Table 3 – Logistic analysis of 11β-HSD1 SNPs with the risk of metabolic syndrome adjusted by age and sex (both) or by age (men or women).

	MS n/n' (%)	Control n/n' (%)	OR (95%C.I.)	P	Pcorr
Both					
+9410T>A	61/370 (14.2)	91/686 (11.7)	1.5 (1.0–2.2)	0.041	NS
+17925C>T	249/182 (57.8)	453/324 (58.3)	1.0 (0.7–1.2)	0.683	NS
+27447G>C	118/313 (27.4)	199/578 (25.6)	1.1 (0.8–1.4)	0.632	NS
Men					
+9410T>A	45/286 (13.6)	16/182 (8.1)	1.9 (1.1–3.5)	0.029	NS
Women					
+9410T>A	16/84 (16.0)	75/504 (13.0)	1.1 (0.6–2.1)	0.683	NS

MS: metabolic syndrome, n: number of minor homozygote and heterozygote, n': number of major homozygote, %: $n/(n+n') \times 100$, 95%C.I: 95% confidence interval.
Odds ratio and 95%C.I. is expressed as per copy of the minor allele for additive model.
Pcorr is P values after Bonferroni correction.
NS means not significance.

and age (OR = 1.5 for allelic effect, 95%C.I., 1.0–2.2; P = 0.041 and Bonferroni corrected P = 0.123). In only men, +9410T>A SNP was nominally associated with metabolic syndrome, while the higher frequency of metabolic syndrome in men lead to the higher power in comparison with women. Taken together all, after considering multiple comparisons we could not find any statistically significant association between metabolic syndrome and SNPs in the HSD11B1 gene. Furthermore, we could not find any significant association between metabolic syndrome of the ATP III criteria and these three SNPs, respectively. We next performed the covariance analysis of the traits related to metabolic syndrome including BMI, waist circumference, systolic and diastolic blood pressures, fasting glucose, HbA1c, and triglyceride and HDL-cholesterol levels in person with or without the +9410T>A SNP, but the SNP did not affect these clinical parameters in total population or only men (Table 4).

Next we studied haplotypes of the HSD11B1. The association between haplotypes comprising SNP-5, -6, and -7 and metabolic syndrome revealed that any haplotype could not have a significant susceptibility to metabolic syndrome

(Table 5). The ATG haplotype was nominally associated with a increased risk of metabolic syndrome in men (metabolic syndrome 7.1%, control 4.0%; OR = 1.82, 95%C.I., 1.01–3.25; P = 0.042 and Bonferroni corrected P = 0.168), while the TTG haplotype was nominally associated with a decreased risk of metabolic syndrome in men (metabolic syndrome 26.4%, control 32.6%; OR = 0.74, 95%C.I., 0.57–0.98; P = 0.033 and Bonferroni corrected P = 0.132). Although only the TTG haplotype had decreased risk effect among haplotypes with +9410T, the association of these haplotypes with metabolic syndrome is mainly explained by +9410T>A SNP.

4. Discussion

This study was a case–control study using a population-based urban Japanese cohort. Metabolic syndrome was diagnosed according to the 2005 Japanese definition [18] and the control subjects were defined as having none of the components of this syndrome. Using these criteria, we obtained 431 individuals with metabolic syndrome and 777 control subjects for

Table 4 – Comparison of clinical parameters in urban Japanese men and women (n = 3005) according to 11β-HSD1 gene +9410T>A genotype.

	Men (n = 1370)			Women (n = 1635)		
	TT (n = 1180)	TA + AA (n = 189)	P	TT (n = 1400)	TA + AA (n = 235)	P
BMI (kg/m²)	24.1 ± 0.1	23.0 ± 2.9	0.502	22.9 ± 0.1	23.0 ± 0.2	0.622
Waist (cm)	87.7 ± 0.2	87.5 ± 0.6	0.800	84.4 ± 0.3	84.1 ± 0.6	0.695
SBP (mmHg)	132.6 ± 0.6	133.8 ± 1.5	0.442	129.8 ± 0.5	129.3 ± 1.3	0.686
DBP (mmHg)	79.9 ± 0.3	80.4 ± 0.8	0.587	77.1 ± 0.3	77.2 ± 0.7	0.834
FBG (mmol/l)	5.83 ± 0.04	5.96 ± 0.11	0.256	5.47 ± 0.03	5.55 ± 0.07	0.309
HbA1c (%)	5.61 ± 0.03	5.70 ± 0.07	0.233	5.45 ± 0.02	5.47 ± 0.05	0.607
TG (mmol/l)	1.47 ± 0.03	1.44 ± 0.07	0.732	1.12 ± 0.02	1.15 ± 0.04	0.582
HDLc (mmol/l)	1.35 ± 0.01	1.38 ± 0.03	0.369	1.64 ± 0.01	1.62 ± 0.03	0.418

SBP: systolic blood pressure, DBP: diastolic blood pressure, FBG: fasting blood glucose, TG: triglycerides, HDLc: HDL cholesterol. Data are shown as the adjusted mean ± SE. These values were obtained after adjusting for age by the least squares method. The laboratory data reported in milligram per deciliter can be converted to SI units as follows: total cholesterol, HDL cholesterol: mg/dl × 0.02586 = mmol/l; triglycerides: mg/dl × 0.01129 = mmol/l; fasting blood glucose: mg/dl × 0.05556 = mmol/l.
P-values for the comparison between TT and TA + AA genotype groups.

Gender	Haplotype	Total	MS	Control	x^2	OR	95%C.I.	P	Pcorr
Both	TCG	0.515	0.514	0.510	0.040	1.02	0.86–1.20	0.841	NS
	TTG	0.277	0.266	0.290	1.653	0.88	0.73–1.07	0.199	NS
	TCC	0.133	0.147	0.140	0.225	1.06	0.84–1.34	0.635	NS
	ATG	0.074	0.073	0.060	1.609	1.24	0.89–1.73	0.205	NS
Men	TCG	0.509	0.526	0.477	2.323	1.21	0.95–1.56	0.128	NS
	TTG	0.280	0.264	0.326	4.563	0.74	0.57–0.98	0.033	NS
	TCC	0.138	0.139	0.157	0.617	0.87	0.61–1.23	0.432	NS
	ATG	0.073	0.071	0.040	4.141	1.82	1.01–3.25	0.042	NS
Women	TCG	0.521	0.475	0.521	1.427	0.83	0.62–1.12	0.232	NS
	TTG	0.275	0.270	0.278	0.055	0.96	0.69–1.35	0.814	NS
	TCC	0.129	0.175	0.135	2.290	1.36	0.91–2.04	0.130	NS
	ATG	0.075	0.075	0.066	0.488	1.22	0.70–2.14	0.485	NS

Table 5 – Frequency of 11β-HSD1 gene haplotypes constructed by SNPs + 9410T>A, +17925C>T, and +27447G>C and their association with metabolic syndrome.

MS: metabolic syndrome; 95%C.I.: 95% confidence interval.
Pcorr is P values after Bonferroni correction.
NS means not significance.

the case–control study. We could not find any significant association between SNPs in the HSD11B1 gene and metabolic syndrome.

11β-HSD1 is expressed abundantly in adipose tissue and reactivates cortisone to cortisol [1]. Recent experiments using transgenic and knockout mice suggest that 11β-HSD1 plays a critical role in metabolic deterioration [6–9]. When cortisol generation is increased in peripheral tissues, the overall cortisol reaction is also increased. In humans, 11β-HSD1 expression is heightened in the adipose tissue of obese individuals [10]. Therefore, 11β-HSD1 is a promising target for the pharmacological inhibition in metabolic syndrome patients [11–13].

A previous study showed that a HSD11B1 gene polymorphism is associated with BMI and insulin resistance in a group of obese Pima Indian children [16] with type 2 diabetes mellitus and hypertension and reported that 11β-HSD1 mRNA concentrations were associated with adiposity [14,15]. The T → G polymorphism in the 3rd intron (rs12086634) protects against diabetes in Pima Indians [14] and reduces 11β-HSD1 gene transcription in vitro [19], which is consistent with reduced cortisol generation within cells. The rs12086634 polymorphism was completely in linkage disequilibrium with the rs932335 SNP (+27447G>C) that was analyzed in this study. We did not find a positive association between the +27447G>C SNP and metabolic syndrome in Japanese men.

There are some limitations in this study. First limitation was the Japanese criteria for metabolic syndrome, which is different from the ATP III criteria. Third, there were deviations in social factors, including age, gender, race/ethnicity, geographic location, and socioeconomic status. This cohort consisted of urban citizens residing in a subtropical area with a temperate climate. Most subjects were Asian with a higher percentage of elderly people.

In summary, the HSD11B1 gene is not associated with metabolic syndrome in Japanese. However, taken together with previous results, 11β-HSD1 might be involved in metabolic syndrome pathogenesis by modulating lipid metabolism and gluconeogenesis. Further studies are needed to investigate the role of 11β-HSD1 in metabolic syndrome.

Conflict of interest

The authors state that they have no conflict of interest.

Acknowledgments

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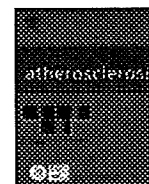
Triglycerides and non-high-density lipoprotein cholesterol and the incidence of cardiovascular disease in an urban Japanese cohort: The Suita study

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ABSTRACT

Objective: The impact of elevated triglycerides (TG) and non-high density lipoprotein cholesterol (non-HDLC) on the incidence of stroke and myocardial infarction (MI) has not been well evaluated in Asian populations such as in Japan, which have a lower incidence of myocardial infarction, but a higher risk of stroke than Western populations.
Methods: The authors conducted an 11.7-year prospective study ending in 2005 of 5098 Japanese aged 30–79 living in an urban population, initially free of stroke or MI. The relationship between serum lipids and the risk for stroke and MI was determined by dividing the participants into four groups stratified by the combination of serum levels of TG and non-HDLC. The cut-off value was 1.7 mmol/L for TG and 4.9 mmol/L for non-HDLC.
Results and conclusion: The total person-years were 59,774 (27,461 for men and 32,313 for women). During the follow-up period, there were 113 cases of MI and 180 of stoke (with 116 cerebral infarctions). Compared with the low TG/low non-HDLC group, the hazard ratio (95% confidence interval) for MI in the high TG/high non-HDLC group was 2.55 (1.53–4.24) after adjustment for other cardiovascular risk factors. The hazard ratio for cerebral infarction in the high TG alone group was 1.63 (1.03–2.56); however, the risk of cerebral infarction was not significantly increased in the other groups. High serum levels of TG and non-HDLC are both important targets for the prevention of cardiovascular disease in Japan.

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1. Introduction

Previous studies suggested that high levels of serum total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) are causal risk factors for coronary artery disease (CAD) [1–4] and possibly for ischemic stroke [5]. However, less attention has been paid to high serum levels of triglycerides (TG) [6–8]. Furthermore, although the US National Cholesterol Education Program Adult Treatment Panel guideline III (NCEP-ATP III) has set goals for non-high-density lipoprotein cholesterol (non-HDLC) after the achievement of LDL-C goals in patients with elevated TG [9], the impact of TG and non-HDLC on the incidence of cardiovascular disease (CVD) has not been evaluated in the Japanese population, which has a lower incidence of CAD but a higher risk of stroke than Western populations [10].

Therefore, our a priori hypothesis was that the coexistence of high serum TG and non-HDLC increases the risk of CAD and stroke in the Japanese population. To investigate this hypothesis, we performed a long-term prospective study in an urban, community-dwelling Japanese population.

2. Methods

2.1. Populations

The Suita study, a cohort study for CVD of urban residents was established in 1989. The details of this study have been described elsewhere [4,11–14]. Briefly, 6485 men and women aged 30–79 years had a baseline survey at the National Cardiovascular Center between September 1989 and March 1994. Of these, a total of 1387 were excluded for the following reasons: past history of coronary heart disease or stroke (n = 210), lack of participation in the baseline survey (n = 79), non-fasting visit (n = 166), use of lipid-lowering agents (n = 125), missing data (n = 109), and lost to follow-up (n = 698). Data from the remaining 5098 participants (2404 men and 2694 women) were included in the analysis. This

cohort study was approved by the Institutional Review Board of the National Cardiovascular Center.

2.2. Baseline examination

Blood samples were collected after the participants had fasted for at least 10 h. The samples were centrifuged immediately and a routine blood examination was performed that included serum total cholesterol (TC), HDL cholesterol, TG and glucose levels.

Blood pressure was measured in triplicate on the right arm after 5 min of rest by well-trained physicians using a standard mercury sphygmomanometer. The average of the second and third measurements was used for analysis. Hypertension was defined as either a systolic blood pressure (SBP) ≥ 140 mmHg, a diastolic blood pressure (DBP) ≥ 90 mmHg or the use of antihypertensive agents. Diabetes was defined as a fasting serum glucose ≥ 7.0 mmol/L (126 mg/dL), the use of anti-diabetic agents, or both. Height with bare feet and weight in light clothing were measured. Waist circumference (WC) was measured at umbilical level in a standing position. Metabolic syndrome (MetS) was defined using modified NCEP-ATP III criteria [13], of which abdominal obesity was defined according to the International Obesity Task Force central obesity criteria for Asia [15].

Public health nurses obtained information on the smoking, drinking and medical histories.

2.3. Endpoint determination

The endpoint determination was previously reported [4,11–14]. The endpoints of the present study were: (1) the first myocardial infarction (MI) or stroke event; (2) death; (3) leaving Suita city; or (4) December 31, 2005.

The first step in the survey for MI and stroke involved checking the health status of all participants by repeated clinical visits every two years and yearly questionnaires by mail or telephone. In the second step, in-hospital medical records of participants who were suspected of having an MI or stroke were reviewed by registered hospital physicians or research physicians, who were blinded to the baseline information. The criteria for stroke were defined according to the US National Survey of Stroke criteria [16]. For each stroke subtype [i.e., cerebral infarction, intracerebral hemorrhage, and subarachnoid hemorrhage], a definite diagnosis was established based on the computed tomography, magnetic resonance imaging, or autopsy. The criteria for definite and probable MI were defined according to the criteria of the MONICA (Monitoring Trends and Determinants of Cardiovascular Disease) project [17]. Sudden deaths of unknown origin that occurred within 24 h of the onset were classified as MI in the present study.

2.4. Statistical analysis

The relationship between serum lipids and the risk of MI and stroke was described by dividing the participants into four groups stratified by the combination of serum levels of TG and non-HDLC. We used 1.7 mmol/L (150 mg/dL) of serum TG as a cut-off point for high serum TG according to the classification of NCEP-ATP III [9] and that of the Japan Atherosclerosis Society [3]. The category of non-HDLC ≥ 4.9 mmol/L (190 mg/dL) was defined as a high serum non-HDLC, which was equivalent to 6.2 mmol/L (240 mg/dL) of TC or 4.1 mmol/L (160 mg/dL) of LDL-C, because non-HDLC was usually 0.8 mmol/L (30 mg/dL) higher than LDL-C [9,18–19].

Continuous variables between groups were compared by analysis of variance and categorical variables were compared by a chi-square test. The hazard ratio (HR) for MI or stroke was calculated using a proportional hazards model adjusted for age, hypertension (dichotomous variable), diabetes, HDL-C, body mass index (BMI), smoking (never-smoked; ex-smoker; current smoker) and drinking (never-drank; ex-drinker; regular drinker) (model 1). Sex-combined analysis with further adjustment for sex was also performed. Another statistical model after replacement of BMI and hypertension with WC and SBP level (continuous variable) was also performed (model 2).

All confidence intervals were estimated at the 95% level and significance was set at a P value of <0.05. The Statistical Package for the Social Sciences (SPSS Japan Inc. version 15.0J, Tokyo, Japan) was used for all the analyses.

3. Results

The median and interquartile range of serum TG in the baseline survey was 1.29 mmol/L (0.90, 1.90) in men and 0.98 mmol/L (0.73, 1.41) in women. The mean baseline serum non-HDLC was 3.93 ± 0.91 mmol/L in men and 4.03 ± 1.03 mmol/L in women.

The means or prevalence of major cardiovascular risk factors in each group stratified by the combination of serum levels of TG and non-HDLC are summarized in Table 1. There was no significant difference in mean age and the prevalence of smoking among the TG and non-HDLC groups for men. There were significant differences in all other variables. Mean BMI, waist circumference and the prevalence of hypertension and diabetes were highest in the high-TG/high non-HDLC group, whereas the values of these parameters were lowest in the low-TG/low-non-HDLC group for both sexes. The prevalence of Mets was much higher in the high-TG groups than in the low-TG groups irrespective of non-HDLC level.

The total person-years were 59,774 (27,461 for men and 32,313 for women), with a mean follow-up period of 11.7 years. During the follow-up period, there were 113 first MIs and 180 first strokes. The strokes consisted of 28 intracerebral hemorrhages, 116 cerebral infarctions, 21 subarachnoid hemorrhages and 15 unclassified cases.

Table 2 shows the number of cases, age and multivariable-adjusted HRs for MI stratified by TG and non-HDLC. Compared with the low TG/low non-HDLC group, the HR for MI in the high TG/high non-HDLC group was 2.05 (95% confidence interval, CI, 1.08–3.90) in men, 3.79 (95% CI, 1.58–9.14) in women and 2.55 (95% CI, 1.53–4.24) in both sexes combined in multivariable adjusted model 1. We did not observe a significant increase in the HR for MI in the other groups. Similar results were observed after replacement of BMI and hypertension with WC and SBP level (model 2).

Table 3 shows the multivariable-adjusted HRs for cerebral infarction stratified by levels of TG and non-HDLC. Compared with the low TG/low non-HDLC group, the HR for cerebral infarction in the high TG alone group (high TG/low non-HDLC group) was 1.45 (95% CI, 0.84–2.50) in men, 2.09 (95% CI, 0.92–4.73) in women and 1.63 (95% CI, 1.03–2.56) in both sexes combined in statistical model 1. There was no significant increase of cerebral infarction in the other groups. Similar results were also observed in statistical model 2.

The incidence of total stroke, intracerebral hemorrhage and subarachnoid hemorrhage was not related to TG and non-HDLC levels in either sex. When the participants were divided into two groups by age (<60 and ≥60), the results of all the analyses listed above were similar in both age groups (data not shown).

4. Discussion

To our knowledge, this is the first cohort study in Japan to clarify the risk for MI and ischemic stroke of high serum level of TG, non-HDLC and both. The risk for MI of both high serum TG and non-HDLC was considerably higher than the risk without both or with only one. This relationship was similarly observed in both men and

Table 1

Means and prevalence of major cardiovascular risk factors in each group stratified by the combination of serum levels of triglycerides (TG) and non-high-density lipoprotein cholesterol (non-HDLC).

Variables	LowTG/lowNon-HDLC		LowTG/highNon-HDLC		HighTG/lowNon-HDLC		HighTG/highNon-HDLC		P value
Men									
No. of subjects	1532		117		550		205		
Non-HDLC (stratum mean), mmol/L	3.6	(0.7)	5.4	0.4	4.0	0.6	5.5	0.5	
Triglycerides (stratum median), mmol/L	1.0	(0.8, 1.3)*	1.3	(1.0, 1.5)*	2.2	(1.9, 2.9)*	2.4	(2.0, 3.7)*	
Age, years	55.8	(13.5)	57.4	(12.9)	54.8	(12.7)	54.8	(11.8)	0.16
HDLC, mmol/L	1.4	(0.3)	1.3	(0.3)	1.1	(0.3)	1.1	(0.2)	<0.01
BMI, kg/m²	22.2	(2.8)	23.1	(3.1)	23.8	(2.6)	24.2	(2.6)	<0.01
Waist circumference, cm	80.8	(7.9)	82.7	(8.6)	85.7	(7.0)	86.3	(6.9)	<0.01
Systolic blood pressure, mmHg	127	(21)	129	(20)	130	(20)	132	(21)	<0.01
Diastolic blood pressure, mmHg	78	(12)	79	(12)	81	(11)	82	(11)	<0.01
Hypertension, %	30.0		35.0		36.4		38.0		0.01
Diabetes, %	4.8		4.3		7.5		9.3		0.02
Metabolic syndrome, %**	4.5		4.3		45.1		47.8		<0.01
Smoking, %									
Current smoker	49.9		43.6		53.5		47.3		0.51
Ex-smoker	30.3		35.0		28.4		32.7		
Never-smoker	19.8		21.4		18.2		20.0		
Drinking, %									
Current drinker	76.0		63.2		76.4		69.3		0.02
Ex-drinker	3.6		6.0		2.9		5.4		
Never-drinker	20.4		30.8		20.7		25.4		
Women									
No. of subjects	1956		290		256		192		
Non-HDLC (stratum mean), mmol/L	3.6	(0.7)	5.5	(0.5)	4.2	(0.5)	5.8	(0.8)	
Triglycerides (stratum median), mmol/L	0.9	(0.7, 1.1)*	1.2	(0.9, 1.4)*	2.0	(1.8, 2.4)*	2.4	(2.0, 3.0)*	
Age, years	51.5	(12.9)	59.3	(9.6)	57.9	(11.2)	60.7	(8.8)	<0.01
HDLC, mmol/L	1.5	(0.3)	1.4	(0.3)	1.2	(0.3)	1.1	(0.3)	<0.01
BMI, kg/m2	21.7	(3.1)	22.9	(3.1)	23.6	(3.3)	24.2	(3.1)	<0.01
Waist circumference, cm	75.5	(9.8)	79.8	(9.7)	82.7	(10.0)	83.5	(9.7)	<0.01
Systolic blood pressure, mmHg	121	(21)	131	(21)	132	(21)	137	(21)	<0.01
Diastolic blood pressure, mmHg	73	(12)	79	(12)	79	(12)	80	(13)	<0.01
Hypertension, %	20.4		37.9		37.1		48.4		<0.01
Diabetes, %	2.4		4.5		6.6		7.8		<0.01
Metabolic syndrome, %**	7.5		19.3		66.8		74.5		<0.01
Smoking, %									
Current smoker	11.8		8.6		14.5		16.1		0.04
EX-smoker	3.5		2.8		2.7		6.3		
Never-smoker	84.7		88.6		82.8		77.6		
Drinking, %									
Current drinker	34.9		29.3		28.5		24.5		<0.01
Ex-drinker	1.8		0.3		0.8		4.2		
Never-drinker	63.3		70.3		70.7		71.4		

TG, triglycerides; non-HDLC, non-high-density lipoprotein cholesterol; BMI, body mass index. Brackets indicate standard deviation.

Analysis of variance was used for comparisons of multiple group means and the chi-square test was used to compare proportions.

* Inter-quartile range.

** MetS was defined using modified NCEP-ATP III. Abdominal obesity was defined as a waist circumference ≥0.90 m in men and ≥ 0.80 m in women. High blood pressure was defined as average systolic/diastolic blood pressures of ≥ 130/85 mm Hg and/or current medication for hypertension. High triglyceride was defined as serum triglycerides of ≥1.7 mmol/L. Low HDL cholesterol was defined as serum HDL cholesterol levels of <1.03 mmol/L in men and of <1.29 mmol/L in women. High blood glucose was defined as fasting blood glucose of ≥6.1 mmol/L and/or current use of anti-diabetic medication. MetS was defined as the presence of three or more of these components.

women. In contrast, the risk for ischemic stroke was highest in the participants with high TG alone.

TG-rich lipoproteins have been shown to be atherogenic, and thus, they are associated with coronary atherosclerosis [9,19–20]. As NCEP-ATP III pointed out [9], elevated non-HDLC is a good therapeutic target in patients with high TG, because the serum concentration of non-HDLC reflects not only LDL-C but also the cholesterol content of all other TG-rich and apolipoprotein B containing lipoproteins, such as very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), small dense LDL particles and their remnant lipoproteins [19–20]. In the Helsinki Heart study [21], most of the risk for coronary heart disease (CHD) was confined to participants with high levels of both TG and LDL-C. In the West of Scotland Coronary Prevention Study [22], a higher incidence of CHD was observed in men in both the pravastatin and placebo groups when TG was at or above the median level. Pischon et al. suggested that TG added significant information to non-HDLC

for CAD risk prediction in a nested case-control study [23]. Our findings are consistent with previous studies.

Similar to previous studies in Japan [4,10], we found no association between non-HDLC and cerebral infarction even in the presence of high serum TG, which may be due to a lower prevalence of atherothrombotic infarction than in Western populations. The ARIC study indicated that TC was associated with increased risk of non-lacunar, non-embolic stroke (atherothrombotic infarction), but not with lacunar or embolic stroke [24]. A recent report from a Japanese rural population showed that LDL-C is a risk factor for only atherothrombotic infraction [25]. Unfortunately, due to the relatively small stroke cases in our study, we were not able to demonstrate an association between any subtype of cerebral infarction and non-HDLC.

It is not clear why participants with high TG alone showed the increased risk for cerebral infarction in the present study. In a meta-analysis of 26 cohort studies in Asia-Pacific area, partici-

Table 2

Age and multivariable-adjusted hazard ratios (95% confidence intervals) for myocardial infarction stratified by TG and non-HDLC groups in an 11.7-year prospective study of 5098 Japanese men and women.

	Low TG/low Non-HDLC	Low TG/high Non-HDLC	High TG/low Non-HDLC	High TG/high Non-HDLC
Men				
Person-years	17410	1288	6358	2404
Case, n	45	6	11	14
Age adjusted	1.00	1.63 (0.70-3.83)	0.76 (0.39-1.48)	2.74 (1.50-5.02)
Model 1[a]	1.00	1.48 (0.62-3.49)	0.63 (0.32-1.26)	2.05 (1.08-3.90)
Model 2[b]	1.00	1.55 (0.66-3.66)	0.64 (0.32-1.29)	2.10 (1.10-3.98)
Women				
Person-years	23652	3455	2936	2270
Case, n	14	5	6	12
Age adjusted	1.00	1.59 (0.57-4.40)	2.28 (0.88-5.94)	4.88 (2.25-10.6)
Model 1[a]	1.00	1.63 (0.58-4.26)	1.99 (0.71-5.57)	3.79 (1.58-9.14)
Model 2[b]	1.00	1.55 (0.55-4.38)	1.92 (0.69-5.34)	3.18 (1.34-7.52)
Men and women				
Person-years	41062	4743	9294	4674
Case, n	59	11	17	26
Age adjusted	1.00	1.51 (0.79-2.89)	1.04 (0.60-1.78)	3.42 (2.15-5.44)
Model 1[a]	1.00	1.42 (0.74-2.74)	0.86 (0.49-1.53)	2.55 (1.53-4.24)
Model 2[b]	1.00	1.45 (0.75-2.79)	0.87 (0.49-1.54)	2.48 (1.49-4.10)

TG, triglycerides; non-HDLC, non high-density lipoprotein cholesterol.

[a] Multivariable adjusted for age, body mass index, hypertension, diabetes, HDL (high-density lipoprotein) cholesterol, cigarette smoking and alcohol intake by a Cox proportional hazard model. Sex was also adjusted in the men and women combined model.

[b] Replacement of body mass index and hypertension as covariates in model 1 with waist circumference and systolic blood pressure level.

pants grouped in the highest fifth of serum TG had a 50% increased risk of stroke compared with those in the lowest fifth [26]. Recent reviews have also concluded that hypertriglyceridemia seems to be a causal risk factor for ischemic stroke [7–8]. However, above-mentioned findings were not able to explain the low incidence of cerebral infarction in the high TG/high non-HDLC group in the present study. An elevated risk for MI might mask the relationship between TG and cerebral infarction; because there would be no further follow-up after a first MI. Another large study concerning about the relationship between serum TG and stroke should be needed.

Recently, we have reported that high serum LDLC and non-HDLC are both associated with an increased risk of MI; and the predictive value of non-HDLC for MI is almost similar to that of LDL-C [4]. However, we did not use serum TG as a covariate to avoid over-adjustment, because difference between serum level of LDLC and

non-HDLC was automatically determined by serum TG level when serum LDLC value was calculated by the Friedewald formula [27]. Considering all the findings together, non-HDLC and TG may be recommended as beneficial screening markers for primary prevention of CAD in the Japanese community, as they are less expensive and more convenient because non-HDLC can be calculated irrespective of serum TG level.

The present study has some limitations. First, the single TG and non-HDLC measurement at the baseline survey may have underestimated the relationship between these lipids and cardiovascular disease due to regression dilution bias. Furthermore, we did not evaluate longitudinal trend for each risk factor and its medication status after baseline survey. Especially, hypertriglyceridemia is associated with not only present existence of metabolic components, such as hypertension and diabetes, but also new onset

Table 3

Age and multivariable-adjusted hazard ratios (95% confidence intervals) for cerebral infarction stratified by TG and non-HDLC groups in an 11.7-year prospective study of 5098 Japanese men and women.

	Low TG/low Non-HDLC	Low TG/high Non-HDLC	High TG/low Non-HDLC	High TG/high Non-HDLC
Men				
Person-years	17410	1288	6358	2404
Case, n	46	2	22	5
Age adjusted	1.00	0.53 (0.13-2.19)	1.51 (0.91-2.52)	0.99 (0.39-2.51)
Model 1[a]	1.00	0.54 (0.13-2.25)	1.45 (0.84-2.50)	0.92 (0.35-2.38)
Model 2[b]	1.00	0.56 (0.14-2.31)	1.48 (0.86-2.56)	0.75 (0.26-2.14)
Women				
Person-years	23652	3455	2936	2270
Case, n	20	8	10	3
Age adjusted	1.00	1.77 (0.78-4.02)	2.62 (1.23-5.60)	0.81 (0.24-2.72)
Model 1[a]	1.00	1.52 (0.66-3.50)	2.09 (0.92-4.73)	0.69 (0.20-2.44)
Model 2[b]	1.00	1.54 (0.67-3.54)	2.10 (0.93-4.73)	0.77 (0.22-2.71)
Men and women				
Person-years	41062	4743	9294	4674
Case, n	66	10	32	8
Age adjusted	1.00	1.14 (0.58-2.23)	1.82 (1.19-2.79)	0.94 (0.45-1.95)
Model 1[a]	1.00	1.12 (0.57-2.20)	1.63 (1.03-2.56)	0.79 (0.37-1.69)
Model 2[b]	1.00	1.12 (0.57-2.21)	1.62 (1.03-2.55)	0.69 (0.62-1.88)

TG, triglycerides; non-HDLC, non high-density lipoprotein cholesterol.

[a] Multivariable adjusted for age, body mass index, hypertension, diabetes, HDL (high-density lipoprotein) cholesterol, cigarette smoking and alcohol intake by a Cox proportional hazard model. Sex was also adjusted in the men and women combined model.

[b] Replacement of body mass index and hypertension (prevalence) as covariates in model 1 with waist circumference and systolic blood pressure levels.

of them in the future [28,29]. Second, we did not measure serum apolipoprotein B (apoB) [22], apolipoprotein A1 (ApoA1) and LP(a) [30], which some previous studies have shown to be strong risk factors for CAD [22]. Third, a recent study indicated that non-fasting TG is a better predictor of CAD than fasting TG [31]. However, in a large individual based meta-analysis in the Asia-Pacific region [26], most blood samples were collected during fasting, and there was a significant positive relationship between serum TG and CAD or stroke.

In conclusion, a combination of higher serum levels of TG and non-HDLC is associated with an increased risk of MI in a Japanese population. Furthermore, the risk for ischemic stroke was highest in the participants with high TG alone; however, further research should be needed. High serum levels of TG and non-HDLC are both important targets for the prevention of cardiovascular disease, which requires evidence-based guidelines for management in the primary care setting.

Acknowledgements

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ORIGINAL ARTICLE

Metabolic syndrome is a significant and independent risk factor for increased arterial stiffness in Japanese subjects

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Metabolic syndrome (MetS) has been recognized as a risk factor for cardiovascular disease; however, the impact of MetS on arterial stiffness has not been fully established in the general Japanese population. We analyzed the relationship between MetS and the severity of arterial stiffness using brachial-ankle pulse wave velocity (baPWV) in 2744 male and 358 female subjects aged 38–62 years, adjusted for conventional risk factors and C-reactive protein. The prevalence rates of MetS identified by Japanese criteria were 22.7% (n=624) and 7.8% (n=28) in male and female subjects, respectively. The subjects with MetS had significantly greater mean values of baPWV than those without MetS among both male and female subjects (1444 ± 209 vs. 1294 ± 165 cm/s in male subjects, $P<0.001$; 1379 ± 151 vs. 1220 ± 171 cm/s in female subjects, $P<0.001$). After adjustment for atherosclerotic variables such as age, smoking habits, total cholesterol and C-reactive protein, the odds ratio (OR) of MetS for increased baPWV was 3.65 in male subjects (95% confidence interval (CI): 2.99–4.47, $P<0.001$) and 8.02 in female subjects (95% CI: 3.18–20.25 $P<0.001$). In conclusion, MetS was identified as a significant and independent risk factor for increased arterial stiffness in both the male and female general population in Japan.
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INTRODUCTION

Metabolic syndrome (MetS) is an accumulation of risk factors such as visceral obesity, hypertension, dyslipidemia and glucose intolerance,[1] and has been closely associated with increased risk of cardiovascular disease,[2–4] diabetes mellitus[5] and mortality.[6–8] MetS is defined according to the diagnostic criteria set forth by the National Cholesterol Education Program Adults Treatment Panel III (NCEP-ATPIII)[9] and the International Diabetes Federation (IDF).[10] In Japan, MetS is diagnosed by criteria proposed for the Japanese population in 2005.[11]

Arterial stiffness has been identified as an independent risk factor for cardiovascular disease and subsequent mortality.[12–15] The brachial–ankle pulse wave velocity (baPWV) obtained by a non-invasive automatic device is an indicator of arterial stiffness.[16,17] Several studies have indicated that MetS defined by the NCEP-ATPIII and IDF criteria is closely associated with increased arterial stiffness.[18–21] The impact of MetS defined by Japanese criteria on arterial stiffness, however, has not been fully examined in the general population in Japan. Moreover, previous studies have not examined the effects of MetS on arterial stiffness after adjustment for other atherogenic risk factors such as age, smoking, total cholesterol and C-reactive protein (CRP).

The aim of this study was to investigate the relationship between MetS and arterial stiffness measured by baPWV in the general Japanese population.

METHODS

Study subjects

The study subjects included 3144 Japanese subjects employed by two companies in Hokkaido, aged 38–62 years old, who had an annual health checkup during the period from April 2007 to March 2008. A total of 42 subjects (37 male and 5 female subjects) were excluded from the analysis because of the following reasons: prior coronary heart disease or stroke (n=28, 24 male and 4 female subjects), peripheral artery disease (n=9, 8 male subjects and 1 female subject), hemodialysis (n=2, 2 male subjects), and atrial fibrillation (n=3, 3 male subjects). Thus, a total of 3102 subjects remained in the present analysis. The two companies having study subjects approved the study protocol and informed consent was obtained from all participants.

Data collection

Body weight, height and waist circumference were measured in the morning in the fasting state. Body mass index was calculated as body weight (kilograms) divided by height (meters) squared. Smoking habits, alcohol intake and exercise

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habits were evaluated by interviews. Subjects who had never smoked and ex-smokers were classified as 'nonsmokers'. Subjects were divided into two groups by the frequency of exercise; <1 time per week or ≥1 time per week. Blood pressure was measured by a trained nurse using a standard mercury sphygmomanometer with the study subjects in the sitting position and after at least 5 min of rest. Blood samples were obtained from the antecubital vein in the morning after an overnight fast, and the serum was separated. After precipitation by heparin manganese, total cholesterol and high-density lipoprotein

(HDL)-cholesterol were measured by the phosphotungstate method. Triglycerides were measured enzymatically. Fasting plasma glucose was enzymatically determined by the hexokinase method. CRP was measured by nephelometry with a latex particle-enhanced immunoassay.

We used the definition and diagnostic criteria of MetS in Japan;[11] subjects with waist circumference ≥85 cm in male and ≥90 cm in female subjects with at least two of the other three criteria, including systolic blood pressure ≥130 mm Hg and/or diastolic blood pressure ≥85 mm Hg, triglycerides

Table 1 Baseline characteristics of male subjects

	MetS (n=624)	No MetS (n=2120)	P-value
Age (years)	52.6±6.1	51.2±6.6	<0.001
Body mass index (kg m⁻²)	25.6±2.7	23.1±2.7	<0.001
Waist circumference (cm)	92±6	85±7	<0.001
Systolic blood pressure (mm Hg)	139±14	123±14	<0.001
Diastolic blood pressure (mm Hg)	87±9	77±10	<0.001
Total cholesterol (mg per 100 ml)	209±36	201±30	<0.001
Triglyceride (mg per 100 ml)	176 (137–245)	105 (75–141)	<0.001
HDL-cholesterol (mg per 100 ml)	54±13	62±16	<0.001
Fasting plasma glucose (mg per 100 ml)	114±28	97±16	<0.001
Heart rate (b.p.m.)	68±11	63±9	<0.001
CRP (mg per 100 ml)	0.08 (0.05–0.13)	0.06 (0.04–0.10)	<0.001
Current smoking (%)	44.1	47.2	0.17
Alcohol intake (%)	74.7	69.8	<0.05
Frequency of exercise			
≥1/week (%)	27.6	28.0	0.82
Medical history			
Hypertension (%)	32.1	8.4	<0.001
Diabetes mellitus (%)	9.6	1.8	<0.001
Hyperlipidemia (%)	12.8	4.5	<0.001

Abbreviations: CRP, C-reactive protein; HDL, high-density lipoprotein; MetS, metabolic syndrome.
Variables are presented as mean±s.d., median (interquartile range) for skewed variables, or percentage.

Table 2 Baseline characteristics of female subjects

	MetS (n=28)	No MetS (n=330)	P-value
Age (years)	51.2±5.0	50.1±7.4	0.50
Body mass index (kg m⁻²)	28.3±4.0	21.6±3.1	<0.001
Waist circumference (cm)	99±7	83±9	<0.001
Systolic blood pressure (mm Hg)	138±11	118±17	<0.001
Diastolic blood pressure (mm Hg)	84±7	72±11	<0.001
Total cholesterol (mg per 100 ml)	210±37	206±33	0.54
Triglyceride (mg per 100 ml)	167 (105–281)	74 (58–99)	<0.001
HDL-cholesterol (mg per 100 ml)	63±16	75±17	<0.01
Fasting plasma glucose (mg per 100 ml)	117±32	93±18	<0.01
Heart rate (b.p.m.)	69±11	64±9	<0.01
CRP (mg per 100 ml)	0.09 (0.05–0.21)	0.04 (0.04–0.07)	<0.01
Current smoking (%)	28.0	28.5	0.96
Alcohol intake (%)	56.0	40.9	0.14
Frequency of exercise			
≥1/week (%)	8.0	24.2	0.06
Medical history			
Hypertension (%)	40.0	5.2	<0.001
Diabetes mellitus (%)	16.0	1.5	<0.001
Hyperlipidemia (%)	6.2	5.5	0.34

Abbreviations: CRP, C-reactive protein; HDL, high-density lipoprotein; MetS, metabolic syndrome.
Variables are presented as mean±s.d., median (interquatile range) for skewed variables, or percentage.

≥150 mg per 100 ml and/or HDL-cholesterol <40 mg per 100 ml, and fasting plasma glucose ≥110 mg per 100 ml.

Arterial stiffness was assessed using baPWV measured by a volume-plethysmographic apparatus (Form PWV/ABI; Colin, Komaki, Japan).[22] baPWV was recorded after at least 5 min of rest. This device was able to measure the phonocardiogram, electrocardiogram, volume pulse form and arterial blood pressure at left and right brachia and ankles, and time intervals between the wave front of the right brachium and that of both ankles were calculated. Ankle/brachial pressure is the ratio of ankle to brachial systolic blood pressure, and right and left ankle/brachial pressures were measured simultaneously. We used the mean of right and left baPWV values in the analysis.

Statistical analysis

All analyses were performed separately for male and female subjects. The clinical and biochemical data of the study subjects are expressed as mean ± s.d., as median (and interquartile range) for variables with a skewed distribution, and as a percentage. The differences in variables between the two groups were examined by Student's unpaired t-test for approximately normally distributed variables; by the Wilcoxon rank-sum test for triglycerides and CRP; and by the χ^2-test for smoking habits, alcohol intake, exercise habits and medical history. Multiple logistic regression analysis was performed to investigate the relationship between MetS and arterial stiffness with adjustment for other variables such as age, smoking habits, total cholesterol, and CRP.

A P-value of less than 0.05 was considered to indicate statistical significance. All statistical analyses were performed using SPSS software version 11.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Baseline characteristics of the male and female study subjects are shown in Tables 1 and 2, respectively. The prevalence of MetS was 22.7% in male subjects and was 7.8% in female subjects. Male subjects with MetS had significantly higher age, body mass index, systolic and diastolic blood pressure, total cholesterol, triglycerides, fasting plasma glucose, heart rate and CRP, along with lower HDL-cholesterol values. The prevalence of alcohol intake, hypertension, diabetes mellitus and hyperlipidemia was higher in male subjects with MetS than in those without MetS. Female subjects with MetS had significantly greater body mass index, systolic and diastolic blood pressure, triglycerides, fasting plasma glucose, heart rate and CRP, along with lower HDL-cholesterol values. The prevalence of hypertension and diabetes mellitus was higher in female subjects with MetS than in those without MetS.

Figure 1 shows the mean values of baPWV in male and female subjects with or without MetS. Male and female subjects with MetS had significantly higher mean values of baPWV than subjects without MetS (male subjects; 1444 ± 209 vs. 1294 ± 165 cm/s, female subjects; 1379 ± 151 vs. 1220 ± 171 cm/s).

Baseline characteristics of the study subjects according to low and high baPWV values are shown in Table 3. High baPWV was designated as more than 1429 and 1308 cm/s in male and female subjects, respectively, which was the cutoff value between the third and fourth quartiles. The prevalence of MetS was significantly higher in subjects with a high baPWV than in those with a low baPWV among both male and female subjects (42.3 vs. 16.2% in male subjects, $P<0.001$; 18.9 vs. 3.0% in female subjects, $P<0.001$). Male subjects with a high baPWV had significantly greater age, smoking and CRP values than those with low baPWV. Female subjects with high baPWV had significantly greater age than those with low baPWV.

Multiple logistic regression analysis was performed to examine the relationship between high baPWV and dependent variables in Table 4. After adjustment for age, smoking habits, total cholesterol, CRP and heart rate, the odds ratio (OR) of MetS for high baPWV values was 3.65 in male subjects (95% confidence interval (CI): 2.99–4.47, $P<0.001$) and 8.02 in female subjects (95% CI: 3.18–20.25 $P<0.001$).
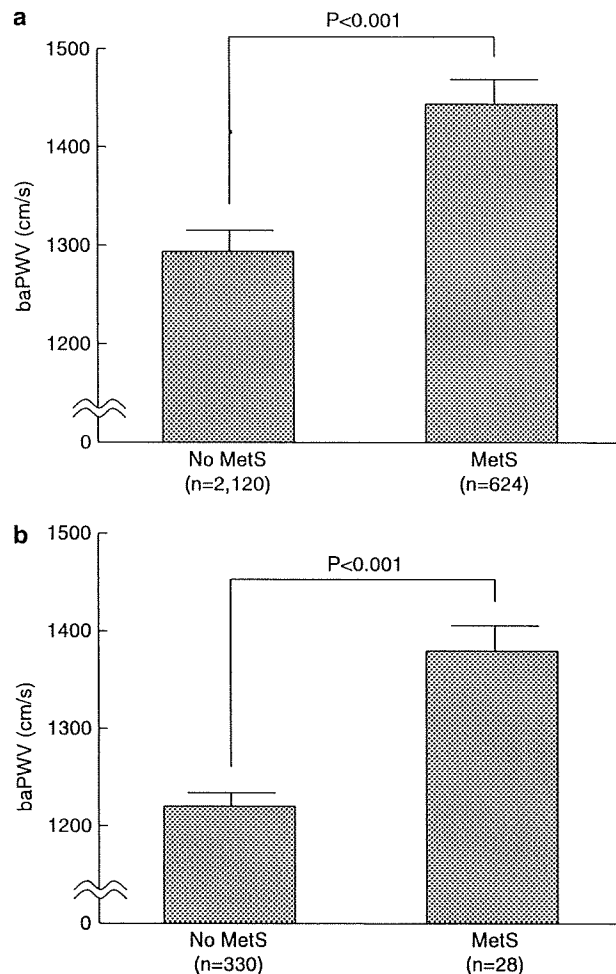

Figure 1 Brachial–ankle pulse wave velocity (baPWV) in male (a) and female (b) subjects with and without metabolic syndrome (MetS). baPWV, brachial–ankle pulse wave velocity; MetS, metabolic syndrome.

DISCUSSION

This study shows that MetS is associated with increased arterial stiffness in the general Japanese population, independent of other atherogenic risk factors.

The prevalence of MetS subjects defined by Japanese criteria in this study was 21.0%; 22.7% in male subjects and 7.8% in female subjects. Thus, the prevalence rate was about 3-fold higher in male subjects than in female subjects. The prevalence rate of MetS in this study was similar to that found in the latest National Health and Nutrition survey in 2004.[23] In that survey, the incidence of MetS in male and female subjects was reported to be 23.0 and 8.9%, respectively.

The previous studies have reported that MetS is closely associated with increased arterial stiffness[18–21] Schillaci et al.[20] found that MetS, according to the NCEP-ATP III criteria, was associated with arterial stiffness measured by carotid–femoral PWV. Li et al.[19] found that baPWV values increased with increasing components of MetS as defined by the NCEP-ATP III criteria. Sipilä et al.[21] showed a similar relationship between MetS and arterial stiffness using the IDF criteria. Conventional risk factors such as age, smoking, total cholesterol and CRP are closely associated with arterial stiffness;[24–27] however, these

Table 3 Baseline characteristics of male and female subjects according to baPWV

	High baPWV	Low baPWV	P-value
Male			
n	775	2327	
MetS (%)	42.3	16.2	<0.001
Age (years)	54.4 ± 5.1	50.6 ± 6.6	<0.001
Total cholesterol (mg per 100 ml)	203 ± 31	203 ± 32	0.92
Current smoking (%)	48.8	39.7	<0.001
CRP (mg per 100 ml)	0.07 (0.05–0.13)	0.06 (0.04–0.10)	<0.001
Female			
n	92	266	
MetS (%)	18.9	3.0	<0.001
Age (years)	53.0 ± 4.4	49.3 ± 7.8	<0.001
Total cholesterol (mg per 100 ml)	207 ± 33	206 ± 33	0.83
Current smoking (%)	27.8	28.7	0.87
CRP (mg per 100 ml)	0.04 (0.04–0.10)	0.05 (0.04–0.07)	0.31

Abbreviations: baPWV, brachial–ankle pulse wave velocity; CRP, C-reactive protein; MetS, metabolic syndrome.
High baPWV was designated as greater than $1429\,cm\,s^{-1}$ and $1308\,cm\,s^{-1}$ in male and female subjects, respectively.
Variables are presented as mean ± s.d., median (interquartile range) for skewed variables, or percentage.

Table 4 Multiple logistic regression analysis with the relationship between high baPWV and risk variables

Variables	OR	95% CI	P-value
Male			
MetS	3.65	2.99–4.47	<0.001
Age	1.11	1.09–1.13	<0.001
Total cholesterol	0.98	0.95–1.01	0.21
Smoking	1.26	1.04–1.52	0.02
CRP	2.09	1.43–3.07	<0.001
Female			
MetS	8.02	3.18–20.25	<0.001
Age	1.12	1.06–1.18	<0.001
Total cholesterol	0.95	0.87–1.03	0.20
Smoking	1.02	0.58–1.81	0.95
CRP	1.77	0.60–5.29	0.30

Abbreviations: baPWV, brachial–ankle pulse wave velocity; CI, confidence interval; CRP, C-reactive protein; MetS, metabolic syndrome; OR, odds ratio.

studies could not exclude the influence of these atherogenic conventional risk factors. In this study, Japanese subjects with MetS had greater baPWV values than those without MetS, and importantly, MetS was a significant risk for increased arterial stiffness in both genders, even after adjustment for other risk factors such as age, smoking habits, CRP and total cholesterol.

The baPWV is a non-invasive index of arterial stiffness.[22] Increased arterial stiffness is one of the pathological states of vascular damage and has been closely associated with the development of cardiovascular diseases. Nagano et al.[28] showed that age and blood pressure were major determinants of baPWV values, and other risk

factors such as diabetes mellitus, dyslipidemia, smoking and high CRP were also associated with increased baPWV.[29] In this study, risk factors such as age and CRP, which are not involved as risk components in the MetS definition, were also associated with increased arterial stiffness.

MetS has been established as an endocrine and inflammatory disorder related to insulin resistance.[30] Nakanishi et al.[31] showed that insulin resistance was closely associated with the risk of increased arterial stiffness. These results indicate that hyperinsulinemia may be involved in the increased arterial stiffness in MetS found in this study. Hyperinsulinemia has been shown to promote the synthesis of collagen, stimulate hyperplasia and hypertrophy of vascular smooth cells[32] and cause endothelial dysfunction by interfering with the generation of vasodilatory and vasoconstrictive substances such as nitric oxide and endothelin-1.[33]

There are several limitations that should be acknowledged in this study. First, medications such as anti-hypertensive drugs and lipid-lowering agents were not examined in our study subjects. Second, baPWV is an indirect marker of increased arterial stiffness or decreased arterial compliance, and we did not examine structural changes of the arterial wall using ultrasound technology.

In conclusion, this study has identified MetS as a significant and independent risk factor for increased arterial stiffness in both male and female subjects. As such, MetS should be recognized to have a crucial impact on public health in the general Japanese population.

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ORIGINAL ARTICLE

Combination of conventional biomarkers for risk stratification in chronic heart failure

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Summary
Background: Although there is substantial interest in the use of newer biomarkers to identify patients with chronic heart failure (CHF), recently few investigations have evaluated the incremental usefulness of multiple conventional biomarkers. Combination of several biomarkers simultaneously could enhance risk stratification in CHF.

Methods and results: We analyzed 7 biomarkers (brain natriuretic peptide, uric acid, sodium, hemoglobin, creatinine, creatinine clearance, high-sensitivity C-reactive protein), which were known as established prognostic markers for CHF, in 154 consecutive CHF patients, and patients were prospectively followed with endpoints of cardiac death or re-hospitalization. When there was an abnormal value, we scored it for one point to calculate multimarker score. Patients were categorized into 3 strata according to multimarker score. There were 83 cardiac events during the follow-up period. A Cox proportional hazard model showed that patients in the high stratum were associated with the highest risk of cardiac events among the 3 strata. Kaplan—Meier analysis revealed that patients in the high stratum had a significantly higher cardiac event rate compared with lower strata.

Conclusion: The combination of conventional biomarkers could potentially improve the risk stratification of CHF patients for the prediction of cardiac events with low cost and wide availability.

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Introduction

Chronic heart failure (CHF) still represents the major cause of death and hospitalization and has a poor prognosis despite the significant reduction in mortality achieved in clinical trials [1—3]. The prognostic evaluation of CHF patients involves a complex assessment of multiple interacting variables. There is currently no simple clinical criterion or score for predicting early outcome and identifying patients who require such caution. New York Heart Association (NYHA) functional classification and several tests including chest X-ray, echocardiography, radionuclide ventriculography, cardiopulmonary exercise test, and hemodynamic measurements, while helping to estimate the degree of CHF, are subject to interobserver variations in interpretation. Therefore, the ability to identify CHF patients at higher risk for adverse outcomes has led to optimize therapeutic interventions and improve the ominous prognosis [4].

Although there has been substantial interest in the use of newer biomarkers for identification of CHF patients, risk assessment, and prevention of cardiac events recently, few investigations have evaluated the incremental usefulness of multiple conventional biomarkers. Measurement of several biomarkers simultaneously could enhance risk stratification. Previous studies demonstrated that elevated levels of brain natriuretic peptide (BNP) [5], uric acid [6], and high-sensitivity C-reactive protein (hs-CRP) [7], and decreased levels of sodium [8] and hemoglobin [9], and renal insufficiency [10] were associated independently with increased risk of cardiac events in patients with CHF. Importantly, each of these markers assesses different pathophysiological mechanisms. We hypothesized that simultaneous assessment of these biomarkers will provide complimentary information and enable clinicians to stratify risk more effectively among CHF patients. We therefore evaluated the combination of 7 biomarkers (BNP, uric acid, hs-CRP, sodium, hemoglobin, creatinine, creatinine clearance) for predicting cardiac events in 154 consecutive patients hospitalized for CHF from various etiologies during a mean follow-up period of 526 ± 313 days.

Methods

Study population

Between November 2001 and September 2007, we prospectively studied 154 consecutive CHF patients (62 men and 92 women, mean age 71 ± 12 years,

Table 1 Clinical and laboratory characteristics of 154 patients with chronic heart failure.

	All chronic heart failure patients ($n = 154$)
Age (years)	71 ± 12
Gender (male/female)	62/92
NYHA functional class (III/IV)	113/41
Hypertension	87 (56%)
Diabetes mellitus	40 (26%)
Hyperlipidemia	28 (18%)
Smoking	28 (18%)
Etiology of chronic heart failure	
Dilated cardiomyopathy	52 (34%)
Ischemic heart disease	34 (22%)
Valvular heart disease	33 (21%)
Hypertensive heart disease	20 (13%)
Others	15 (10%)
Laboratory markers	
Creatinine (mg/dl)	1.15 ± 0.86
Hemoglobin (g/dl)	12.0 ± 2.2
Sodium (mmol/L)	139.2 ± 3.8
Uric acid (mg/dl)	6.9 ± 2.4
BNP (pg/ml)	1019 ± 1140
hs-CRP (mg/dl)	0.46 ± 0.35
Creatinine clearance (ml/min)	44.5 ± 30.8
Multimarker score	4.27 ± 1.44
Echocardiography	
LVEDD (mm)	56 ± 11
LVEF (%)	42 ± 19
Medications at discharge	
ACE inhibitors and/or ARBs	108 (70%)
β-Blockers	48 (31%)
Calcium channel blockers	26 (17%)
Spironolactone	51 (33%)
Loop diuretics	119 (77%)
Digoxin	55 (36%)
Statins	17 (11%)

NYHA, New York Heart Association; BNP, brain natriuretic peptide; hs-CRP, high-sensitivity C-reactive protein; LVEDD, left ventricular dimension at end-diastole; LVEF, left ventricular ejection fraction; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker.

range 37—93 years) who had been admitted to the Yamagata University Hospital for the treatment of worsening CHF. The diagnosis of CHF was made by two senior cardiologists using the generally accepted Framingham criteria. Baseline characteristics of the population are shown in Table 1. Exclusion criteria in this study were patients with clinical or electrocardiographic evidence suggestive of acute coronary syndrome within 3 months prior to admission, those with renal insufficiency characterized by a serum creatinine concentration >1.5 mg/dl, and those with active hepatic dis-

ease, active pulmonary disease, and degenerative disease of the muscles. Patients who underwent percutaneous coronary intervention or coronary artery bypass graft within 3 months prior to admission were also excluded. Informed consent was obtained from all patients before participation in this study, and the protocol was approved by the Human Investigations Committee of our institution.

Blood samples were obtained at admission from all patients. The optimal cut-off values for 7 biomarkers were determined as those with the largest sum of sensitivity plus specificity on each of the receiver operating characteristic (ROC) curves. When there was an abnormal value, we scored it for one point to calculate multimarker score. Patients were categorized into 3 strata according to multimarker score. Transthoracic echocardiography was performed by experienced echocardiologists without knowledge of the biochemical data using an ultrasound instrument (Hewlett Packard SONOS 5500 and 7500) equipped with a sector transducer (carrier frequency of 2.5 or 3.75 MHz) within 1 week of admission. Demographics and clinical data, including age, sex, and NYHA functional class at admission, were collected from hospital medical records and patient interviews. Physicians were kept blind to the results of the biochemical markers, and optimal medical therapy was administered independently based on improvement in symptoms, physical examination findings, and pulmonary congestion on chest X-ray [11]. Diuretics were given in flexible dosages on the basis of body weight and daily diuresis. Spironolactone was administered as 25 or 50 mg/day. Angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), and β-blockers were gradually increased to the maximum dosage possible. The discharge was decided by two senior cardiologists using clinical examination, electrocardiogram, and chest X-ray film. The etiologies of CHF were dilated cardiomyopathy in 52 patients, ischemic heart disease in 34 patients, valvular heart disease in 33 patients, and hypertensive heart disease in 20 patients. The diagnosis of dilated cardiomyopathy was based on the definition of the WHO/ISFC task force [12]. The diagnoses of hypertension, diabetes, and hyperlipidemia were obtained from medical records or patient history of currently or previously received medical therapy.

Endpoints and follow-up

Patients were prospectively followed-up and no patients were lost to follow-up (mean follow-up 526 ± 313 days) after discharge from the Yamagata University Hospital. The endpoints were (1) cardiac death, defined as death from worsening CHF or sudden cardiac death, and (2) worsening CHF requiring readmission. Sudden cardiac death was defined as death without definite premonitory symptoms or signs and was established by the attending physician. A review of medical records and follow-up telephone interviews were conducted by senior cardiologists to survey cardiac events, who were blinded to blood examination data. Cardiac events were adjudicated using electrocardiograms, chest X-ray reports, autopsy reports, death certificates, and witness statements.

Statistical analysis

Results are presented as mean ± standard deviation (S.D.) for continuous variables and as the percentage of total patients for categorical variables. The independent samples t-test or Mann–Whitney test and chi-square test were used for comparisons of continuous and categorical variables, respectively. A Cox proportional hazard analysis was performed to evaluate the associations between cardiac events and blood measurements. The cardiac event-free curve was computed according to the Kaplan–Meier method and compared by the log-rank test. All p-values reported are two-sided, and a p-value < 0.05 was considered significant. Statistical analysis was performed with a standard statistical program package (StatView, version 5.0, SAS Institute Inc, Cary, NC, USA).

Results

The mean age of study subjects was 71 ± 12 years old. As shown in Table 1, 62 patients were men, 41 were in NYHA functional class IV, and 34 had ischemic heart disease. Mean multimarker score was 4.27 ± 1.44.

Associations with subsequent clinical outcomes

There were 9 noncardiac deaths (3 cerebral infarction, 2 pneumonia, 2 lung cancer, 1 septic shock, and 1 suicide) and 83 cardiac events, including 43 cardiac deaths (5 in-hospital deaths) and 40 readmissions for worsening heart failure during the follow-up period. The causes of cardiac death were worsening CHF in 34 patients, fatal acute myocardial infarction in 3 patients, and sudden cardiac death in 6 patients.

As shown in Table 2, patients with cardiac events were older, and had a higher multimarker score

Table 2 Comparisons of clinical characteristics between patients with and without cardiac events.

	Event free (n = 71)	Cardiac events (n = 83)	p-Value
Age (years)	68 ± 15	74 ± 10	0.0100
Gender (male/female)	29/42	33/50	0.8911
NYHA functional class (III/IV)	54/17	59/24	0.4856
Hypertension	43 (61%)	44 (53%)	0.3456
Diabetes mellitus	21 (30%)	19 (23%)	0.3461
Hyperlipidemia	10 (14%)	18 (22%)	0.2193
Current smoking	12 (17%)	16 (19%)	0.6270
Etiology of chronic heart failure			
Dilated cardiomyopathy	27 (38%)	25 (30%)	
Ischemic heart disease	13 (18%)	21 (25%)	
Valvular heart disease	18 (25%)	15 (18%)	
Hypertensive heart disease	6 (9%)	14 (17%)	
Others	7 (10%)	8 (10%)	0.2951
Laboratory markers			
Creatinine (mg/dl)	1.04 ± 0.64	1.25 ± 1.01	0.0436
Hemoglobin (g/dl)	12.9 ± 2.4	11.9 ± 2.2	0.0328
Sodium (mmol/L)	140.7 ± 2.6	138.2 ± 4.4	0.0008
Uric acid (mg/dl)	6.4 ± 1.9	7.2 ± 2.7	0.0254
BNP (pg/ml)	604 ± 1156	1032 ± 1134	<0.0001
hs-CRP (mg/dl)	0.33 ± 0.30	0.57 ± 0.34	0.0094
Creatinine clearance (ml/min)	56.8 ± 38.5	35.2 ± 19.3	0.0031
Multimarker score	3.85 ± 1.32	4.63 ± 1.45	0.0007
Echocardiography			
LVEDD (mm)	55 ± 9	56 ± 11	0.6403
LVEF (%)	39 ± 18	43 ± 18	0.2741

Abbreviations as in Table 1.

compared with those without cardiac events. Furthermore, as reported in previous studies [5—10], patients with cardiac events showed renal dysfunction, anemia, hyponatremia, hyperuricemia, and higher levels of BNP and hs-CRP compared with those without cardiac events. Other parameters including gender, NYHA functional class, and numbers of patients with hypertension, diabetes mellitus, hyperlipidemia, or currently smoking were not significantly different between patients with and without cardiac events. In addition, there was no difference in etiology of heart failure, left ventricular end-diastolic diameter (LVEDD), and left ventricular ejection fraction (LVEF).

Strata of multimarker score in CHF patients

The optimal cut-off values for 7 biomarkers were determined as those with the largest sum of sensitivity plus specificity on each of the ROC curves, respectively (Fig. 1). When there was an abnormal value, we scored it for one point to calculate multimarker score. Patients were categorized into 3 strata according to multimarker score: low stratum (multimarker score 0—3, n = 48), intermediate stratum (multimarker score 4, n = 40), and high

stratum (multimarker score 5—7, n = 66). Table 3 summarizes the comparisons of the clinical characteristics of the 3 strata. Patients in the high stratum were older, had more severe NYHA functional class, and higher rate of use of loop diuretics compared with lower strata. Furthermore, patients in the high stratum had significantly higher rates of re-hospitalization and cardiac deaths than those in the lower strata (Fig. 2). Average periods of follow-up days for the 3 strata were 817 ± 632 (low stratum), 696 ± 586 (intermediate stratum), and 444 ± 492 (high stratum) days. The period was significantly shorter in the high stratum compared with lower strata (p < 0.01). Whereas, other parameters including gender, etiology of heart failure, LVEDD, and LVEF were not significantly different among the 3 strata. In addition, there was no difference in the numbers of patients who had hypertension, diabetes mellitus, hyperlipidemia, or were currently smoking, and rate of use of ACE inhibitors, ARBs, and β-blockers at discharge among the 3 strata.

Risk stratification by the stratum analysis

Prognostic results by the univariate Cox proportional hazard analysis to predict cardiac events are
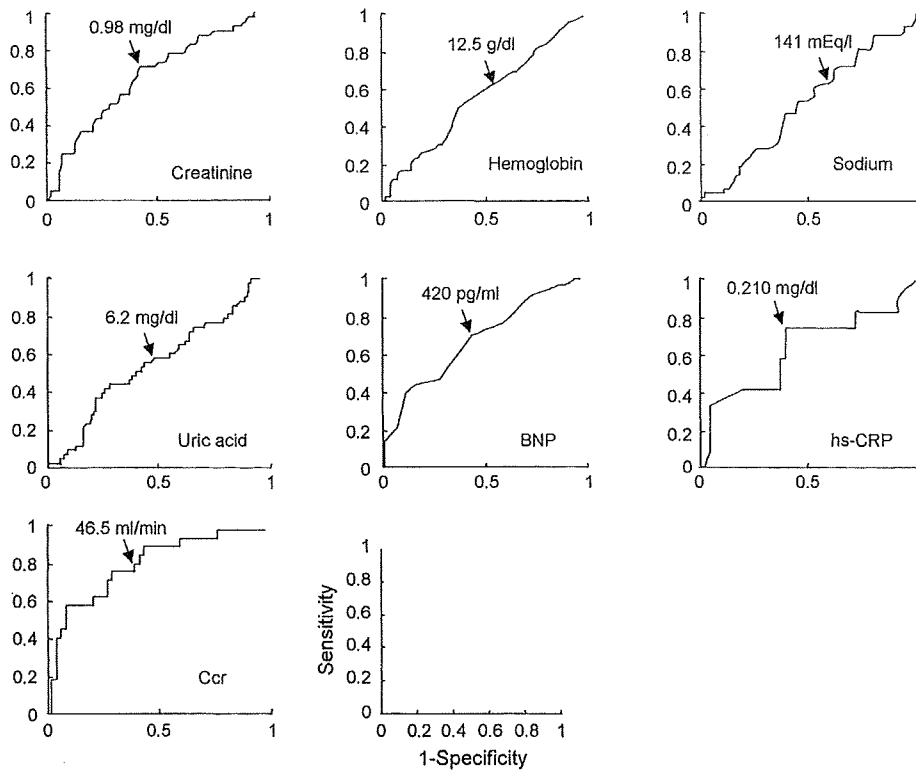
Figure 1 Receiver operating characteristic (ROC) curve analysis of 7 biomarkers. BNP, brain natriuretic peptide; hs-CRP, high-sensitivity C-reactive protein; and Ccr, creatinine clearance.

shown in Fig. 3. The univariate Cox proportional hazard analysis showed that risk of total cardiac events (Fig. 3A) and cardiac deaths (Fig. 3B) were significantly higher in patients in the high stratum compared with low stratum patients. In addition, patients in the high stratum were at higher risk of total cardiac events (hazard ratio 1.934,

Figure 2 Cardiac mortality and cardiac events among the 3 strata. Patients in the high stratum had higher rates of re-hospitalization and cardiac deaths compared with lower strata. **$p < 0.001$ by chi-square test.

$p = 0.0144$) and cardiac death (hazard ratio 1.393, $p = 0.0313$) compared with intermediate-stratum patients. The hazard ratios of total cardiac events (Fig. 4A) and cardiac deaths (Fig. 4B) adjusted for age and sex were significantly higher in patients in the high stratum compared with low stratum patients.
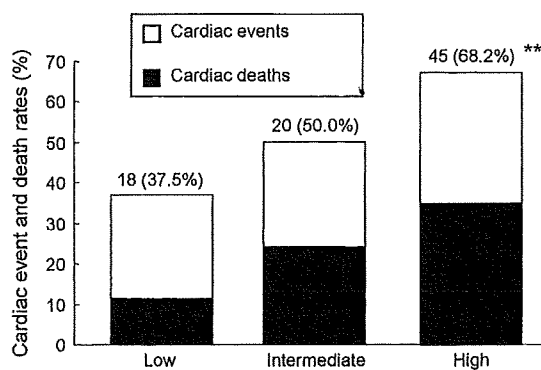
We performed univariate and multivariate Cox proportional hazard regression analyses of predicting cardiac events among multimarker score, age, NYHA functional class, and LVEF (Table 4). In the univariate Cox proportional hazard regression analysis, multimarker score and age were significantly associated with cardiac events. Furthermore, only multimarker score was an independent predictor of cardiac events among these variables by the multivariate Cox proportional hazard regression analysis.

Kaplan—Meier analysis demonstrated that patients in the high stratum had a significantly higher total cardiac events rate (Fig. 5A) and cardiac death rate (Fig. 5B) compared with lower strata. One- and 2-year total cardiac event rates were 14.6% and 29.2% in low stratum, 27.5% and 37.5% in intermediate stratum, and 51.5% and

Table 3 Comparisons of clinical characteristics among the 3 strata in chronic heart failure patients.

	Low stratum ($n = 48$)	Intermediate stratum ($n = 40$)	High stratum ($n = 66$)
Age (years)	68 ± 14	70 ± 12	74 ± 10*'##
Gender (male/female)	23/25	14/26	25/41
NYHA functional class (III/IV)	39/9	28/12	46/20$$
Hypertension	26 (54%)	24 (60%)	37 (56%)
Diabetes mellitus	18 (38%)	9 (23%)	13 (20%)
Hyperlipidemia	8 (17%)	6 (15%)	14 (21%)
Current smoking	9 (19%)	8 (20%)	11 (17%)
Etiology of chronic heart failure			
Dilated cardiomyopathy	17 (35%)	12 (30%)	23 (35%)
Ischemic heart disease	13 (27%)	8 (20%)	13 (20%)
Valvular heart disease	4 (8%)	12 (30%)	17 (26%)
Hypertensive heart disease	6 (13%)	4 (10%)	10 (15%)
Others	8 (17%)	4 (10%)	3 (4%)
Laboratory markers			
Creatinine (mg/dl)	0.76 ± 0.23	1.08 ± 0.67*	1.48 ± 1.10*'##
Hemoglobin (g/dl)	12.7 ± 2.1	12.8 ± 2.0*	10.9 ± 2.0*'##
Sodium (mmol/L)	141.1 ± 2.6	139.6 ± 3.9*	138.5 ± 4.1*'#
Uric acid (mg/dl)	5.3 ± 1.8	6.9 ± 2.3**	7.9 ± 2.3*'#
BNP (pg/ml)	566 ± 587	962 ± 844**	1380 ± 1447*'#
hs-CRP (mg/dl)	0.34 ± 0.30	0.49 ± 0.40*	0.55 ± 0.32*'#
Creatinine clearance (ml/min)	77.9 ± 44.2	50.3 ± 28.3**	32.4 ± 15.8*'##
Multimarker score	2.60 ± 0.64	4.00 ± 0.00**	5.66 ± 0.73*'##
Echocardiography			
LVEDD (mm)	56 ± 10	55 ± 10	56 ± 11
LVEF (%)	39 ± 18	42 ± 18	42 ± 19
Medications at discharge			
ACE inhibitors and/or ARBs	33 (69%)	30 (75%)	45 (68%)
β-Blockers	12 (25%)	14 (35%)	22 (33%)
Calcium channel blockers	10 (21%)	4 (10%)	12 (18%)
Spironolactone	12 (25%)	19 (48%)	20 (30%)
Loop diuretics	31 (65%)	35 (88%)	53 (80%)#
Digoxin	16 (33%)	15 (38%)	24 (36%)
Statins	5 (10%)	4 (10%)	8 (12%)

*$p < 0.05$ and **$p < 0.01$ vs low, and #$p < 0.05$ and ##$p < 0.01$ vs intermediate. $$p < 0.05$ and $$$p < 0.01$ by chi-square test. Abbreviations as in Table 1.

(A) Hazard ratio of total cardiac events relative to Low stratum

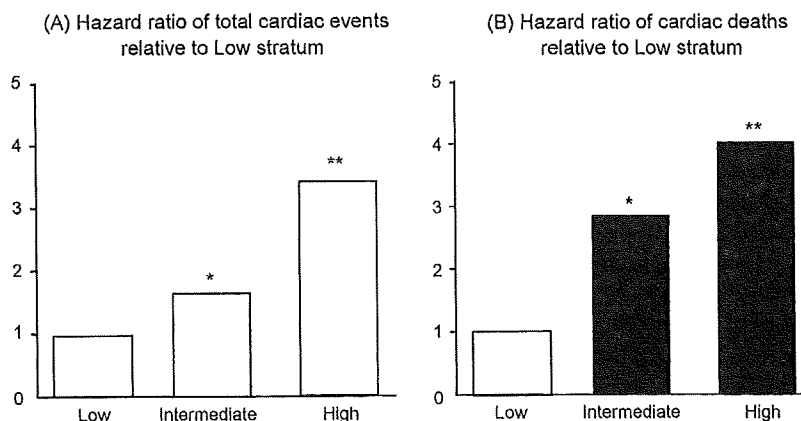(B) Hazard ratio of cardiac deaths relative to Low stratum

Figure 3 Hazard ratios to predict cardiac events among the 3 strata. The univariate Cox proportional hazard analysis demonstrated that the high stratum was associated with the highest risk for total cardiac events (A) and cardiac deaths (B) among the 3 strata. **$p < 0.001$ vs low stratum patients.
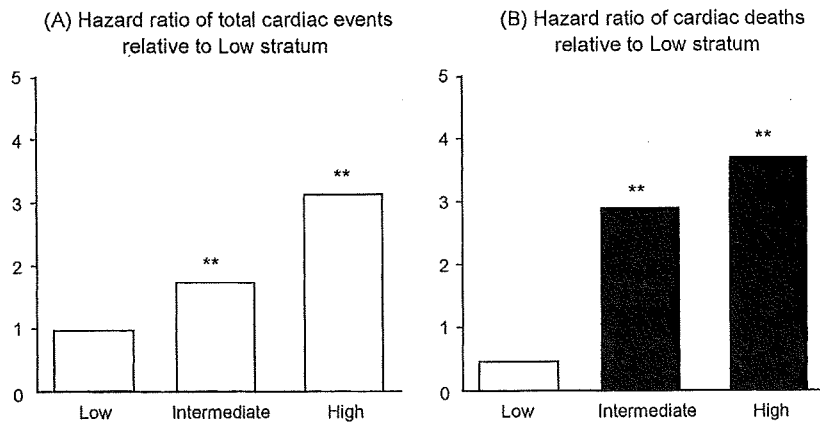
(A) Hazard ratio of total cardiac events relative to Low stratum

(B) Hazard ratio of cardiac deaths relative to Low stratum

Figure 4 Hazard ratios adjusted for age and sex by univariate Cox proportional hazard analysis. The high stratum was associated with the highest risk for total cardiac events (A) and cardiac deaths (B) among the 3 strata. **$p < 0.001$ vs low stratum patients.

Table 4 Univariate and multivariate analyses of predicting cardiac events.

Variable	Hazard ratio	95% confidence interval	p-Value
Univariate analysis			
Age (per 1 S.D. increase), (0.026, 12)	1.366	1.074–1.715	0.0111
NYHA functional class	1.305	0.810–1.117	0.0932
LVEF (per 1 S.D. increase), (0.011, 19)	0.812	0.625–1.039	0.0985
Multimarker score	1.764	1.342–2.320	<0.0001
Multivariate analysis			
Age (per 1 S.D. increase), (0.019, 12)	1.256	0.964–1.657	0.0963
NYHA functional class	1.156	0.656–1.447	0.2159
LVEF (per 1 S.D. increase), (0.0008, 19)	0.985	0.649–1.122	0.2624
Multimarker score	1.689	1.239–2.304	0.0009

Abbreviations as in Table 1.
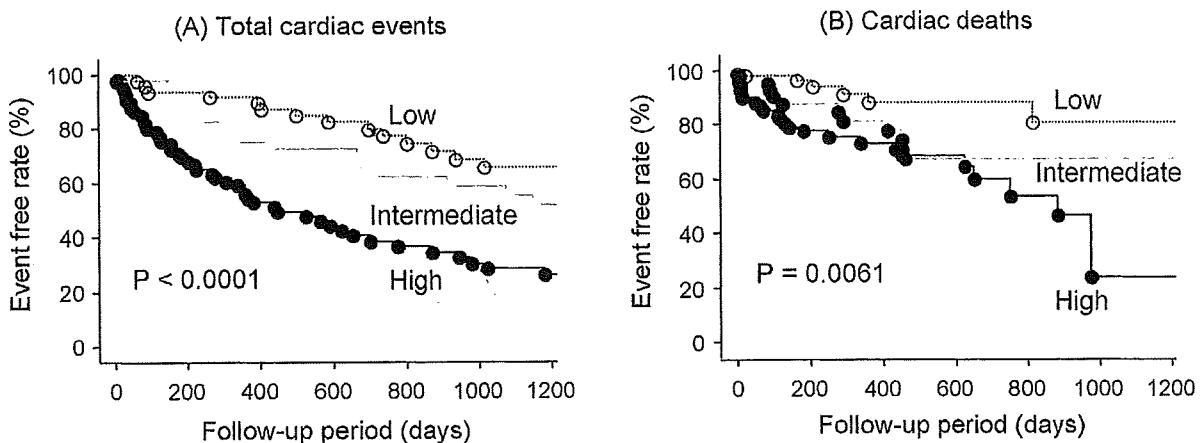

(A) Total cardiac events

(B) Cardiac deaths

Figure 5 Kaplan–Meier analysis in chronic heart failure patients stratified into 3 strata based on multimarker score. Patients in the high stratum had significantly higher rates of total cardiac events (A) and cardiac deaths (B) compared with lower strata.