is sufficient such as in Japan [33]. Nonetheless, a strong inverse relationship between serum selenium and infectious disease-related death was clearly indicated; therefore, we should pay more attention to selenium deficiency in hemodialysis patients and should examine why serum selenium levels in hemodialysis patients are decreased.

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Conflict of interest statement. None declared.

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Original Article

Seropositivity for Anti-HCV Core Antigen is Independently Associated With Increased All-Cause, Cardiovascular, and Liver Disease-Related Mortality in Hemodialysis Patients

Masaki Ohsawa[1], Karen Kato[2], Kozo Tanno[1], Kazuyoshi Itai[1], Yosuke Fujishima[1,3], Akira Okayama[4], Tanvir Chowdhury Turin[5], Toshiyuki Onoda[1], Kazuyuki Suzuki[6], Motoyuki Nakamura[7], Kazuko Kawamura[8], Takashi Akiba[9], Kiyomi Sakata[1], and Tomoaki Fujioka[3]

[1]Department of Hygiene and Preventive Medicine, Iwate Medical University, Iwate, Japan
[2]Division of Urology, San-ai Hospital, Iwate, Japan
[3]Department of Urology, Iwate Medical University, Morioka, Japan
[4]The First Institute of Health Service, Japan Anti-Tuberculosis Association, Tokyo, Japan
[5]Department of Medicine, University of Calgary, Calgary, Alberta, Canada
[6]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Iwate Medical University, Morioka, Japan
[7]Division of Cardiovascular Medicine, Nephrology and Endocrinology, Department of Internal Medicine, Iwate Medical University, Morioka, Japan
[8]Iwate Health Service Association, Morioka, Japan
[9]Division of Blood Purification, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan

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ABSTRACT

Background: It is not known whether chronic or past hepatitis C virus (HCV) infection contributes to the high mortality rate in hemodialysis patients.

Methods: This prospective study of 1077 adult hemodialysis patients without hepatitis B virus infection used Poisson regression analysis to estimate crude and sex- and age-adjusted rates (per 1000 patient-years) of all-cause, cardiovascular, infectious disease-related and liver disease-related mortality in patients negative for HCV antibody (group A), patients positive for HCV antibody and negative for anti-HCV core antigen (group B), and patients positive for anti-HCV core antigen (group C). The relative risks (RRs) for each cause of death in group B vs group C as compared with those in group A were also estimated by Poisson regression analysis after multivariate adjustment.

Results: A total of 407 patients died during the 5-year observation period. The sex- and age-adjusted mortality rate was 71.9 in group A, 80.4 in group B, and 156 in group C. The RRs (95% CI) for death in group B vs group C were 1.23 (0.72 to 2.12) vs 1.60 (1.13 to 2.28) for all-cause death, 0.75 (0.28 to 2.02) vs 1.64 (0.98 to 2.73) for cardiovascular death, 1.64 (0.65 to 4.15) vs 1.58 (0.81 to 3.07) for infectious disease-related death, and 15.3 (1.26 to 186) vs 28.8 (3.75 to 221) for liver disease-related death, respectively.

Conclusions: Anti-HCV core antigen seropositivity independently contributes to elevated risks of all-cause and cause-specific death. Chronic HCV infection, but not past HCV infection, is a risk for death among hemodialysis patients.

Key words: hepatitis C virus; hemodialysis; mortality; population-based cohort study

INTRODUCTION

Hepatitis C virus (HCV) infection, currently the most common blood-borne infection, is an emerging public health problem.[1] Only 20% to 30% of patients with acute HCV infection spontaneously recover; the rest develop chronic HCV infection. Most patients who recover from HCV infection do not develop liver cirrhosis or hepatocellular carcinoma (HCC), whereas patients with chronic HCV infection develop liver cirrhosis or HCC within 20 to 30 years of initial infection.[2]

Hemodialysis patients are especially vulnerable to HCV infection, because of exposure associated with dialysis and blood transfusion.[3–5] The prevalence of HCV in hemodialysis patients is very high (2.7%–30.0%).[6–21] Studies suggest that HCV infection independently contributes to increased mortality among hemodialysis patients.[22–27] However, it is not known whether chronic HCV infection or a history of past

Address for correspondence. Masaki Ohsawa, MD, Department of Hygiene and Preventive Medicine, Iwate Medical University, 2-1-1 Nishitokuta, Yahaba-cho, Iwate 028-3694, Japan (e-mail: masakio@iwate-med.ac.jp).

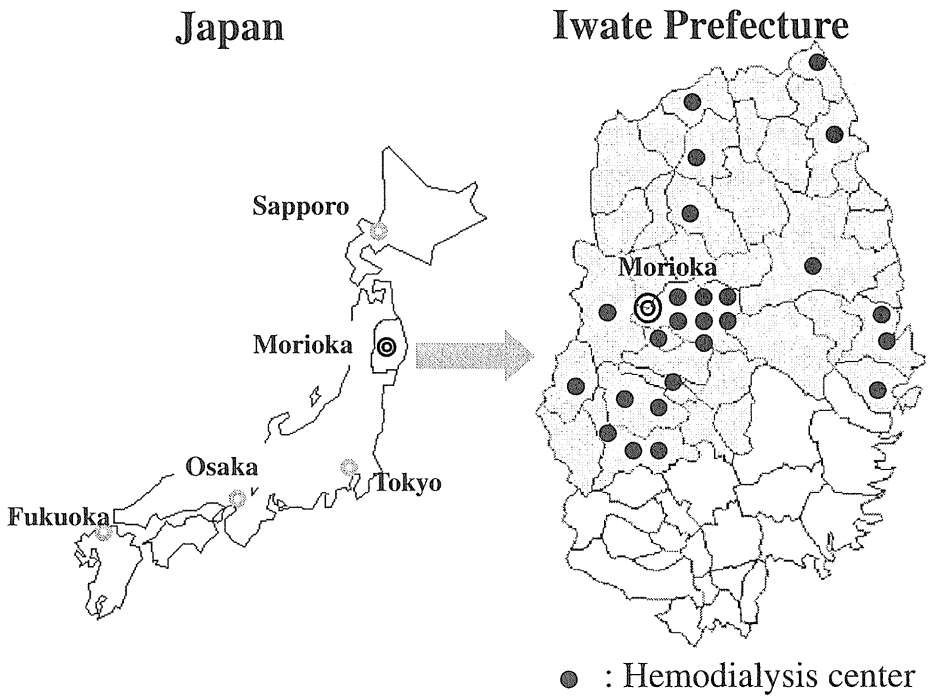

Figure 1. Map of the KAREN Study area. A map of Japan. Morioka, the capital of Iwate Prefecture, is located in northeast Honshu island. The KAREN Study area (the shaded area covering about two-thirds of Iwate Prefecture) has 26 hemodialysis centers. Only 1 center, which treats 7 patients, was not included in the study. Each closed circle represents a hemodialysis center.

HCV infection increases mortality. Moreover, it has not been established whether the elevated mortality risk due to HCV infection is mostly attributable to an increase in liver disease-related deaths.

To assess the contribution of past and chronic HCV infection among hemodialysis patients, we estimated the relative risks of all-cause and cause-specific death attributable to HCV antibody seropositivity and anti-HCV core antigen seropositivity.

METHODS

Participants

The eligible participants were adult hemodialysis patients who participated in the KAREN study, a population-based prospective study that has been conducted since 2003 in northern Japan (Figure 1).[28] A total of 1214 adult hemodialysis patients (80.6% of all hemodialysis patients in the study area; age 22 to 95 years; 779 men and 435 women) are included in the KAREN study. The participants in the KAREN study are patients who were undergoing adult hemodialysis in April 2003. A total of 137 patients who were positive for hepatitis B surface antigen were excluded. Ultimately, data from 1077 patients were analyzed. We ascertained the vital status of all subjects in a 5-year follow-up survey (Figure 2). All participants gave written informed consent to participate. This study was approved by the Medical Ethics Committee of Iwate Medical University and conducted in accordance with the guidelines of the Declaration of Helsinki.

Data collection

The initial investigations in the KAREN Study consisted of a questionnaire, review of medical records, measurements of blood pressure and anthropometric data, and blood testing. The data gathering methodology was previously described.[21,28] Information on HCV antibody serology testing was collected by reviewing medical charts.[21]

Results of anti-HCV antibody tests could not be obtained from chart review for 50 patients. Frozen serum samples from those patients were thawed and anti-HCV antibody tests were performed using a second-generation assay (Architect HCV, Abbott Laboratories, Japan). Frozen samples from patients who were positive for anti-HCV antibody were thawed, and HCV core antigen tests were performed using a chemiluminescent enzyme immunoassay (Lumispot Eiken HCV antigen, Eiken Chemical Co., LTD, Japan).[21]

Outcomes

Follow-up studies were performed annually at each center. Members of the KAREN Study team reviewed all the medical records of study participants. The medical records of deceased patients were summarized. Cause of death was independently determined by physicians on the KAREN Outcome Review

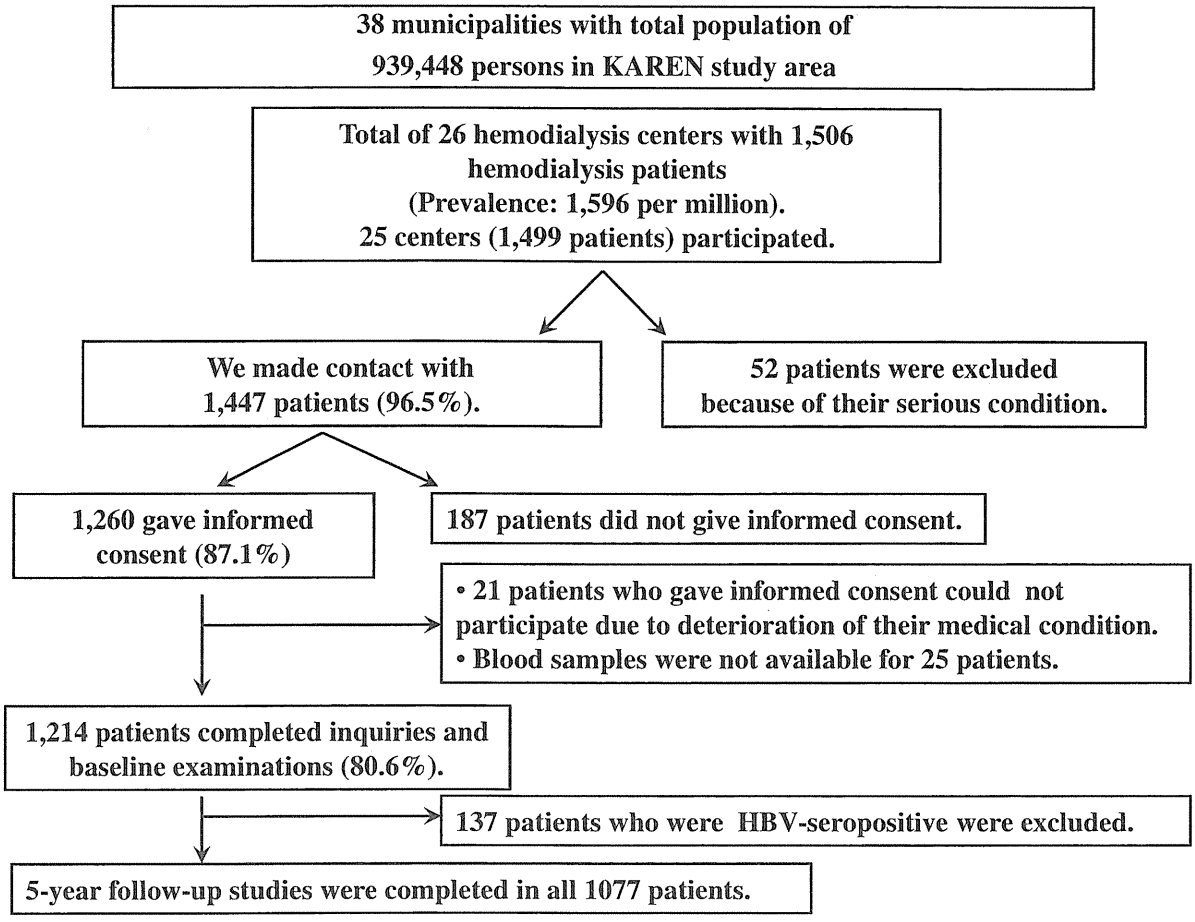

Figure 2. Flow chart of the procedure used to select patients for participation in the KAREN Study. There were 1506 adults receiving hemodialysis in 26 centers in the study area. We were able to contact 1447 patients (96.5%). Fifty-two patients were excluded because of their serious clinical condition. A total of 1260 patients (87.1%) provided written informed consent for participation in the study, and 1214 patients (80.6%) completed the baseline examinations. A total of 137 patients who were positive for hepatitis B surface antigen were excluded. Finally, data from 1077 patients were analyzed. We ascertained the vital status of all participants after completion of a 5-year follow-up survey.

Committee, based on the summaries. Disagreements regarding cause of death were discussed, and the final determination was reached by consensus. We identified the 3 major causes of death (cardiovascular, infectious disease-related, and liver disease-related) using codes form the Tenth Revision of the International Classification of Diseases (ICD-10; Table 1).

Classification and definition

The participants were divided into 3 groups based on the results of HCV antibody testing and anti-HCV core antigen testing at the baseline survey. Group A consisted of patients who were negative for anti-HCV antibodies ($n = 968$). Group B consisted of patients who were positive for anti-HCV antibodies and negative for anti-HCV core antigen antibodies ($n = 55$). Group C consisted of patients who were positive for both anti-HCV antibodies and anti-HCV core antigen antibodies ($n = 79$). These 3 groups were selected because they roughly corresponded to patients without HCV infection (group A), patients with past HCV infection (group B), and patients with chronic HCV infection (group C).[21]

High blood pressure was defined as systolic blood pressure (SBP) in the highest quartile of this study population (SBP ≥169 mm Hg). Low blood pressure was defined as SBP in the lowest quartile (<140 mm Hg). Diabetes was defined as a nonfasting plasma glucose level of 200 mg/dL or higher, a plasma HbA1c of 6.5% or higher, use of antidiabetic medication, or a combination thereof. Dyslipidemia was defined as serum total cholesterol (TC) of 220 mg/dL or higher, serum high-density lipoprotein cholesterol (HDL-C) level less than 40 mg/dL, use of antidyslipidemic medication, or a combination thereof. High body mass index (BMI) was defined as a BMI of 27.5 kg/m^2 or higher. Low BMI was defined as a BMI less than 18.5 kg/m^2. High-sensitivity C reactive protein (hs-CRP) level was considered high if it was in the highest quartile (≥3.6 mg/L). Hypoalbuminemia was

Table 1. Criteria for determining causes of death in the KAREN Study (based on ICD-10)

Cardiovascular death: I01–I99 plus R96		
cardiac death: I20–I25, I27, I29, I30–I52		
	I20–I25	coronary artery disease
	I33	Acute and subacute endocarditis
	I50	heart failure
pulmonary embolism: I26		
stroke death: I60–I69		
	I60	subarachnoidal hemorrhage
	I61, I62	intracerebral hemorrhage
	I63	cerebral infarction
	I64, I67	other type of stroke
vascular death: I70–I77		
	I70	Atherosclerosis
	I71	aortic anuerysm and dissection
	I72, I73	other peripheral artery disease
	I74	arterial embolism and thrombosis
	I77	other arterial disease
sudden cardiac death: I46, I49, R96		
cardiac arrest: I46		
	I46.0	Cardiac arrest with successful resuscitation
	I46.1	Sudden cardiac death, so described
	I46.9	Cardiac arrest, unspecified
ventricular fibrillation and flutter: I49		
	I49.0	Ventricular fibrillation and flutter
other sudden death, cause unknown R96		
	R96.0	Instantaneous death
	R96.1	Death occurring less than 24 hours from onset of symptoms, not otherwise explained
Infectious disease-related death:		
A: bacterial infection: A00–A09, A15–A19, A40–A41 (septicemia)		
B: viral infection, fungal and other microorganism infection		
G: infectious diseases in nervous system		
	G00	Bacterial meningitis, not elsewhere classified
	G04.2	Bacterial meningoencephalitis and meningomyelitis, not elsewhere classified
J: infectious diseases in respiratory tract		
	J10–J11	influenza
	J12–J18	pneumonia
	J20	acute bronchitis
	J69	Pneumonitis due to solids and liquids
	J86	Pyothorax
K: infectious diseases in gastrointestinal tract and digestive organ		
	K65	Peritonitis
	K80.3	Calculus of bile duct with cholangitis
	K81	Cholecystitis
L: infectious diseases in skin and subcutaneous tissue		
	L03	Cellulitis
	L89	Decubitus ulcer
Liver disease-related death		
	K71.2	Toxic liver disease with acute hepatitis
	K72	Acute and subacute hepatic failure
	K73	Chronic hepatitis, not elsewhere classified
	K74	Fibrosis and cirrhosis of liver
	C22.0	Liver cell carcinoma

defined as a serum albumin level less than 3.5 mg/dL. A smoking habit was defined as current smoking. Regular drinking was defined as alcohol consumption on 5 or more days per week.

Statistical analysis

Risk factor-related variables were expressed as sex- and age-adjusted means plus 95% CI and compared across HCV infection status groups using analysis of covariance (ANCOVA). The hs-CRP level was expressed as a sex- and age-adjusted geometric mean plus 95% CI. The χ^2 test was used to compare frequencies.

We defined the follow-up period as the period from the initial survey to the first outcome or the end of observation. Individuals who were free of outcomes in the 5-year follow-up study were administratively censored. The cumulative probability of each cause of death was estimated using the Kaplan-Meier method, and differences in the cumulative probability of death were assessed by the log-rank test. Crude mortality rates and sex- and age-adjusted mortality rates were estimated in the 3 groups (groups A, B, and C) by Poisson regression analysis in which multivariate-adjusted mortality rate ratios and their 95% CIs were calculated in groups B and C, with those of group A serving as reference. The variables used in the multivariate adjustment were traditional risk factors, including age, male sex, high BMI, dyslipidemia, diabetes, high blood pressure, history of myocardial infarction, stroke, or malignant disease, smoking habit, and regular drinking habit (model A). Hemodialysis-related risk factors, including low BMI, low blood pressure, high CRP level, and hypoalbuminemia, were also additionally used as explanatory variables in model B. All P values were 2-tailed, and values less than 0.05 were considered to indicate statistical significance. The statistical package PASW (version 18.0, IBM Japan Inc., Tokyo, Japan) was used for the statistical analysis.

RESULTS

Table 2 shows the baseline characteristics of the patients, stratified by HCV infection status. The proportions of patients in groups A, B, and C were 90.0%, 3.6%, and 6.5%, respectively. As compared with patients in group A, those in group C had significantly lower serum TC, serum low-density lipoprotein cholesterol (LDL-C), serum albumin, and serum creatinine levels, and lower platelet and white blood cell (WBC) counts ($P < 0.05$ for all tests). Patients in group B had significantly lower systolic blood pressure, TC, and LDL-C levels, and lower platelet and WBC counts than did patients in group A ($P < 0.05$ for all tests).

The proportion of current smokers in group C was the highest of the 3 groups ($\chi^2 = 6.47$, $P = 0.03$). There was no significant difference among groups in the proportions of patients with chronic glomerulonephritis ($\chi^2 = 5.66$, $P = 0.06$), diabetic nephropathy ($\chi^2 = 3.06$, $P = 0.22$), diabetes mellitus ($\chi^2 = 5.41$, $P = 0.07$), or past histories of myocardial infarction ($\chi^2 = 1.65$, $P = 0.44$), stroke ($\chi^2 = 0.93$, $P = 0.63$), or malignancy ($\chi^2 = 4.12$, $P = 0.13$). There were 4233 observed patient-years, after 5 years of follow-up. The mean and median follow-up periods were 3.9 and 4.9 years, respectively. A total of 406 patients died during the 5-year observation period.

Figure 3 shows the Kaplan-Meier estimated cumulative probabilities of death for patients in the 3 groups. The cumulative probability of all-cause death (upper left) in group C was significantly higher as compared with group A

Table 2. Baseline characteristics of patients stratified by HCV seropositivity

HCV seropositivity status groups (number of subjects)	group A HCV Ab(−) *n* = 968	group B HCV Ab(+) Ag(−) *n* = 39	group C HCV Ab(+) Ag(+) *n* = 70
male *n*, (%)	605 (62.5%)	25 (64.1%)	53 (75.7%)
mean age (SD) (yrs)	61.2 (13.3)	61.1 (13.6)	58.8 (10.9)
median vintage of HD (25–75%) (yrs)	4.5 (1.9–8.3)	8.9 (3.6–21.2)	8.3 (2.4–21.8)
Sex- and age-adjusted mean levels and their 95% CIs of anthropometrical and blood test measurements			
body mass index (kg/m^2)	21.0 (20.8–21.2)	20.6 (19.6–21.5)	20.2 (19.5–20.9)
SBP (mm Hg)	156 (154–157)	146 (138–153)[b]	155 (149–161)
total cholesterol level (mg/dl)	157 (155–159)	140 (129–150)[c]	136 (128–144)[c]
HDLC (mg/dl)	47.1 (46.1–48.0)	43.4 (38.7–48.1)	44.7 (41.2–48.2)
LDLC (mg/dl)	86.1 (84.4–87.8)	74.2 (65.9–82.5)[b]	71.8 (65.6–78.0)[c]
total protein (g/dl)	6.48 (6.46–6.52)	6.48 (6.33–6.63)	6.66 (6.55–6.78)[c]
serum albumine (g/dl)	3.78 (3.76–3.80)	3.74 (3.63–3.84)	3.50 (3.42–3.58)[c]
serum creatinine (mg/dl)	11.2 (11.1–11.4)	10.7 (9.94–11.5)	10.4 (9.87–11.0)[b]
hemoglobin (g/dl)	10.2 (10.1–10.3)	9.9 (9.50–10.4)	10.2 (9.93–10.5)
platelet count (10^4/μl)	18.7 (18.2–19.1)	15.0 (13.0–17.0)[c]	16.1 (14.5–17.6)[c]
white blood count (/μl)	5814 (5706–5923)	5025 (4487–5562)[b]	5120 (4718–5522)[c]
hsCRP[a] (mg/l)	1.16 (1.06–1.28)	1.40 (0.89–2.20)	1.20 (0.85–1.69)
Causes of renal filure, comorbid conditions and habits expressed as numbers (%)			
CGN	276 (28.5%)	18 (46.2%)	20 (28.6%)
DMN	241 (24.9%)	5 (12.8%)	16 (22.9%)
HTN	97 (10.0%)	4 (10.3%)	9 (12.9%)
PCK	37 (3.8%)	1 (2.6%)	0 (0.0%)
Lupus N	4 (0.4%)	0 (0.0%)	0 (0.0%)
Others	62 (6.4%)	4 (10.3%)	6 (8.6%)
unknown	251 (25.9%)	7 (17.9%)	19 (27.1%)
MI	42 (4.3%)	1 (2.6%)	1 (1.4%)
stroke	159 (16.4%)	5 (12.8%)	9 (12.9%)
malignancy	67 (6.9%)	6 (15.4%)	6 (8.6%)
DM	289 (29.9%)	5 (12.8%)	19 (27.1%)
dyslipidemia	440 (45.5%)	19 (48.7%)	32 (45.7%)
current smoker	254 (26.2%)	12 (30.8%)	28 (40.0%)[d]
past smoker	247 (25.5%)	8 (20.5%)	16 (22.9%)
regular drinker	71 (7.3%)	2 (5.1%)	2 (2.9%)

[a]hsCRP levels are expressed as adjusted geometric means (95% CIs) estimated by ANCOVA.
[b]$P < 0.05$ [c]$P < 0.01$ estimated by multiple comparisons using Bonferroni adjustment.
[d]$P < 0.05$ estimated by chi squared test.
Abbreviations: SD, standard deviation; HD, hemodialysis; CI, confidence interval; SBP, systolic blood pressure; HDLC, high-density lipoprotein cholesterol level; LDLC, low-density lipoprotein cholesterol level; hsCRP, serum high-sensitive C reactive protein; CGN, chronic glommerulonephritis; DMN, diabetic nephropathy; HTN, hypertensive nephrosclerosis; PCK, congenital polycystic kidney disease; Lupus N, lupus nephritis; MI, myocardial infarction; DM, diabetes mellitus; ANCOVA, analysis of covariance.

($P = 0.007$, log rank test), but not as compared with group B ($P = 0.174$). Group C also had higher probabilities of cardiovascular death (upper right, $P = 0.033$) and liver disease-related death (lower right, $P < 0.001$) as compared with group A. Group B did not have significantly higher probabilities of cardiovascular death ($P = 0.118$), infectious disease-related death ($P = 0.775$), or liver disease-related death ($P = 0.457$).

Table 3 shows the number of deaths, crude mortality rates, and sex- and age-adjusted mortality rates per 1000 patient-years (95% CIs), and relative risks for death expressed as sex- and age-adjusted relative mortality rate ratios (95% CIs) in group B and group C as compared with the reference (group A). The crude mortality rates in groups A, B, and C were 92.7, 94.0, and 147, respectively. Sex- and age-adjusted mortality rates (95% CI) in groups A, B, and C were 71.9 (62.6 to 81.3), 80.4 (37.9 to 123), and 156 (104 to 207), respectively. The relative risks (95% CI) for all-cause, cardiovascular, infectious disease-related, and liver disease-related death in group C were 2.16 (1.53 to 3.07), 1.98 (1.19 to 3.28), 2.46 (1.27 to 4.76), and 30.8 (5.34 to 178), respectively. In contrast, group B did not have significantly higher risks for death, with the exception of liver disease-related death (RR, 13.7; 95% CI, 1.24 to 152).

Table 4 shows the relative risks for each cause of death in groups B and C, as compared with the reference (group A), expressed as multivariate-adjusted mortality rate ratios. The RRs for all-cause and cardiovascular death in group C were approximately 2.0, which indicated significantly higher risks for such deaths in group C. The RR for infectious disease-related death in group C was also approximately 2.0, although the result was of marginal significance ($P = 0.051$ after

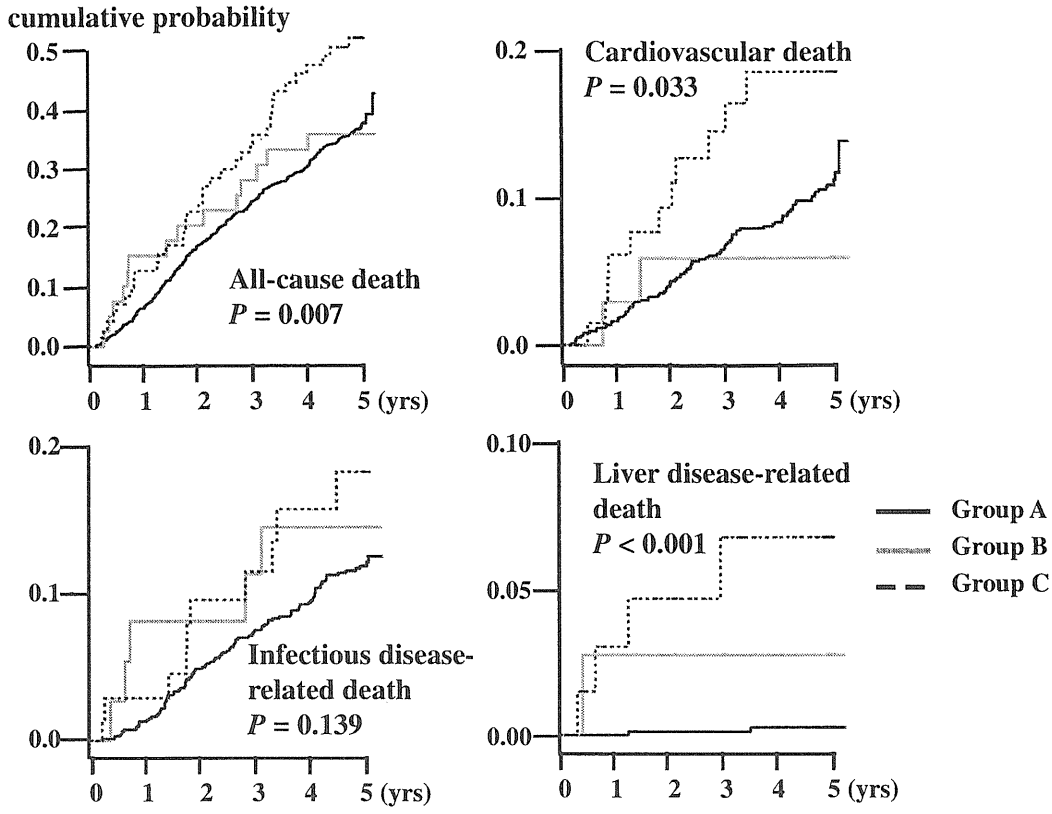

Figure 3. Estimated Kaplan-Meier cumulative probability of death in the 3 groups. The upper left graph shows the cumulative probability of all-cause death. Patients in group C had a significantly higher probability of death than did patients in group A ($P = 0.007$, log rank test), but there was no significant difference in probability of death between groups A and B ($P = 0.174$). The upper right graph shows the cumulative probability of cardiovascular death. Patients in group C had a significantly higher probability of cardiovascular death than did patients in group A ($P = 0.033$); the probability of cardiovascular death did not significantly differ between groups A and B ($P = 0.118$). The lower left graph shows the cumulative probability of infectious disease-related death, which did not significantly differ among the 3 groups. The lower right graph shows the cumulative probability of liver disease-related death. Patients in group C had a significantly higher probability of liver disease-related death as compared with patients without HCV infection ($P < 0.001$). The probability of liver disease-related death in group B did not significantly differ from that of group A ($P = 0.457$).

model A adjustment; $P = 0.14$ after model B adjustment). In contrast, the RRs for all-cause, cardiovascular, and infectious disease-related death in group B ranged from 0.75 to 1.66, and there was no significant increase in the risk of such deaths. The risk of liver disease-related death was 15.3 in group B and 28.8 in group C, which were significantly higher as compared with the reference group.

DISCUSSION

In this study, we estimated crude and sex- and age-adjusted rates for all-cause death and cause-specific death in hemodialysis patients who were negative for HCV antibodies, those who were positive for HCV antibodies, and those who were positive for both HCV antibodies and anti-HCV core antigen antibodies. We also calculated the relative risks of all-cause death and cause-specific death in patients positive for HCV antibodies only and patients positive for both HCV antibodies and anti-HCV core antigen antibodies as compared with patients who were negative for anti-HCV antibodies. These 3 groups roughly correspond to patients without HCV infection (group A), patients with past HCV infection (group B), and patients with chronic HCV infection (group C). Therefore, the results showed higher risks of all-cause, cardiovascular, infectious disease-related, and liver disease-related death among the chronic HCV subgroup, whereas past HCV infection was not associated with increased risk of any cause of death, except liver disease-related death.

Most prior studies investigated only the relative risks of all-cause and/or cause-specific death attributable to HCV infection among hemodialysis patients, without further differentiating between past and chronic HCV infection.[19,22,24,25] In a meta-analysis, Fabrizi et al found that the adjusted RR for all-cause mortality due to HCV infection was 1.34.[27] However, Stehman-Breen et al used quantitative estimation of HCV RNA levels to determine whether chronic

Table 3. Number of deaths, crude and sex- and age-adjusted mortality rates, and relative risks (RRs) for death compared with references by groups according to HCV seropositivity

HCV seropositivity status groups (number of subjects)	group A HCV Ab(−) *n* = 968	group B and C HCV Ab(+) *n* = 109	group B	group C	All subjects *n* = 1077
			HCV Ab(+) Ag(−) *n* = 39	HCV Ab(+) Ag(+) *n* = 70	
all-cause death (crude mortality)	356 (92.7)	50 (127)	14 (94.0)	36 (147)	406 (95.9)
adjusted mortality (95% CI)	71.9 (62.6–81.3)	123 (88.6–158)	80.4 (37.9–123)	156 (104–207)	96.5 (75.5–118)
RR (95% CI)	REF	1.71 (1.27–2.30)	1.12 (0.66–1.91)	2.16 (1.53–3.07)	
cardiovascular (crude mortality)	173 (45.1)	21 (53.4)	4 (26.9)	17 (69.5)	194 (45.8)
adjusted mortality (95% CI)	37.2 (30.5–43.9)	53.0 (30.0–76.0)	24.3 (0.40–48.2)	73.5 (38.0–109)	40.5 (25.3–55.7)
RR (95% CI)	REF	1.42 (0.90–2.44)	0.65 (0.24–1.76)	1.98 (1.19–3.28)	
infectious disease-related (crude mortality)	97 (25.3)	15 (38.1)	5 (33.6)	10 (40.9)	112 (26.5)
adjusted mortality (95% CI)	17.8 (13.1–22.5)	35.6 (17.1–54.1)	25.7 (2.70–48.7)	43.6 (16.3–71.0)	27.1 (16.5–37.7)
RR (95% CI)	REF	1.99 (1.15–3.43)	1.45 (0.50–3.56)	2.46 (1.27–4.76)	
liver disease-related (crude mortality)	2 (0.52)	5 (12.7)	1 (6.71)	4 (16.4)	7 (1.65)
adjusted mortality (95% CI)	0.40 (0.00–1.10)	9.80 (0.00–21.5)	5.70 (0.00–17.4)	12.8 (0.00–29.6)	3.10 (0.00–6.60)
RR (95% CI)	REF	24.2 (4.56–128)	13.7 (1.24–152)	30.8 (5.34–178)	

Crude and sex- and age-adjusted mortality rates (95% confidence intervals) are expressed as /1000 person-years. Adjusted mortalities and relative risks (expressed as relative mortality rate ratios) were estimated by Poisson regression analysis.

Table 4. Relative risks (RRs) for each cause of death compared with references by groups according to HCV seropositivity

HCV seropositivity status groups	group A HCV Ab(−)	group B and C HCV Ab(+)	group B	group C
			HCV Ab(+) Ag(−)	HCV Ab(+) Ag(+)
all-cause death				
model A	Ref	1.62 (1.20–2.18)	1.24 (0.72–2.12)	1.83 (1.29–2.59)
model B		1.48 (1.09–2.00)	1.23 (0.72–2.12)	1.60 (1.13–2.28)
cardiovascular death				
model A	Ref	1.42 (0.89–2.24)	0.75 (0.28–2.04)	1.79 (1.08–2.97)
model B		1.33 (0.84–2.11)	0.75 (0.28–2.02)	1.64 (0.98–2.73)
infectious disease-related death				
model A	Ref	1.83 (1.05–3.19)	1.66 (0.66–4.13)	1.94 (0.99–3.75)
model B		1.60 (0.91–2.80)	1.64 (0.65–4.15)	1.58 (0.81–3.07)
liver disease-related death				
model A	Ref	18.6 (3.51–98.1)	8.55 (0.75–98.1)	26.6 (4.57–155)
model B		22.9 (3.53–149)	15.3 (1.26–186)	28.8 (3.75–221)

Crude and adjusted mortalities (95% confidence intervals) are expressed as /1000 person-years.
Adjusted mortalities were estimated by Poisson regression analysis after adjusting for risk factors.
Adjustment for risk factors
model A: age, male gender, high BMI, dyslipidemia, diabetes, high blood pressure, history of myocardial infarction, stroke, or malignant diseases, smoking habit and regular drinking habit.
model B: model A plus low BMI, low blood pressure, high CRP level, and hypoalbuminemia.

HCV infection increased mortality among hemodialysis patients: the multivariate-adjusted RR for all-cause death attributable to chronic HCV infection was 2.0.[23] Other studies reported a multivariate-adjusted RR of death attributable to HCV infection (including past HCV infection) between 1.2 and 1.6.[27] The lower relative risks in studies that assessed HCV status using antibody-based techniques may be due to underestimation related to the inclusion of patients with past HCV infection only. In our study, the RR of all-cause death due to chronic HCV infection, as determined by quantitative estimation of HCV core antigen, was 1.83 after traditional risk factor adjustment, which is similar to the RR of 2.0 reported by Stehman-Breen et al. Taken together, both previous studies and the present study suggest that it is mainly chronic HCV infection that increases the risks of all-cause and cause-specific death.

The causes of death that contribute to increased mortality among hemodialysis patients with chronic HCV infection were not fully identified in previous studies. In a meta-analysis, Fabrizi et al showed that HCV-seropositive hemodialysis patients had higher rates of liver disease-related death than their seronegative counterparts, but that

cardiovascular and infectious disease-related morality rates were similar.[27] The studies included in their meta-analysis all cited cardiovascular death as the most common cause of death in dialysis patients. Excess deaths attributable to HCV infection cannot be explained by an increase in the number of HCV-attributable liver disease-related deaths. Whether cardiovascular death (the most common cause of death) and infectious disease-related death (the second most common cause of death) increase mortality among hemodialysis patients is also important, as is the contribution of liver disease-related death.

We are unable to explain the increased risks of cardiovascular death and infectious disease-related death among hemodialysis patients who were positive for anti-HCV core antigen antibodies in the present study. Cross-sectional analysis of baseline data provides some clues regarding possible mechanisms that might explain the association between anti-HCV core antigen positivity and increased cardiovascular and infectious disease-related mortality risk. Despite being younger, patients who were positive on the anti-HCV core antigen test had lower levels of serum lipids and albumin as compared with patients who were negative on the HCV antibody test. These findings suggest that hemodialysis patients who were positive for anti-HCV core antigen antibodies had hypocholesterolemia and hypoalbuminemia. Thus, insufficient levels of serum cholesterol and albumin might be associated with a malnutrition-inflammation syndrome activated by chronic HCV infection. Such a syndrome might lead to immune dysfunction, resulting in an increased risk of cardiovascular and infectious disease-related death.[19–31]

Several limitations in our study should be noted. Because we enrolled only 70 patients who were positive for anti-HCV core antigen, we were not able to perform an accurate sex-stratified risk assessment of cause-specific death. Sex differences in the risk of each cause of death might exist, and the relationships should therefore be re-examined in larger cohort studies or in meta-analyses using data from patients whose chronic HCV infection status is precisely defined. Lack of high-sensitivity, quantitative HCV-RNA data from patients who were positive for HCV antibody and negative for HCV core antigen is a major limitation of this study. It is possible that hemodialysis patients who are negative for HCV core antigen nevertheless have very low levels of HCV-RNA; however, the possibility of missing such cases is very low because none of the population-based controls in our previous study were simultaneously positive for HCV-RNA and HCV antibody and negative for HCV core antigen.[21] Therefore, we believe that the results of the current study are not distorted by the lack of HCV-RNA data.

Because second-generation ELISA became available as a clinical diagnostic tool in 1992, patients who began hemodialysis treatment before 1992 might have had more exposures to infection and a higher incidence of HCV infection. It remains to be clarified whether HCV infection, and a long history of hemodialysis treatment, independently increase the risk of death. In a separate analysis, we estimated the risk of each cause of death attributable to HCV infection only in patients who started hemodialysis treatment after 1992. The results were similar to those from analyses of all subjects (data not shown).

We determined HCV infection status based on baseline information only. Changes in HCV infection status (eg, incident HCV infection during the observation period) were not considered. Previous studies showed that the incidence of HCV infection in hemodialysis patients was lower than 0.5% per year.[11,32] The risk of death attributable to HCV infection may have been underestimated, and putative underestimation of relative risks of death is not negligible.

Despite its limitations, our simple and economic method of determining HCV infection status provided sufficient results to discern a difference in mortality between patients with past versus chronic HCV infection. Furthermore, limiting the analysis to patients with chronic HCV infection enabled us to show that an increased risk of death among hemodialysis patients with HCV infection was due to an increased risk of cardiovascular and infectious disease-related deaths, as well as the increased risk of liver disease-related death. We conclude that more attention should be paid to chronic HCV infection in hemodialysis patients.

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Conflicts of interest: None declared.

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Sex-Specific Threshold Levels of Plasma B-Type Natriuretic Peptide for Prediction of Cardiovascular Event Risk in a Japanese Population Initially Free of Cardiovascular Disease

Motoyuki Nakamura, MD[a,*], Fumitaka Tanaka, MD[a], Tomohiro Takahashi, MD[a], Shinji Makita, MD[a], Takenori Ishisone, MD[a], Masayuki Onodera, MD[a], Yasuhiro Ishibashi, MD[a], Kazuyoshi Itai, PhD[b], Toshiyuki Onoda, MD, PhD[b], Masaki Ohsawa, MD, PhD[b], Kozo Tanno, MD, PhD[b], Kiyomi Sakata, MD, PhD[b], Omama Shinichi, MD[c], Kuniaki Ogasawara, MD[c], Akira Ogawa, MD[c], Toru Kuribayashi, PhD[d], and Akira Okayama, MD, PhD[e]

Elevated plasma B-type natriuretic peptide (BNP) levels have been reported to be related to a high risk for cardiovascular (CV) disease in the general population. However, there has been no accurate determination of the threshold levels of plasma BNP that indicate an increased potential for the development of general CV events (i.e., heart failure, stroke, and myocardial infarction) and the validity of these levels for predicting CV events compared to classic risk markers. To establish gender-specific thresholds of plasma BNP levels associated with increased risk for CV disease in the general population, baseline BNP levels were determined in community-dwelling adults (n = 13,209, mean age 62 ± 10 years,) and CV events in the cohort were captured prospectively. The cohort was divided by deciles of plasma BNP level in each gender. A Cox proportional-hazards model was used to determine the relative hazard ratios among the deciles. In addition, to compare the utility of plasma BNP to the Framingham 10-year risk score for predicting general CV events, receiver-operating characteristic analysis was performed. During follow-up, CV events were identified in 429 patients in the cohort. Compared to the reference decile level (first to fourth), the hazard ratio was significantly increased from the ninth decile in men (greater than approximately 37 pg/ml) and the highest decile in women (greater than approximately 55 pg/ml). The area under the curve generated on receiver-operating characteristic analysis of plasma BNP testing was comparable to that for the Framingham risk scoring system (0.67 vs 0.68 in men, 0.63 vs 0.68 in women; p = NS for both). In conclusion, within a community-based general population with no CV history, plasma BNP levels higher than defined thresholds show increased risk for general CV events, and the predictive ability for CV events occurring within several years may be comparable to that of an established long-standing risk score. (Am J Cardiol 2011;108:1564–1569)

In the present study, we measured plasma B-type natriuretic peptide (BNP) in a large-scale population-based sample of >13,000 men and women. This cohort was followed prospectively for >5 years to ascertain the incidence of cardiovascular (CV) events, including heart failure, stroke, and myocardial infarction. To determine gender-specific threshold levels of plasma BNP, the relation between plasma BNP deciles and risk for CV events was determined. In addition, to validate plasma BNP testing for the prediction of general CV events, its predictive ability was compared to an established CV risk scoring system.

Methods

This study is part of the Iwate-KENCO study, a population-based prospective cohort study to investigate heath status and CV risks in Japanese residents living in the Iwate prefecture, northeast Honsyu, Japan. Details about this cohort are provided elsewhere.[1] In brief, the original cohort (n = 26,469) was recruited from April 2002 and January 2005 in 3 districts (Ninohe, Kuji, and Miyako in the Iwate prefecture). The baseline survey included routine anthropometric measurements, blood pressure measurement, electrocardiography, routine laboratory assessment, and a self-administered lifestyle questionnaire. This study protocol was approved by our institutional ethics committee. All participants gave written informed consent.

Of the original cohort living in the Ninohe and Kuji districts (n = 15,927), 15,394 subjects (96.6%) agreed to provide additional blood samples for the measurement of

Departments of [a]Internal Medicine, [b]Preventive Medicine, and [c]Neurosurgery, Iwate Medical University; [d]Department of Health and Physical Education, Iwate University, Morioka; and [e]The First Anti-Tuberculosis Association, Tokyo, Japan. Manuscript received May 26, 2011; revised manuscript received and accepted July 6, 2011.

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*Corresponding author: Tel: 81-19-651-5111; fax: 81-19-651-0401.
E-mail address: nkmrmoto@iwate-med.ac.jp (M. Nakamura).

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plasma BNP levels, and these are designated as the BNP cohort in the present study. Subjects were excluded from this cohort on the basis of the following characteristics: age <40 years (n = 575) or >80 years (n = 330), serum creatinine level ≧2.0 mg/dl (n = 10), and missing data for blood pressure (n = 3), anthropometrics (n = 47), and/or routine blood tests (n = 4). The final statistical analysis was therefore performed on 13,209 subjects (4,365 men, 8,844 women; mean age 62.1 years).

A follow-up survey assessing mortality, migration, and the incidence of CV events was carried out after the baseline study. We defined CV events as stroke, congestive heart failure, and myocardial infarction requiring hospitalization. Hospital admissions for congestive heart failure and myocardial infarction in the cohort were identified by accessing data from the Northern Iwate Heart Disease Registry Consortium, which has been collecting data since 2002. Heart failure was defined by Framingham criteria,[2] and registration of myocardial infarction was based on criteria used in the Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) study.[3] Stroke events were identified by accessing the prefecture stroke registration program conducted by the Iwate Medical Association. Stroke diagnostic criteria in this registry are based on those published by the World Health Organization and defined as the sudden onset of neurologic symptoms.[4] To ensure that nearly all appropriate cases had been identified, researchers in each registration study periodically retrieved and reviewed medical charts and/or discharge summaries for patients admitted to the cardiology, neurology, neurosurgery, and internal medicine wards of all hospitals located within the study district.

In the baseline survey, all participants underwent routine anthropometric measurements, electrocardiography, blood pressure measurements, and laboratory assessments. In addition, a self-administered questionnaire was used to ascertain lifestyle factors such as smoking habits and medical history, including stroke, congestive heart failure, and myocardial infarction. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Systolic and diastolic blood pressure were determined with an automatic device with the subject in a sitting position for ≧5 minutes before measurement. Measurement was performed twice, with the mean value used for statistical analysis. Hypertension was defined as systolic blood pressure ≧140 mm Hg and/or diastolic blood pressure ≧90 mm Hg and/or current antihypertensive therapy. Diabetes was defined as a nonfasting glucose concentration ≧200 mg/dl, and/or a glycosylated hemoglobin value ≧6.5%, and/or current antidiabetic therapy. Hypercholesterolemia was defined as total cholesterol level ≧240 mg/dl and/or current lipid-lowering therapy. Enzymatic methods were used to measure serum total cholesterol levels, serum creatinine, and blood glucose. Glycosylated hemoglobin was measured quantitatively using high-performance liquid chromatography. Smoking was defined as current smoking. Estimated glomerular filtration rate was calculated using an equation (estimated glomerular filtration rate [ml/min/1.73 m^2] = 194 × serum creatinine$^{-1.094}$ × age$^{-0.287}$) from the Modification of Diet in Renal Disease (MDRD) study for the Japanese population.[5] The 10-year risk for general CV disease was calculated using the Framingham 10-year risk

Table 1
Baseline characteristics according to plasma B-type natriuretic peptide deciles in men

Variable	Total	D1–D4	D5	D6	D7	D8	D9	D10
Number	4,365	1,741	441	441	434	436	436	436
BNP (pg/ml)	14.2 (6.3–28.3)	5 (2.1–7.6)	12.3 (11.4–13.2)	16.3 (15.3–17.5)	21.3 (19.8–22.8)	28.3 (26.5–30.5)	41.4 (37.5–46.5)	76.5 (63.4–116.7)
Age (years)	63.3 ± 9.8	58.3 ± 10.0	62.9 ± 9.0	65.5 ± 8.4	65.8 ± 8.1	67.6 ± 7.2	68.1 ± 7.4	69.7 ± 6.2
BMI (kg/m^2)	23.9 ± 2.9	24.1 ± 2.9	24.0 ± 3.0	23.9 ± 2.8	23.7 ± 2.9	23.6 ± 2.8	23.5 ± 2.9	23.7 ± 3.0
Hypertension	43.8%	35.1%	41.0%	46.5%	45.6%	49.8%	56.6%	57.6%
Diabetes mellitus	9.6%	9.8%	8.2%	11.6%	9.0%	10.1%	8.9%	9.2%
Smoker	33.9%	39.1%	33.1%	30.2%	32.7%	31.7%	28.0%	27.1%
Hypercholesterolemia	10.5%	14.5%	9.1%	9.3%	7.1%	8.5%	7.6%	5.7%
eGFR (ml/min/1.73 m^2)	77.2 ± 15.3	80.0 ± 15.3	77.3 ± 15.1	76.4 ± 15.6	76.5 ± 15.0	74.8 ± 14.7	75.8 ± 15.2	71.3 ± 13.3
Antihypertensive drugs	23.3%	15.8%	21.8%	25.9%	26.0%	27.5%	32.8%	35.3%
Framingham risk score	13.8 ± 4.4	12.8 ± 4.5	13.7 ± 4.3	14.5 ± 4.3	14.5 ± 4.1	14.8 ± 4.1	14.9 ± 4.2	15.1 ± 4.1

Data are expressed as median (interquartile range), as mean ± SD, or as percentages.
D = decile; eGFR = estimated glomerular filtration rate.

Table 2
Baseline characteristics according to plasma B-type natriuretic peptide deciles in women

Variable	Total	D1–D4	D5	D6	D7	D8	D9	D10
Number	8,844	3,539	880	880	893	882	885	885
BNP (pg/ml)	16.9 (8.8–29.8)	7.3 (3.8–10.4)	15.0 (14.1–15.9)	18.7 (17.8–19.7)	23.5 (22.2–25.0)	29.8 (28.0–31.9)	40.4 (37.1–43.8)	66.1 (55.1–88.0)
Age (years)	61.6 ± 9.7	58.1 ± 9.5	60.7 ± 9.4	60.9 ± 9.6	63.2 ± 9.0	64.3 ± 8.7	65.3 ± 8.3	68.7 ± 7.2
BMI (kg/m^2)	24.2 ± 3.4	24.2 ± 3.4	24.0 ± 3.3	24.0 ± 3.4	24.1 ± 3.4	24.0 ± 3.3	24.0 ± 3.5	24.4 ± 3.7
Hypertension	38.2%	29.5%	35.1%	36.0%	43.8%	43.2%	47.2%	59.0%
Diabetes mellitus	5.4%	5.3%	4.7%	4.8%	5.0%	6.2%	5.2%	7.2%
Smoker	2.5%	3.5%	1.7%	2.5%	1.9%	2.0%	2.3%	0.8%
Hypercholesterolemia	20.3%	23.3%	18.0%	18.9%	21.2%	19.7%	14.5%	17.2%
eGFR (ml/min/1.73 m^2)	75.8 ± 15.0	78.6 ± 14.9	76.5 ± 14.8	76 ± 13.9	74.7 ± 14.1	73.9 ± 15.2	73.2 ± 14.7	69.5 ± 14.8
Antihypertensive drugs	23.8%	16.8%	22.8%	21.5%	28.4%	27.4%	29.9%	41.2%
Framingham risk score	11.9 ± 4.6	10.8 ± 4.6	11.2 ± 4.5	11.7 ± 4.5	12.4 ± 4.5	12.6 ± 4.4	13.1 ± 4.4	14.3 ± 4.0

Data are expressed as median (interquartile range), as mean ± SD, or as percentages.
D = decile; eGFR = estimated glomerular filtration rate.

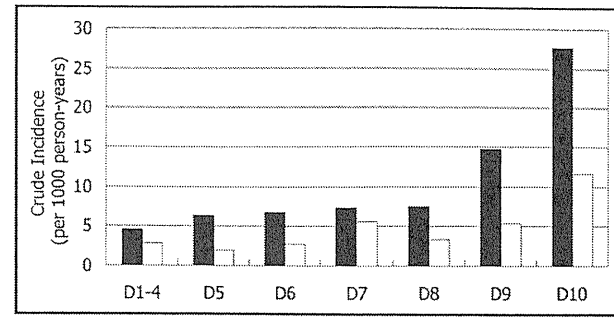

Figure 1. Crude incidence of CV events per 1,000 person-years among baseline plasma BNP deciles in men *(closed bars)* and women *(open bars)*.

score, including age, gender-specific cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diabetes, and cigarette smoking.[6]

Blood samples for routine laboratory testing were drawn from the antecubital vein with the subject in a sitting position. While blood samples were being collected into vacuum tubes, an additional 2-ml sample of venous blood was collected into a test tube containing ethylenediaminetetraacetic acid sodium. Tubes were stored immediately after sampling in an icebox and were transported to the laboratory <8 hours after collection. They were then centrifuged at 1,500g for 10 minutes. After separation, the plasma samples were stored frozen at −20°C until the time of assay. Plasma BNP levels were measured by direct radioimmunoassay using monoclonal antibodies specific for human BNP (Shionogi, Osaka, Japan) <4 months after blood sampling. Cross-reactivity of the antibodies was 100% for human BNP and 0.001% for human atrial natriuretic peptide. Intra- and interassay coefficients of variation were 5% and 6%, respectively. The lower detection limit of the assay was 0.05 pg/ml.

Continuous variables are expressed as mean ± SD. The cohort was divided into deciles according to baseline plasma BNP levels. To compare baseline data among the BNP deciles, 1-way analysis of variance and chi-square tests were used as appropriate. Differences in clinical characteristics between men and women were tested using unpaired Student's *t* test or Mann-Whitney U tests. We defined the end point as general CV events (i.e., a composite of stroke, heart failure, and myocardial infarction). The association between baseline plasma BNP levels and the end point was evaluated using a Cox proportional-hazards regression model. The gender-specific hazard ratios (HR) for each BNP decile's end point were assessed. In this multivariate regression model, adjustments were made in the analysis for age, BMI, diabetes, hypertension, hypercholesterolemia, atrial fibrillation, estimated glomerular filtration rate, and current smoking. For analyses of CV incidence, person-years were censored at the date of CV events, the date of emigration from the study area, the date of death, or the end of the follow-up period, whichever came first. To compare the predictive abilities of plasma BNP testing to the Framingham 10-year risk scoring system, receiver-operating-characteristic curves were constructed. The area under the curve (AUC) and 95% confidence interval (CI) for each ROC curve were calculated to provide a measure of the overall diagnostic accu-

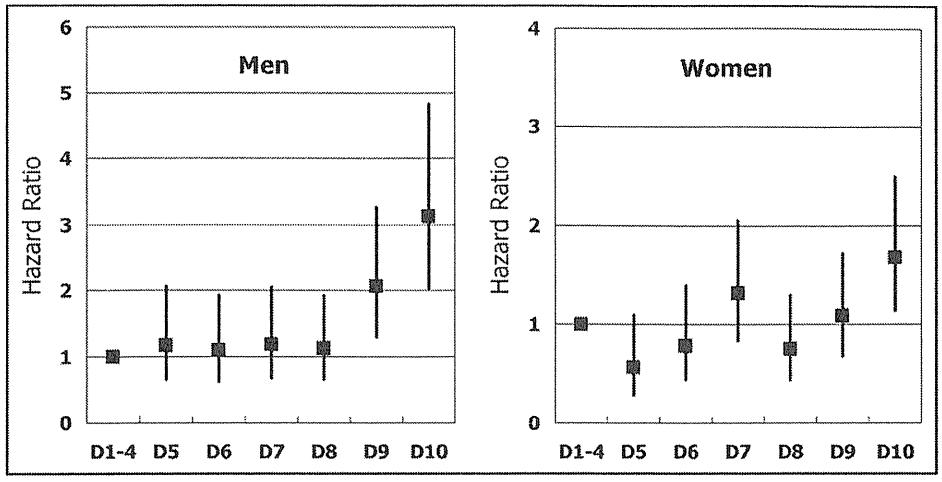

Figure 2. Multivariate-adjusted HRs and 95% CIs for risk for CV events according to plasma BNP decile in men *(left)* and women *(right)*.

racy of the test. The follow-up survey for congestive heart failure, stroke, and myocardial infarction was carried out after the baseline study through to March 2009. Migrations were confirmed by official resident registration data issued by the local government offices (October 2009). All statistical analyses were performed using SPSS version 11.0.1J (SPSS, Inc., Chicago, Illinois). A significant difference was defined as $p < 0.05$.

Results

Mean ages were 63.3 ± 9.8 years in men and 61.6 ± 9.7 years in women (Tables 1 and 2). The number of women was approximately twice the number of men. Plasma BNP levels and BMI were higher in women than in men (median BNP 16.9 vs 14.2 pg/ml, $p < 0.001$; mean BMI 24.2 ± 3.4 vs 23.9 ± 2.9 kg/m^2, $p < 0.001$). The prevalence of hypertension (44% vs 38%), atrial fibrillation (2.9% vs 0.6%), diabetes (9.6% vs 5.4%), and current smoking (33.9% vs 2.5%) was higher in men. The incidence of hypercholesterolemia was higher in women (10.5% vs 20.3%). The administration rates for hypertensive drugs was 23.3% in men and 23.8% in women ($p = 0.232$). The mean Framingham risk score in men was higher than that in women (13.8 ± 4.4 vs 11.9 ± 4.6).

During the mean follow-up period of 5.8 years, 430 CV events (215 in men, 215 in women) were recorded. When the lowest 4 (first to fourth) plasma BNP deciles were set to the reference, the crude incidence of CV events per 1,000 person-years increased with deciles in both genders (Figure 1). As shown in Figure 2, after adjustment for potential confounding factors in the Cox regression model, the relative HR for CV events increased according to deciles (p for trend <0.01 in men, p for trend <0.001 in women). Compared to the reference, the HR was significantly elevated in the ninth (HR 2.06, 95% CI 1.30 to 3.27) and tenth (HR 3.15, 95% CI 2.03 to 4.88) deciles in men and in the tenth decile only in women (HR 1.68, 95% CI 1.13 to 2.50). The thresholds for increased CV risk were greater than approximately 37 pg/ml in men and greater than approximately 55 pg/ml in women.

The overall power for predicting general CV events was comparable between plasma BNP level and Framingham risk score. The areas under the curve for plasma BNP were 0.669 (95% CI 0.629 to 0.710) in men and 0.634 (95% CI 0.593 to 0.676) in women. The areas under the curve did not differ significantly from those for the Framingham risk score (men 0.676, 95% CI 0.640 to 0.712; women 0.681, 95% CI 0.649 to 0.713).

Discussion

The present study has demonstrated that in the general population with no CV history or renal dysfunction, plasma BNP levels signaling increased CV risk are greater than the 80th percentile in men and the 90th percentile in women. The predictive ability of plasma BNP testing for general CV events is similar to that of the established total CV risk scoring system. The present study has therefore shown for the first time that increased plasma BNP levels higher than these gender-specific thresholds are a simple and useful marker for elevated risk for CV events in a community-based middle-aged and elderly population.

Several previously published studies have shown a significant association between plasma BNP and N-terminal pro-BNP (NT-proBNP) levels and CV events in the general population.[7–11] The Framingham study conventionally applied a single cutoff point (the 80th percentile) to examine the association between "high" BNP levels and CV events.[7] Linssen et al[10] recently reported that in a selected population mainly with urinary albumin excretion >10 mg/L, multivariate HRs for the risk for all-cause mortality increased gradually with increasing levels of plasma NT-proBNP, with no clear cut-off level in both genders. However, no studies have explored the threshold levels of BNP that indicate an increased risk for the future development of CV events.

Several studies have shown that median plasma BNP and NT-proBNP levels are higher in women,[12,13] although the incidence of CV events in the general population is usually lower in women than in men. This suggests that a gender-stratified analysis should be incorporated when determining cut-off levels of plasma BNP and NT-proBNP for predicting the future onset of CV events in the general population. However, no reports to date have shown which levels of plasma BNP increase the risk for CV events in either gender.

The present study has shown for the first time in an unselected general population that the adjusted HR was significantly increased from the ninth plasma BNP decile in men and the tenth decile in women. The association between plasma BNP and the future development of CV events may be because elevated plasma BNP is a significant biomarker for asymptomatic structural heart disease such as impaired left ventricular function, left ventricular hypertrophy, atrial dilatation and fibrillation, and myocardial ischemia. In accord with this concept, Struthers and Lang[14] suggested that BNP and NT-proBNP testing could be used to identify "pancardiac" target organ damage and may become to the heart what albuminuria is to the kidneys, that is, a useful biomarker for targeting organ damage in the CV system. In our previous cross-sectional study applying transthoracic echocardiography in the general population, plasma BNP concentrations >50 pg/ml showed sensitivity and specificity for several select phenotypes of structural heart disease that are prone to progress into several types of CV events.[15] The threshold plasma BNP levels that increased the HR (greater than approximately 37 pg/ml in men and greater than approximately 55 pg/ml in women) in the present study are lower compared to the previously reported cut-off level for detection of structural heart disease. This apparent discord may be due to the present study being longitudinal and the cut-off level being gender specific.

The present study suggests that the usefulness in terms of sensitivity and specificity of plasma BNP testing for predicting CV events differs little from the Framingham 10-year risk score for general CV events. This finding may indicate that the predictive ability of BNP testing is equivalent to that of the established risk calculation. However, such a conclusion may be premature, because the mean follow-up period of this study was shorter (<6 years) than the Framingham study (10 years). In fact, the established risk scoring includes lipids, blood pressure, smoking, and diabetes, which are long-standing risk factors for CV events. In contrast, plasma BNP may be unique in that it is instead identifying the end process of several types of cardiac damage itself. In view of this, plasma BNP testing could be useful for identifying subjects at high risk for several types of CV events within a few years. BNP may thus be a direct or novel biomarker for various types of intrinsic cardiac abnormalities rather than an additional biomarker for assessing long-term risk.

The present study had several strengths. This study included the largest general population sample in whom plasma BNP levels have been reported. The plasma BNP measurement was performed in fresh plasma samples without long-term freezing and repeated thawing. CV events were captured prospectively according to previously determined standard epidemiologic criteria and confirmed by the research staff at medical chart review. Baseline data including clinical characteristics and biochemical data were determined well before the start of the follow-up study.

Despite these merits, several limitations must be considered when interpreting the results. First, because echocardiographic evaluation was not included in the baseline data, the utility of the BNP testing could not be compared to that of echocardiography. However, several previous studies have reported that BNP testing remained independently predictive of future CV events after adjusting for echocardiographic variables.[7,11] Second, mean BMI was lower in our study than in previous general population reports.[7,9,10] Plasma BNP levels have been reported to be lower in obese subjects than in the lean population,[16,17] with 1 previous study demonstrating that each standard deviation increase in BMI was associated with a 16% to 18% decrement in plasma BNP.[16] It follows that threshold BNP levels may be slightly lower in predominantly obese populations. Third, according to population-based studies, the Japanese population has a lower incidence of CV events than Western countries. Thus, care must be taken before these data can be generalized to other ethnic groups. Fourth, McKie et al[18] recently demonstrated that the use of NT-proBNP as a CV risk predictor is worthless in healthy subjects, as verified by close clinical examination including echocardiography. This observation may not validate our results, because the present population comprises entirely healthy subjects and may include a substantial part of subjects with CV risk factors, as listed in Tables 1 and 2. Finally, the age range of our population may have been relatively narrow, with no subjects aged <40 years or >80 years.

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