E, Fried LP, Guralnik JM. Handgrip strength and cause-specific and total mortality in older disabled women: exploring the mechanism. *J Am Geriatr Soc* 2003; **51**: 636–641.
50 Ling CHY, Taekema D, de Craen AJM, Gussekloo J, Westendorp RGJ, Maier AB. Handgrip strength and mortality in the oldest old population: the Leiden 85-plus study. *CMAJ* 2010; **182**: 429–435.

© 2013 Japan Geriatrics Society

Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/authorsrights

Atherosclerosis 231 (2013) 365–370

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Efficacy of combined use of three non-invasive atherosclerosis tests to predict vascular events in the elderly; carotid intima-media thickness, flow-mediated dilation of brachial artery and pulse wave velocity

Kumiko Nagai [a], Shigeki Shibata [a], Masahiro Akishita [b], Noriko Sudoh [a], Toshimasa Obara [a], Kenji Toba [c], Koichi Kozaki [a,*]

[a] Department of Geriatric Medicine, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka, Tokyo 181-8611, Japan
[b] Department of Geriatric Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan
[c] National Center for Geriatrics and Gerontology, Aichi, Japan

ARTICLE INFO

Article history:
Received 28 December 2012
Received in revised form
25 September 2013
Accepted 30 September 2013
Available online 16 October 2013

Keywords:
Intima-media thickness
Flow-mediated dilation
Pulse wave velocity
Vascular event

ABSTRACT

Background: Intima-media thickness (IMT) of the carotid artery, flow-mediated dilation (FMD) of the brachial artery, and pulse wave velocity of the central artery (PWV) have been widely used to evaluate progression of atherosclerosis. Our previous work has revealed that IMT, FMD and PWV are related to each other, and the combination of these measurements was useful in identifying patients with atherosclerotic disease. The aim of the present study was to investigate whether combination of these measurements would predict future cardiovascular events better than each test alone.

Methods and results: From November 2000 to March 2008, 274 consecutive elderly subjects (men/women; 114/160, mean age; 71 ± 12 years) were enrolled in this study. We measured IMT, FMD, and PWV in all of these subjects and followed them for a mean of 41 ± 28 months. During the follow-up period, vascular events occurred in 42 patients (15.3%). IMT (hazard ratio = 1.28 [95%CI, 1.09–1.50], $p = 0.002$ per 0.1 mm increase in mean IMT) and brachial-ankle (ba) PWV (hazard ratio = 1.06 [95%CI, 1.01–1.10], $p = 0.015$ per 1 m/s increase in baPWV) were independent predictors of future vascular events by Cox proportional hazard analysis, although FMD did not reach statistical significance (hazard ratio = 0.85 [95%CI, 0.72–1.01], $p = 0.062$ per 1% increase in %FMD). Importantly, the number of tests showing results in the worst tertile was a more powerful predictor (hazard ratio = 2.21 [95%CI, 1.42–3.43], $p = 0.0004$ for number of tests showing worst tertile) of future vascular events than either IMT, baPWV, or FMD alone. When both IMT and baPWV (with respective cut-off values of 0.98 mm and 19.1 m/s) were taken into consideration, the efficacy increased as compared with each test alone (odds ratio 4.9).

Conclusion: These results indicate that IMT and baPWV, especially when combined, are useful in predicting future vascular events in elderly subjects.

© 2013 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Recently, noninvasive tests of atherosclerosis have been clinically available, such as common carotid intima-media thickness (IMT), flow-mediated dilation (FMD) of the brachial artery, pulse wave velocity (PWV), beta stiffness index, and systemic arterial stiffness [1]. Several epidemiologic studies have shown that IMT, PWV, and FMD are important, independent determinants of cardiovascular risk in patients with cardiovascular disease [2–5], or diabetes mellitus [6,7] and healthy adults [1,8–12]. These three tests assess different aspects of atherosclerosis; carotid IMT reflects structural changes in the artery wall [13], PWV reflects central arterial stiffness, and FMD reflects endothelial function. While the majority of previous studies utilized a single method to evaluate atherosclerosis, several recent studies showed that combination of two measurements may strengthen the predictive power for future cardiovascular events [14,15].

Consistent with these studies, our previous article showed that a combination of IMT, FMD and PWV was able to predict more reliably the prevalence of atherosclerotic disease in an elderly population than did each test alone [16]. However, it is not clear whether the combination of these three tests is more reliable in predicting future vascular events than is each single test. Thus, the purpose of the present study was to prove the hypothesis that the combination

* Corresponding author. Tel.: +81 422 47 5511; fax: +81 422 44 1917.
E-mail addresses: kozaki-tky@umin.org, kozaki-tky@umin.ac.jp (K. Kozaki).

0021-9150/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.atherosclerosis.2013.09.028

of the three different methods to evaluate atherosclerosis would strengthen the predictive power over each test alone. For this purpose, we followed vascular events in patients in whom IMT, FMD and PWV were measured in advance.

2. Methods

2.1. Subject background

From November 2000 to March 2008, 274 consecutive subjects from outpatients of the Department of Geriatric Medicine, Kyorin University Hospital (Tokyo, Japan) were enrolled in this study (Table 1). Three non-invasive atherosclerosis tests (IMT, FMD and PWV) were performed in these subjects for the study purpose. All participants gave written informed consent to the study, which was approved by our institutional ethics committee. The study was performed in compliance with the Helsinki declaration. We included all subjects who agreed to participate in the study, and in whom IMT, FMD and PWV were available.

Diabetes mellitus was defined as fasting glucose of 126 mg/dL or higher, or the use of hypoglycemic medication. Resting blood pressure was measured three times in the seated position, and the average of the second and third readings was recorded. Hypertension was defined as systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, or use of medication prescribed for hypertension. Body mass index was calculated as weight (kg)/height^2 (m^2). Total and high-density lipoprotein (HDL) cholesterol were measured in blood samples obtained after a 12-h fast. Low-density lipoprotein (LDL) cholesterol was estimated by the Friedewald equation. Framingham risk score was determined from age, sex, smoking, blood pressures, diabetes, total cholesterol and HDL cholesterol based on the report by Wilson et al. [11].

Table 1
Clinical characteristics of study subjects.

	Vascular event		
	With (n = 42)	Without (n = 232)	p
Sex (male/female)	24/18	90/142	0.026
Age, y/o	74 ± 12	71 ± 12	0.087
Body mass index (kg/m^2)	23 ± 4	23 ± 3	0.208
Number of risk factors	1.8 ± 1.1	1.6 ± 1.0	0.281
Hypertension, n (%)	31 (74)	126 (54)	0.019
Hyperlipidemia, n (%)	14 (33)	115 (50)	0.052
Diabetes mellitus, n (%)	12 (29)	57 (25)	0.582
Chronic pulmonary disease, n (%)	0 (0)	2 (0.9)	0.546
Kidney disease, n (%)	1 (2.4)	8 (3.4)	0.721
Chronic systemic inflammatory disease, n (%)	0 (0)	2 (0.9)	0.546
Smokers, n (%)			
Never	22 (52)	139 (60)	0.364
Past	17 (41)	69 (30)	–
Current	3 (7)	24 (10)	–
History of stroke, n (%)			
Cerebral infarction	7 (17)	14 (6)	0.295
Brain hemorrhage	0 (0)	3 (1)	–
Cerebral thrombosis	1 (2)	1 (0)	–
Cerebral infarction & hemorrhage	0 (0)	2 (9)	–
Multiple cerebral infarction	0 (0)	7 (3)	–
Transient ischemic attack	1 (2)	0 (0)	–
Unknown	2 (5)	6 (3)	–
History of IHD, n (%)			
Angina pectoris	3 (7)	7 (3)	0.560
Myocardial infarction	0 (0)	4 (2)	–
Unknown	0 (0)	3 (1)	–
Atherosclerosis measurements			
Mean IMT, mm	1.06 ± 0.21	0.94 ± 0.19	0.000
FMD, %	2.01 ± 1.71	2.83 ± 2.42	0.045
baPWV, m/s	22.5 ± 6.6	19.7 ± 6.5	0.018
Medication			
ACEI/ARB, n (%)	13 (33)	54 (24)	0.210
Ca blocker, n (%)	14 (35)	58 (16)	0.185
β-Blocker, n (%)	3 (8)	10 (4)	0.383
Statin, n (%)	5 (12)	38 (17)	0.426
Anti-platelet agent, n (%)	15 (38)	42 (19)	0.009

Data are expressed as mean ± SD. FMD, flow-mediated dilation of right brachial artery; IMT, intima-media thickness of common carotid artery; baPWV, brachial-ankle pulse wave velocity, IHD, ischemic heart disease; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; Smoker Never, no smoking history; Smoker Past, previously smoked and quit; Smoker Current, currently regularly smoking.
Student's t test for continuous variables and χ^2 test for categorical variables.

2.2. Participant follow-up and CV events

The occurrence of vascular events was investigated by inquiry to the attending doctor (n = 234), examining the patient's clinical record (n = 9), or inquiry to the patient and/or the family by either telephone (n = 13) or mail (n = 18). The majority of cardiovascular events (n = 243/274) was confirmed as follows: angina and myocardial infarction were confirmed by clinical symptoms and/or coronary arteriography; cerebral hemorrhage, subarachnoid hemorrhage, cerebral infarction and transient ischemic attack were confirmed by clinical symptoms followed by computed tomography, MRI and/or angiography; heart failure, renal failure and arteriosclerosis obliterans were diagnosed according to the clinical guidelines; and aortic dissection was confirmed by contrast computed tomography.

2.3. Measurements of atherosclerosis

Measurements of atherosclerosis were performed as previously described [16]. All examinations were performed by the same skilled technician throughout the study. The subject reclined on the examination table for at least 15 min before the examination to obtain hemodynamic stability.

2.3.1. Measurement of carotid IMT

Common carotid IMT was measured by ultrasound (PowerVision6000, Toshiba) with a 7.5 MHz linear-array transducer. The images were recorded on S-VHS videotape. IMT at the far wall of the common carotid artery was measured by B-mode scan within 10 mm proximal to the bifurcation. Four points were measured in one scan, and mean IMT was calculated [2,16]. The typical error as a coefficient of intra-observer variation in the measurement of IMT was 3.7%, and changes in mean were 2.0%.

2.3.2. Measurement of FMD of brachial artery

The diameter of the artery was measured by ultrasound (PowerVision6000, Toshiba) with a 7.5 MHz linear-array transducer. The images were recorded on S-VHS videotape. The mean diameter of the brachial artery was calculated from four cardiac cycles synchronized with the R-wave peaks on ECG. After a 10 min rest in the supine position, the right brachial artery was scanned. After recording the resting diameter, a cuff was placed around the forearm distal to the target artery and inflated to a pressure of 250 mmHg. Inflation was maintained for 5 min. Maximal vasodilation was observed 45–60 s after cuff release. The change in diameter caused by the restoration of blood flow was expressed as the percent change relative to the initial diameter [16–18]. The typical error as a coefficient of intra-observer variation in the measurement of FMD was 7.4%, and changes in mean were 0.1%.

2.3.3. Measurement of PWV

Brachial-ankle (ba) PWV was measured using an automated device (Form PWV/ABI, OMRON-COLIN, Japan). The average measurement of left and right baPWV was used for analysis. The typical error as a coefficient of intra-observer variation in the measurement of PWV was 2.0%, and changes in mean were 0.2%.

2.4. Statistical analysis

All data are expressed as mean ± SD. Patients were classified according to the tertiles of IMT, %FMD and baPWV. Event rate was calculated using the Kaplan–Meier method, and the statistical significance of differences was investigated by log-rank test. A Cox proportional hazard model was used to determine the variables independently associated with vascular events. Odds ratio was calculated by logistic regression analysis to evaluate the association of event occurrence and each atherosclerosis measurement, with adjusted for age and sex as well as FRS. Receiver operating characteristic curve analysis was performed to estimate the best cut-off point in each test for predicting future vascular events. A p value <0.05 was considered statistically significant.

3. Results

3.1. Subjects

In the 274 patients, the mean duration of follow-up was 41 ± 28 months. During this time, 42 (15.3%) patients experienced vascular events: 14 (33.3%) had angina, 13 (31.0%) stroke, 10 (23.8%) heart failure, 6 (14.3%) renal failure, 3 (7.1%) myocardial infarction, 3 (7.1%) transient ischemic attack, 2 (4.8%) arteriosclerosis obliterans, 2 (4.8%) cerebral hemorrhage, 1 (2.4%) aortic dissection, and 1 (2.4%) subarachnoid hemorrhage.

As shown in Table 1, male sex and hypertension were more frequent in patients with vascular events than in those without events. In addition, patients with vascular events showed thicker mean IMT, smaller %FMD, and greater baPWV than those without events.

3.2. Tertiles and prognostic value of each test

With regard to IMT and baPWV, Kaplan–Meier analysis showed that patients in the worst tertile experienced a higher rate of vascular events than those in the other two tertiles (Fig. 1a, b). A similar trend was also found for %FMD, although not reaching statistical significance ($p = 0.052$ by log-rank test, Fig. 1c). Of note, patients in the three worst tertiles had a markedly higher rate of vascular events than those in the other groups (0, 1, and 2 in Fig. 1d).

In the Cox proportional hazard model, IMT, baPWV, and the number of results in the worst tertiles were significantly associated with vascular events. They remained significant after adjusting for age and sex (Table 2), and FRS (Table 3). FRS alone was not a significant predictive factor ($p = 0.203$, RR 0.987, 95%CI 0.966–1.007).

3.3. Test combination model and vascular events

Receiver operating characteristic curve analysis demonstrated that IMT of 0.98 mm (area under curve = 0.72, sensitivity = 83%, specificity = 57%) and baPWV of 19.1 m/s (area under curve = 0.67, sensitivity = 61%, specificity = 63%) were the best cut-off points for predicting future vascular events.

When the subjects were subdivided into four groups according to the cut-off values of IMT and baPWV, Kaplan–Meier curves showed a stepwise increase in the risk of vascular events (Fig. 2a). Patients with both IMT and baPWV above the cut-off values (group IV) showed the highest rate of vascular events. In addition, the odds ratio of vascular events in group IV was significantly higher than that in group I (Fig. 2b).

4. Discussion

Consistent with the hypothesis, the combination of the three atherosclerosis measurements (IMT, %FMD, and baPWV) was

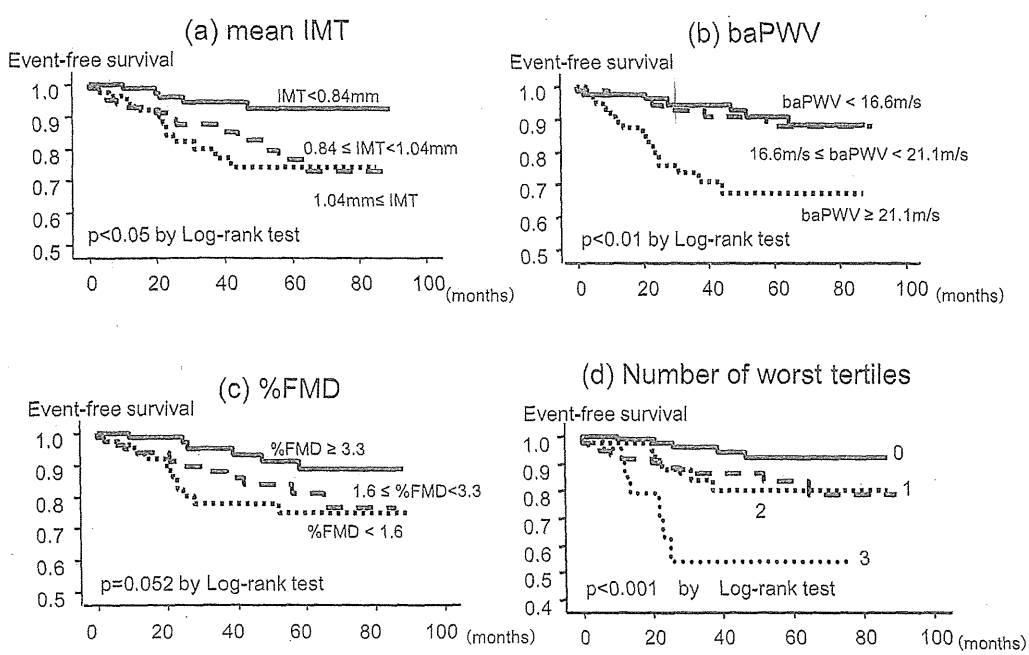


Fig. 1. Kaplan–Meier curves according to tertiles of (a) IMT, (b) baPWV, (c) %FMD, and (d) number of worst tertiles in atherosclerosis tests.

Table 2
Predictive value for future vascular events by Cox proportional hazard analysis adjusted by age and sex.

Variable	Unadjusted ($n = 274$)		Adjusted for age and sex ($n = 274$)	
	HR (95%CI)	p	HR (95%CI)	p
IMT, tertile (increase of 1)	1.836 (1.187–2.840)	0.0064	1.606 (1.002–2.753)	0.0489
IMT, 0.1 mm	1.279 (1.093–1.497)	0.0022	1.226 (1.034–1.454)	0.0191
baPWV, tertile (increase of 1)	2.191 (1.365–3.516)	0.0012	1.969 (1.115–3.476)	0.0195
baPWV, 1.0 m/s	1.055 (1.010–1.101)	0.0152	1.027 (0.970–1.088)	0.3552
FMD, tertile (increase of 1)	1.691 (1.090–2.624)	0.0190	1.631 (0.925–2.342)	0.1029
FMD, 1%	0.849 (0.716–1.008)	0.0615	0.903 (0.756–1.079)	0.2608
Number of worst tertiles	1.930 (1.353–2.754)	0.0003	1.891 (1.229–2.912)	0.0038

HR, hazard ratio; CI, confidence interval; other abbreviations are as in Table 1.

shown to be more powerful in predicting future vascular events as compared with each single test. This finding is consistent with our previous study showing that the combination of these three tests more reliably reflected the prevalence of atherosclerotic disease in the elderly population than did each test alone [16]. Here, IMT, baPWV, and %FMD (although less sensitively) were shown to predict future vascular events in the elderly population. This is in agreement with previous longitudinal studies showing that IMT was a predictor of future vascular disease [2,19], including a study of an elderly population [8], as well as central PWV in a study of subjects over 70 years old [4,11].

With regard to FMD, the present study did not show a significant association with the occurrence of vascular events. Considering the low value and small difference in FMD in the two groups with and without vascular events, one possible explanation for the non-significance is the floor effect. In support of this result, a significant relationship was found between baseline FMD and future cardiovascular events in middle-aged adults [9,10], whereas the prognostic power declined linearly with advancing age from the mid-40s, reaching nearly zero around 70 years of age [20]. Considering the subjects' age in the present study, the non-significance of FMD could be attributable to the advanced age of the patients.

An important point of the present study is that while IMT, baPWV, and %FMD were useful to predict future vascular events, combination of these tests increased the predictive power (Fig. 1d). This finding was consistent with those of our previous cross-sectional study showing that the result of a combination of the three tests was more strongly related to the prevalence of vascular disease in the elderly population than was that of each single test [16]. Several longitudinal studies have shown efficacy of the combination of two different atherosclerosis tests, such as FMD and ankle-brachial pressure index [14], plaque score of the common carotid artery and FMD [15], or carotid IMT and FMD [21], in predicting vascular events. The present study showed strong predictive power of combining three atherosclerosis tests for future vascular events. From our results, it is recommended that three tests should be combined in clinical work to evaluate vascular risk. However, when cost-effectiveness is taken into account, the combination of two tests (IMT and baPWV) would be sufficiently practical for event prediction in the elderly population because FMD requires much more skill and time than does IMT or baPWV. The same idea has been introduced in the recent guidelines from the ACCF/AHA for the assessment of cardiovascular risk in asymptomatic adults [22].

Table 3
Predictive value for future vascular events by Cox proportional hazard analysis adjusted by Framingham Risk Score (FRS).

Variable	Unadjusted ($n = 215$)		Adjusted for FRS ($n = 215$)	
	HR (95%CI)	p	HR (95%CI)	p
IMT, tertile (increase of 1)	1.704 (1.044–2.782)	0.0329	1.669 (1.018–2.735)	0.0422
IMT, 0.1 mm	1.277 (1.069–1.526)	0.0071	1.281 (1.064–1.544)	0.0090
baPWV, tertile (increase of 1)	2.675 (1.522–4.700)	0.0006	2.582 (1.445–4.614)	0.0014
baPWV, 1.0 m/s	1.065 (1.018–1.115)	0.0060	1.060 (1.011–1.111)	0.0166
FMD, tertile (increase of 1)	1.785 (1.072–2.973)	0.0260	1.669 (0.989–2.815)	0.0548
FMD, 1%	0.864 (0.716–1.043)	0.1281	0.888 (0.730–1.080)	0.2339
Number of worst tertiles	2.031 (1.350–3.055)	0.0007	1.991 (1.309–3.027)	0.0013

HR, hazard ratio; CI, confidence interval; other abbreviations are as in Table 1.

Increased carotid IMT has been considered as a marker of subclinical atherosclerosis. Although the biological meaning of IMT remains to be debated, it seems more likely to represent target organ damage related to cardiovascular risk [13]. PWV is the mostly widely used index for evaluating central arterial stiffness. FMD is a tool that is proposed for the assessment of endothelial function and is related to cardiovascular risk, but is not yet a commonly applied method to assess CV risk. Our major finding that the combination of three tests was more predictive than each test alone may be attributable to the fact that each test reflects a different aspect of the progression of atherosclerosis.

The best cutoff value of IMT calculated from event prediction was 0.98 mm in the present study. This value was comparable to previously reported values; approximately ~1.00 mm in healthy adults in spite of populations of different ages; middle age [23–25], over 55 yearsold [26], and 60–74 years old [27]. Although the detailed methodologies were slightly different between studies, ~1.00 mm appears to be relevant to the occurrence of vascular events.

The cutoff value of baPWV calculated from the receiver operating characteristic curve was 19.1 m/s in the present study. This value is slightly higher as compared with values reported previously; that for major cardiovascular events in patients with acute coronary syndrome was 18.0 m/s [28], and that for re-hospitalization and cardiac death in patients with heart failure was 17.5 m/s [29]. On the other hand, the cut-off value for cardiovascular death in community-dwelling elderly people (LILAC study [30]) was higher (25 m/s) than that in the present study. This difference could be explained by the susceptibility to arteriosclerosis in different subjects depending on whether they have preexisting cardiovascular disease and how old they are.

Because of the efficacy of the cut-off values of baPWV and IMT, we investigated the significance of the combination of these two measurements. A stepwise increase in the risk of vascular events was evident by Kaplan–Meier analysis and calculated odds ratio. This is important because much higher predictability can be obtained by simple non-invasive tests. Although FMD did not reach statistical significance, the combination of even three tests would strengthen the predictive power. Indeed, our previous results showed higher prevalence of atherosclerotic disease by combining three tests. Considering the efficacy and simplicity of performance of the three tests, combination of baPWV and IMT (with cut-off values of 19.1 m/s and 0.98 mm, respectively) should be of value for prediction of future occurrence of vascular events in elderly patients.

5. Limitations

One of the limitations of this study was that our approach of three tests for atherosclerosis did not follow the most updated

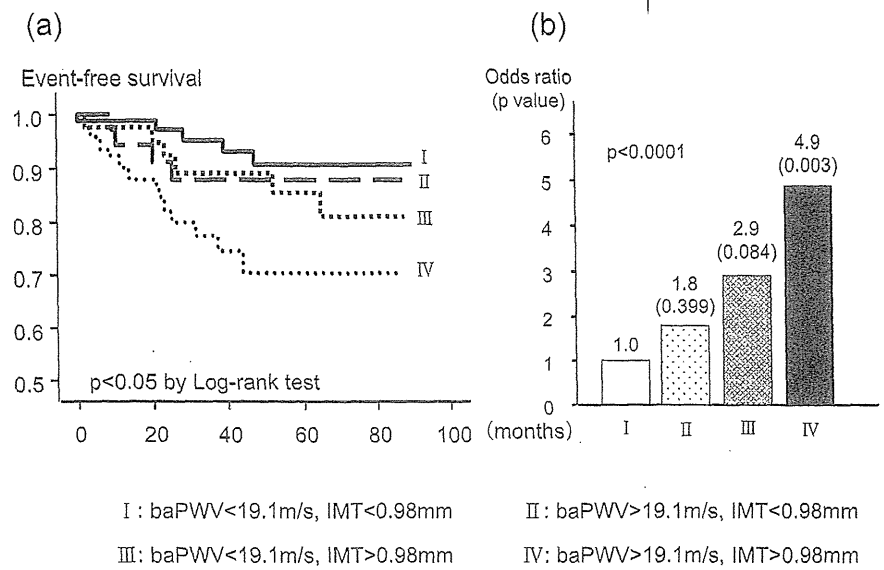


Fig. 2. (a) Kaplan–Meier curves and (b) adjusted relative risks of future vascular events according to cut-off values of baPWV and IMT. Odds ratio and *p* value, in parentheses, are indicated over the bar.

methodologies, because they were not fully established when we started this study. The most recent approaches may provide better predictive values for PWV and IMT, and statistical significance for FMD. Particularly, we manually held the echo probe for FMD measurement, which may have led to low intra-observer reproducibility and no statistical significance.

Second, despite the number of controls, the number of patients was very small.

Third, we included renal failure and heart failure as vascular events since these diseases are thought to be associated with progression of atherosclerosis in the elderly. However, it is possible that heart failure and renal failure may be caused by other etiologies such as collagen disease, infection, valvular disease, etc. Therefore, we also analyzed predictive values excluding heart failure and renal failure as vascular events, and obtained similar results. Thus, the effect of bias in selecting vascular events is considered to be small.

6. Conclusion

IMT, baPWV and, less significantly, FMD, especially when combined, are useful to predict future vascular events in elderly subjects. Because elderly people are at high risk for vascular disease, performing these simple and reliable non-invasive tests will add important clinical information.

Acknowledgments

This study was supported by a Health and Labour Sciences Research Grant (H24-Ninchisho-002) from the Ministry of Health, Labour and Welfare of Japan, and by the Hakujikai Institute of Gerontology. We appreciate Mr. Shinya Ishii for his statistical advice.

References

[1] Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J 2006;27:2588–605.
[2] Simon A, Chironi G, Levenson J. Comparative performance of subclinical atherosclerosis tests in predicting coronary heart disease in asymptomatic individuals. Eur Heart J 2007;28:2967–71.
[3] Boutouyrie P, Tropeano AI, Asmar R, et al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension 2002;39:10–5.
[4] Sutton-Tyrrell K, Najjar SS, Boudreau RM, et al. Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. Circulation 2005;111:3384–90.
[5] Ciccone MM, Scicchitano P, Zito A, et al. Correlation between coronary artery disease severity, left ventricular mass index and carotid intima media thickness, assessed by radio-frequency. Cardiovasc Ultrasound 2011;9:32.
[6] Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? Circulation 2002;106:2085–90.
[7] Folsom AR, Chambless LE, Duncan BB, Gilbert AC, Pankow JS. Prediction of coronary heart disease in middle-aged adults with diabetes. Diabetes Care 2003;26:2777–84.
[8] O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson Jr SK. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med 1999;340:14–22.
[9] Shechter M, Issachar A, Marai I, et al. Long-term association of brachial artery flow-mediated vasodilation and cardiovascular events in middle-aged subjects with no apparent heart disease. Int J Cardiol 2009;134:52–8.
[10] Yeboah J, Folsom AR, Burke GL, et al. Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: the multi-ethnic study of atherosclerosis. Circulation 2009;120:502–9.
[11] Meaume S, Benetos A, Henry OF, Rudnichi A, Safar ME. Aortic pulse wave velocity predicts cardiovascular mortality in subjects >70 years of age. Arterioscler Thromb Vasc Biol 2001;21:2046–50.
[12] Yeboah J, Crouse JR, Hsu FC, Burke GL, Herrington DM. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. Circulation 2007;115:2390–7.
[13] Mancini GB, Dahlof B, Diez J. Surrogate markers for cardiovascular disease: structural markers. Circulation 2004;109:IV22–30.
[14] Brevetti G, Silvestro A, Schiano V, Chiariello M. Endothelial dysfunction and cardiovascular risk prediction in peripheral arterial disease: additive value of flow-mediated dilation to ankle-brachial pressure index. Circulation 2003;108:2093–8.
[15] Chan SY, Mancini GB, Kuramoto L, Schulzer M, Frohlich J, Ignaszewski A. The prognostic importance of endothelial dysfunction and carotid atheroma burden in patients with coronary artery disease. J Am Coll Cardiol 2003;42:1037–43.
[16] Kobayashi K, Akishita M, Yu W, Hashimoto M, Ohni M, Toba K. Interrelationship between non-invasive measurements of atherosclerosis: flow-mediated dilation of brachial artery, carotid intima-media thickness and pulse wave velocity. Atherosclerosis 2004;173:13–8.
[17] Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the