Table 1. Characteristics of Studies Included in the Meta-analysis

Source (Location)	No. of Participants	Men, %	Mean (or Midpoint) Age, y	Mean Follow-up, y	Methods for Outcome Measures	Specific Outcomes (CHD/CVD Criteria)	No. of Events for Each Outcome	Instrument for Assessing CRF	Whether Max or Sub Reached[a]
Aijaz et al,[29] 2008 (US)	8620	73	52	16	Registry	All-cause mortality	535	Treadmill	Max
Aktas et al,[30] 2004 (US)	3554	81	57	8	Registry	All-cause mortality	114	Treadmill	Sub
Allen et al,[31] 1980 (US)									
Men	350	100	NA	1.1	Questionnaire	CHD event (MI, sudden cardiac death)	34	Ergometer	Max
Women	302	0	NA				10		
Arraiz et al,[32] 2004 (Canada)	NA	NA	47	7	Registry	All-cause mortality; CVD death (NA)	55; 37	Canadian Home Fitness Test	Sub
Balady et al,[33] 2004 (US)									
Men	1431	100	45	18.2	Hospital record	CHD event (onset of AP, coronary insufficiency, MI)	224	Treadmill	Sub
Women	1612	0	45				81		
Bruce et al,[34] 1980 (US)	2365	100	45	5.6	Questionnaire	CHD event (NA)	47	Treadmill	Max
Cumming et al,[35] 1975 (Canada)	486[b]	100	53	3	Questionnaire	CHD event (NA)	26	Ergometer	Max
Erikssen et al,[36] 1998 (Norway)	1428	100	57	13	Registry	All-cause mortality; CVD death (CHD, stroke, the other CVD)	238; 120	Ergometer	Max
Erikssen et al,[37] 2004 (Norway)	2014	100	49	26	Questionnaire and registry	CHD death (CHD, sudden cardiac death)	300	Ergometer	Max
Farrell et al,[38] 2004 (US)	6925	0	43	11.4	Registry	All-cause mortality	195	Treadmill	Sub
Gulati et al,[10] 2003 (US)	5721	0	52	8.4	Registry	All-cause mortality	180	Treadmill	Max
Gulati et al,[39] 2005 (US)	5636	0	52	9	Registry	All-cause mortality; CVD death (*ICD-9*, *ICD-10*)	171; 52	Treadmill	Max
Gulati et al,[40] 2005 (US)	5721	0	52	8.4	Registry	CVD death (NA)	180	Treadmill	Max
Gyntelberg et al,[41] 1980 (Denmark)	5249	100	50	5	Registry	CHD event (MI, sudden cardiac death)	170	Ergometer	Sub
Hein et al,[42] 1992 (Denmark)	4999	100	48	17	Registry	All-cause mortality	941	Ergometer	Sub
Jouven et al,[43] 2005 (France)	5713[b]	100	48	23	Hospital record	CHD death (MI death)	210	Ergometer	Sub
Kampert et al,[44] 1996 (US)	25 341	100	43	8.4	Registry	All-cause mortality	601	Treadmill	Sub
Katzmarzyk et al,[45] 2005 (US)	19 173	100	43	10.2	Registry	All-cause mortality	477	Treadmill	Sub
Laukkanen et al,[8] 2007 (Finland)	1639	100	52	16.6	Registry	All-cause mortality; CVD event (*ICD-9*, *ICD-10*)	304; 340	Ergometer	Max
Laukkanen et al,[9] 2008 (Finland)	1639	100	52	16.6	Registry	All-cause mortality; CVD event (*ICD-9*, *ICD-10*)	304; 340	Ergometer	Max
Miller et al,[6] 2005 (UK)	578	100	52	7.3	Questionnaire, registry, and hospital record	All-cause mortality; CVD event (*ICD-9*)	68; 62	Ergometer	Sub
Mora et al,[46] 2003 (US)	2994	0	55	20.3	Questionnaire and registry	All-cause mortality; CVD death (NA)	427; 147	Treadmill	Sub
Myers et al,[47] 2002 (US)	2534[b]	100	56	6.2	Registry	All-cause mortality	288	Treadmill and ergometer	Sub
Peters et al,[48] 1983 (US)	2779	100	45	4.8	Hospital record	CHD event (MI, sudden cardiac death)	36	Ergometer	Sub
Rywik et al,[49] 2002 (US)	1083	57	52	8.8	Registry	CHD event (AP, MI, sudden cardiac death)	76	Treadmill	Max

(continued)

Table 1. Characteristics of Studies Included in the Meta-analysis (continued)

Source (Location)	No. of Participants	Men, %	Mean (or Midpoint) Age, y	Mean Follow-up, y	Methods for Outcome Measures	Specific Outcomes (CHD/CVD Criteria)	No. of Events for Each Outcome	Instrument for Assessing CRF	Whether Max or Sub Reached[a]
Sandvik et al,[50] 1988 (Norway)	1960[b]	100	50	15.9	Registry	All-cause mortality; CVD death (NA)	271; 143	Ergometer	Max
Sawada and Muto,[51] 1999 (Japan)	9986[b]	100	37	14	Questionnaire	All-cause mortality; CHD death (*ICD-10*)	247; 72	Ergometer	Sub
Slattery and Jacobs,[5] 1988 (US)	2431	100	50	18.5	Registry	All-cause mortality; CHD death (*ICD-8*)	631; 258	Treadmill	Sub
Sobolski et al,[52] 1987 (Belgium)	1476	100	48	5	Registry	CHD event (MI, sudden cardiac death)	19	Ergometer	Sub
Stevens et al,[21] 2002 (US)									
Men	2860	100	45	26	Questionnaire and registry	All-cause mortality; CVD death (*ICD-9*)	682; 270	Treadmill	Sub
Women	2506	0	47				484; 179		
Stevens et al,[22] 2004 (US)	1359	100	49	19	Questionnaire and registry	All-cause mortality; CVD death (*ICD-9*)	211; 98	Treadmill	Sub
Sui et al,[7] 2007 (US)									
Men	20 278	100	44	10.4	Questionnaire	CVD event (MI, stroke, coronary revascularization)	1512	Treadmill	Sub
Women	5909	0	45				159		
Villeneuve et al,[53] 1998 (Canada)	7561	48	45	7	Registry	All-cause mortality	129	Canadian Home Fitness Test	Sub

Abbreviations: AP, angina pectoris; CHD, coronary heart disease; CRF, cardiorespiratory fitness; CVD, cardiovascular disease; *ICD-8, International Classification of Diseases, Eighth Revision*; *ICD-9, International Classification of Diseases, Ninth Revision*; *ICD-10, International Statistical Classification of Diseases, 10th Revision*; MI, myocardial infarction; NA, not available.
[a]Max, workload testing was continued until maximal workload; Sub, maximal workload was predicted from findings of submaximal exercise workload.
[b]Including participants with abnormal exercise electrocardiogram (ie, ST elevation/depression).

for >3 factors among obesity, hypertension, total cholesterol or low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and diabetes [yes or no], mean follow-up [≥12 years or <12 years], instrument for assessing CRF [ergometer or others], and maximal exercise testing [yes or no]). To examine the extent to which between-study heterogeneity was explained by these study characteristics, we additionally conducted linear multiple regression analyses by simultaneously entering these confounders as explanatory variables.

Categorical analyses were repeated with multiadjustment for the prespecified confounders to consider the potential heterogeneity of study characteristics among the subgroups (ie, low vs high CRF, low vs intermediate CRF, and intermediate vs high CRF). Tests of interaction were performed to assess whether the association between CRF and the study outcomes varied across these 3 subgroups.

The Begg and Egger tests[25,26] were used for assessment of publication bias (ie, the tendency for positive associations to be published and negative or null associations to be unpublished). We also followed the Duval and Tweedie "trim and fill" procedure[27] as a method of adjustment for suspected publication bias. This method considers the possibility of hypothetical "missing" studies that might exist, imputes their RRs, and recalculates a pooled RR that incorporates the hypothetical missing studies as though they actually existed.

Two-sided $P \leq .05$ was considered statistically significant, except for the test of publication bias for which the recommended levels are $P \leq .10$.[28] Data were analyzed using STATA version 10 (STATA Corp, College Station, Texas).

RESULTS

Literature Search and Study Characteristics

FIGURE 1 shows the number of studies that were identified and excluded at different stages of the selection process. A total of 33 studies[5-9,16,21,22,29-53] were included in our meta-analysis. Characteristics of the 33 selected studies comprising 102 980 participants (range, 486-25 341) and 6910 cases (range, 26-941) for all-cause mortality and 84 323 participants (range, 302-20 278) and 4485 cases (range, 10-1512) for CHD/CVD are shown in TABLE 1. Twenty-one studies* reported all-cause mortality and 24 studies† reported CVD/CHD. Mean age and follow-up duration ranged from 37 to 57 years and 1.1 to 26 years, respectively. Eight studies[8,33,37,39,45,46,49,52] were used for the dose-response analyses only and 4 studies[9,16,40,44] were used for the categorical analyses only. In 20 studies,‡ RRs were adjusted for smoking and in 9 stud-

*References 5, 6, 8, 9, 16, 21, 22, 29, 30, 32, 36, 38, 39, 42, 44-47, 50, 51, 53.
†References 5-9, 21, 22, 31-37, 39-41, 43, 46, 48-52.
‡References 5, 7-9, 16, 21, 22, 30, 32, 33, 37-39, 44-46, 48, 50, 52, 53.

ies,[7-9,16,33,39,46,50,52] there were multiple study confounders (available in an eTable [http://www.jama.com]).

Dose-response Analyses

FIGURE 2 shows the pooled estimates for the reduction in risk of all-cause mortality and CHD/CVD per higher MET of exercise capacity. Pooled RRs of all-cause mortality and CHD/CVD per 1-MET higher level of MAC were 0.87 (95% CI, 0.84-0.90) and 0.85 (95% CI, 0.82-0.88), respectively. There was evidence of statistical heterogeneity of RRs across studies (I^2=82.3%; $P<.001$ for all-cause mortality; I^2=74.7%; $P<.001$ for CHD/CVD).

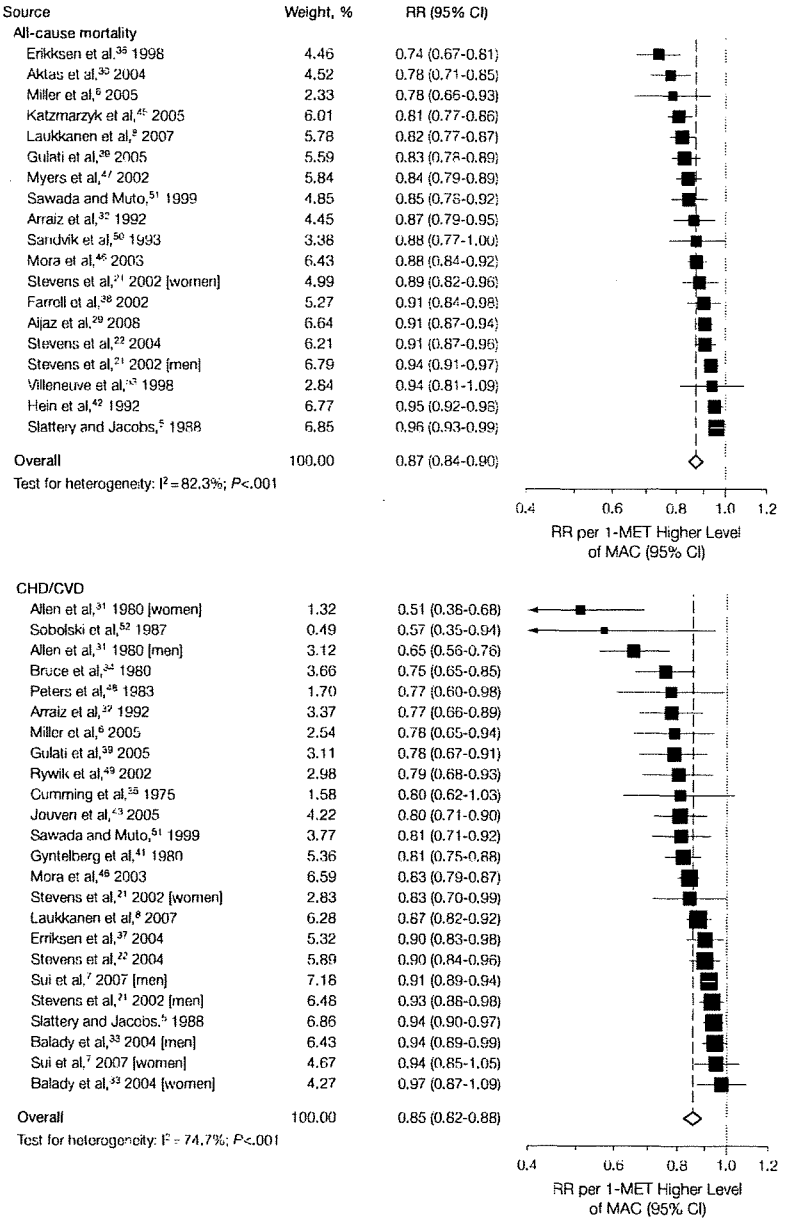
TABLE 2 shows the results of analyses investigating the associations of study characteristics on each outcome. The finding of risk reduction per higher MET for all-cause mortality and CHD/CVD was consistently significant in all of the stratified analyses. However, studies with a follow-up of at least 12 years had weaker associations with study outcomes compared with those that had follow-up of less than 12 years for all-cause mortality ($P=.08$) and CHD/CVD events ($P=.004$). The associations between CRF and risk of CHD/CVD events were stronger in studies that used an ergometer for assessing CRF ($P=.009$) or conducted maximal exercise testing ($P=.02$) and were weaker in studies that were adjusted for smoking ($P=.006$) or multiple metabolic factors ($P=.06$). However, these study characteristics did not influence the associations between MAC and risk of all-cause mortality.

Multiple regression analyses in which all the study characteristics listed in Table 2 were entered as independent variables indicated that study characteristics significantly explained the heterogeneity of the RRs per 1-MET higher level of MAC (all-cause mortality, 79% of total variance; $P=.01$; and CHD/CVD, 67% of total variance; $P=.01$). After adjustment for these study characteristics, there were neither significant differences in risk estimates of CHD/CVD between CHD and CVD (0.89; 95% CI, 0.86-0.92 and 0.89; 95% CI, 0.87-0.90, respectively; $P=.99$) nor between CHD or CVD death and CHD or CVD events (0.88; 95% CI, 0.86-0.90 and 0.90; 95% CI, 0.88-0.91, respectively; $P=.27$).

Categorical Analyses

We performed categorical analyses to summarize the risk of all-cause mortality and CHD/CVD for 3 subgroups (low vs high CRF [FIGURE 3], low vs inter-

Figure 2. Meta-analysis of All-Cause Mortality and CHD/CVD per 1-MET Higher Level of MAC



CHD indicates coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; MAC, maximal aerobic capacity; MET, metabolic equivalent; RR, risk ratio. Area of each square is proportional to study weight.

mediate CRF [FIGURE 4], and intermediate vs high CRF [FIGURE 5]). After adjustment for heterogeneity of study characteristics and compared with high and intermediate CRF, respectively, the pooled RRs for the association of low CRF with all-cause mortality were 1.70 (95% CI, 1.51-1.92) and 1.56 (95% CI, 1.39-1.75), respectively. After adjustment for heterogeneity and compared with high and intermediate CRF, respectively, the pooled RRs for the association of low CRF with CHD/CVD events were 1.40 (95% CI, 1.32-1.48) and 1.47 (95% CI, 1.35-1.61), respectively. The pooled RRs for the associations of intermediate CRF with all-cause mortality and CHD/CVD events compared with high CRF were 1.13 (95% CI, 1.04-1.22) and 1.07 (95% CI, 1.01-1.13), respectively. However, tests of the interaction indicated that these estimates for comparisons between intermediate and high risk were significantly lower than for those between low vs high CRF and low vs intermediate CRF ($P<.001$ for any comparisons). Tests of interaction also indicated that there were no significant differences in risk estimates for low vs high CRF compared with low vs intermediate CRF (all-cause mortality, $P=.28$; CHD/CVD, $P=.33$).

Publication Bias

In risk estimates per 1-MET higher level of MAC, there was a statistically significant publication bias according to Egger test (all-cause mortality, $P=.002$; CHD/CVD, $P=.02$). However, adjustment for publication bias by the trim and fill procedure could not detect hypothetical negative unpublished studies that could influence the study. In some of the categorical analyses, statistically significant publication bias was also observed in risk estimates after adjustment for heterogeneity of study characteristics (pooled RR of all-cause mortality for low vs high CRF and low vs intermediate CRF, $P=.03$ by Egger test and $P=.03$ by Begg test, respectively; pooled RR of CHD/CVD for low vs intermediate CRF, $P<.001$ by Egger test). After incorporating the hypothetical studies using trim and fill methods, the risk estimates were attenuated in risk of all-cause mortality for low vs high CRF (RR, 1.48; 95% CI, 1.31-1.68) and low vs intermediate CRF (RR, 1.35; 95% CI, 1.18-1.54), and CHD/CVD for low vs high CRF (RR, 1.38; 95% CI, 1.30-1.45), which suggested the existence of potentially negative studies. Nevertheless, these biases did not change the general conclusions.

COMMENT

Our meta-analysis is the first to our knowledge to quantify CRF as measured by METs, which is a standard scale for expressing exercise workload, and its relationship to all-cause mortality and CHD or CVD events in healthy men and women. According to the dose-response analyses, a 1-MET higher level of MAC was as-

Table 2. Stratified Analyses of Pooled RR of All-Cause Mortality and CVD/CHD for Each MET Higher Level of Maximal Aerobic Capacity

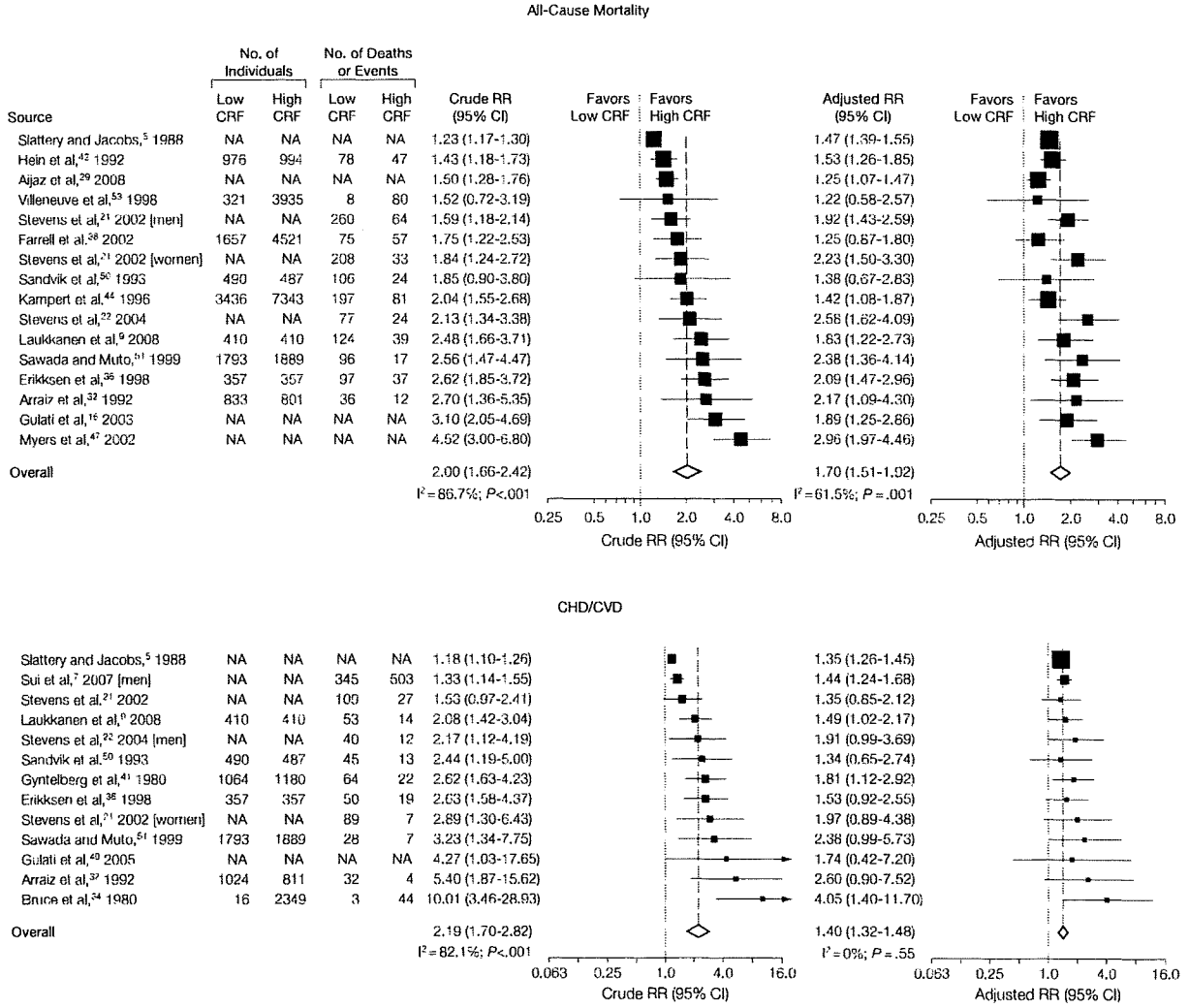
Characteristics	All-Cause Mortality			CHD/CVD		
	No. of Cohorts	RR (95% CI)	*P* Value[a]	No. of Cohorts	RR (95% CI)	*P* Value[a]
Mean age, ≥50 y						
No	10	0.90 (0.86-0.93)	.10	16	0.89 (0.88-0.91)	.80
Yes	9	0.84 (0.80-0.89)		8	0.84 (0.79-0.90)	
Only men						
No	8	0.87 (0.84-0.91)	.88	8	0.84 (0.81-0.87)	.60
Yes	11	0.87 (0.83-0.91)		16	0.86 (0.83-0.89)	
Adjustment for confounders, smoking						
No	7	0.87 (0.83-0.93)	.82	10	0.77 (0.70-0.85)	.006
Yes	12	0.87 (0.84-0.90)		14	0.89 (0.86-0.92)	
>3 Metabolic factors[b]						
No	14	0.86 (0.84-0.89)	.67	16	0.81 (0.77-0.86)	.06
Yes	5	0.86 (0.83-0.89)		8	0.89 (0.85-0.93)	
Patients with abnormal exercise electrocardiogram						
No	10	0.85 (0.81-0.90)	.20	16	0.83 (0.79-0.88)	.40
Yes	9	0.90 (0.86-0.93)		8	0.90 (0.88-0.92)	
Mean follow-up, 12 y						
No	8	0.84 (0.82-0.86)	.08	13	0.78 (0.72-0.84)	.004
Yes	11	0.91 (0.9-0.93)		11	0.89 (0.86-0.92)	
Ergometer used to assess CRF						
No	13	0.90 (0.89-0.92)	.82	13	0.89 (0.86-0.92)	.009
Yes	6	0.88 (0.84-0.91)		11	0.78 (0.73-0.84)	
Whether workload testing was continued until maximal workload						
No	15	0.88 (0.85-0.91)	.24	16	0.88 (0.85-0.91)	.02
Yes	4	0.84 (0.76-0.92)		8	0.77 (0.70-0.85)	

Abbreviations: CI, confidence interval; CHD, coronary heart disease; CRF, cardiorespiratory fitness; CVD, cardiovascular disease; RR, risk ratio.
[a] Represents meta-regression for differences across strata.
[b] Means of adjustment for more than 3 coronary risk factors among obesity (or body mass index or waist-to-hip ratio), systolic blood pressure (or hypertension), total cholesterol (or low-density lipoprotein cholesterol or hyperlipidemia), high-density lipoprotein cholesterol, and diabetes.

sociated with 13% and 15% decrements in risk of all-cause mortality and CHD/CVD, respectively. From the clinical viewpoint, these values may be considerable. For example, based on risk estimates of the components of metabolic syndrome according to the National Cholesterol Education Program,[54] these findings suggest that a 1-MET higher level of MAC is comparable to a 7-cm, 5-mm Hg, 1-mmol/L, and 1-mmol/L decrement in waist circumference,[55] systolic blood pressure,[56] triglyceride level (in men),[57] and fasting plasma glucose,[58] respectively, and a 0.2-mmol/L increment in high-density lipoprotein cholesterol.[59] It is possible that prediction of CHD risk could be improved by including CRF with already established risk factors for CHD.

In categorical analyses, individuals with low CRF (<7.9 METs in MAC) had a substantially higher risk of all-cause mortality and CHD/CVD compared with those with intermediate and high CRF (7.9-10.8 and ≥10.9 METs in MAC, respectively). These risk estimates were higher than those for individuals with intermediate CRF compared with those with high CRF, according to the test of interaction. These analyses suggest that a minimal CRF of 7.9 METs may be important for significant prevention of all-cause mortality and CHD/CVD. A previous review suggested that physical activity yielding 1000 kcal energy expenditure per week is needed for signifi-

Figure 3. Meta-analysis of All-Cause Mortality and CHD/CVD for Individuals With Low vs High CRF

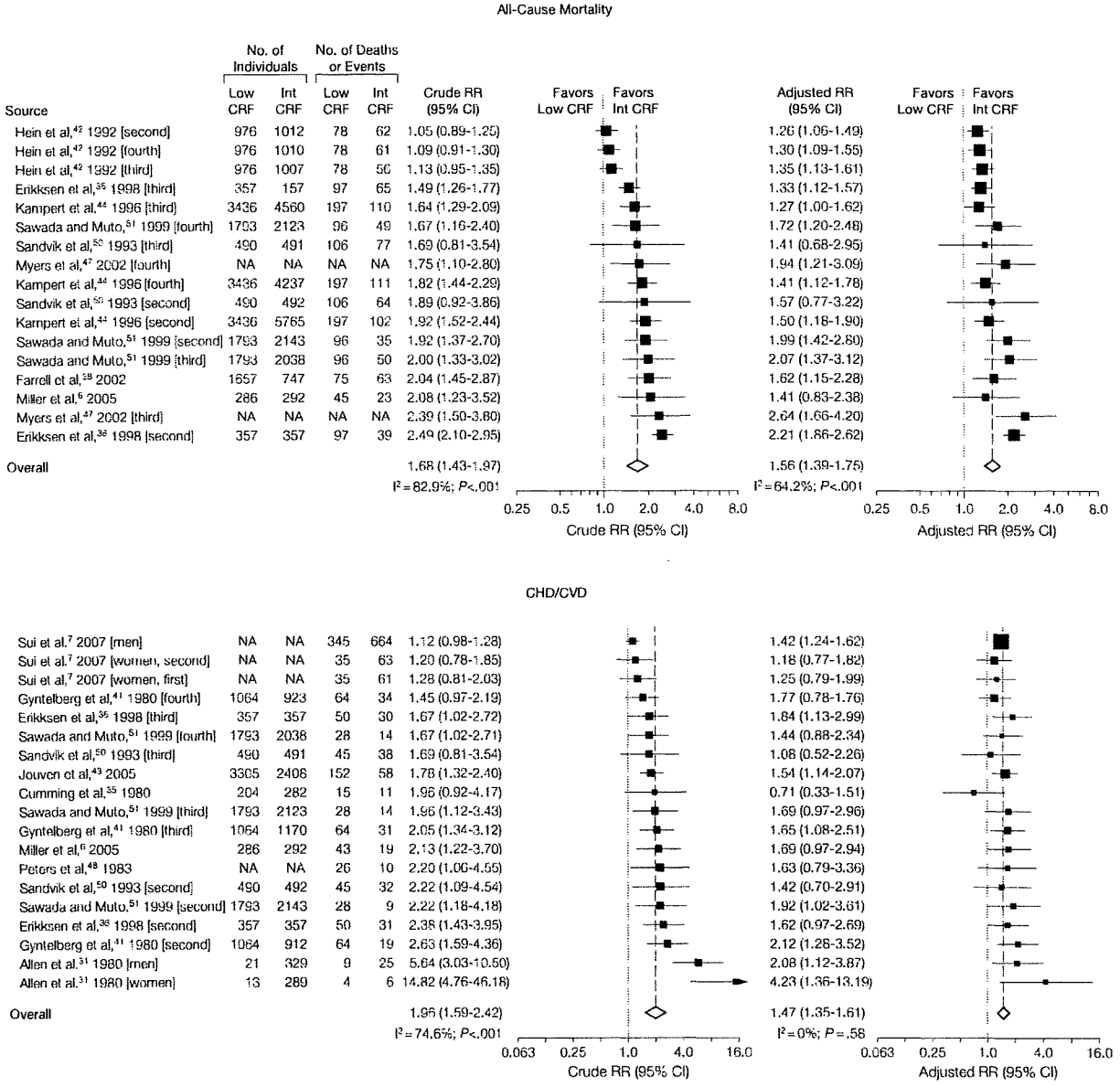


CHD indicates coronary heart disease; CI, confidence interval; CRF, cardiorespiratory fitness; CVD, cardiovascular disease; MET, metabolic equivalent; NA, not available; RR, risk ratio. Low and high CRF categories were defined as less than 7.9 METs and 10.9 METs or more of maximal aerobic capacity, respectively, under the assumption that all participants were 50-year-old men. Crude and adjusted RR indicate RRs before and after adjustment for study heterogeneity among the subgroups, respectively.

cant risk reduction of all-cause mortality.[60] However, using CRF may be preferable to using physical activity as risk predictors because 1 prior study[61] suggested that physical fitness was more strongly correlated with CHD than physical activity.

According to the results reported herein, the minimum CRF level that is associated with significantly lower event rates for men and women is approximately 9 and 7 METs (at 40 years old), 8 and 6 METs (at 50 years), and 7 and 5 METs (at 60 years), respectively. This means that women and men younger than 60 years

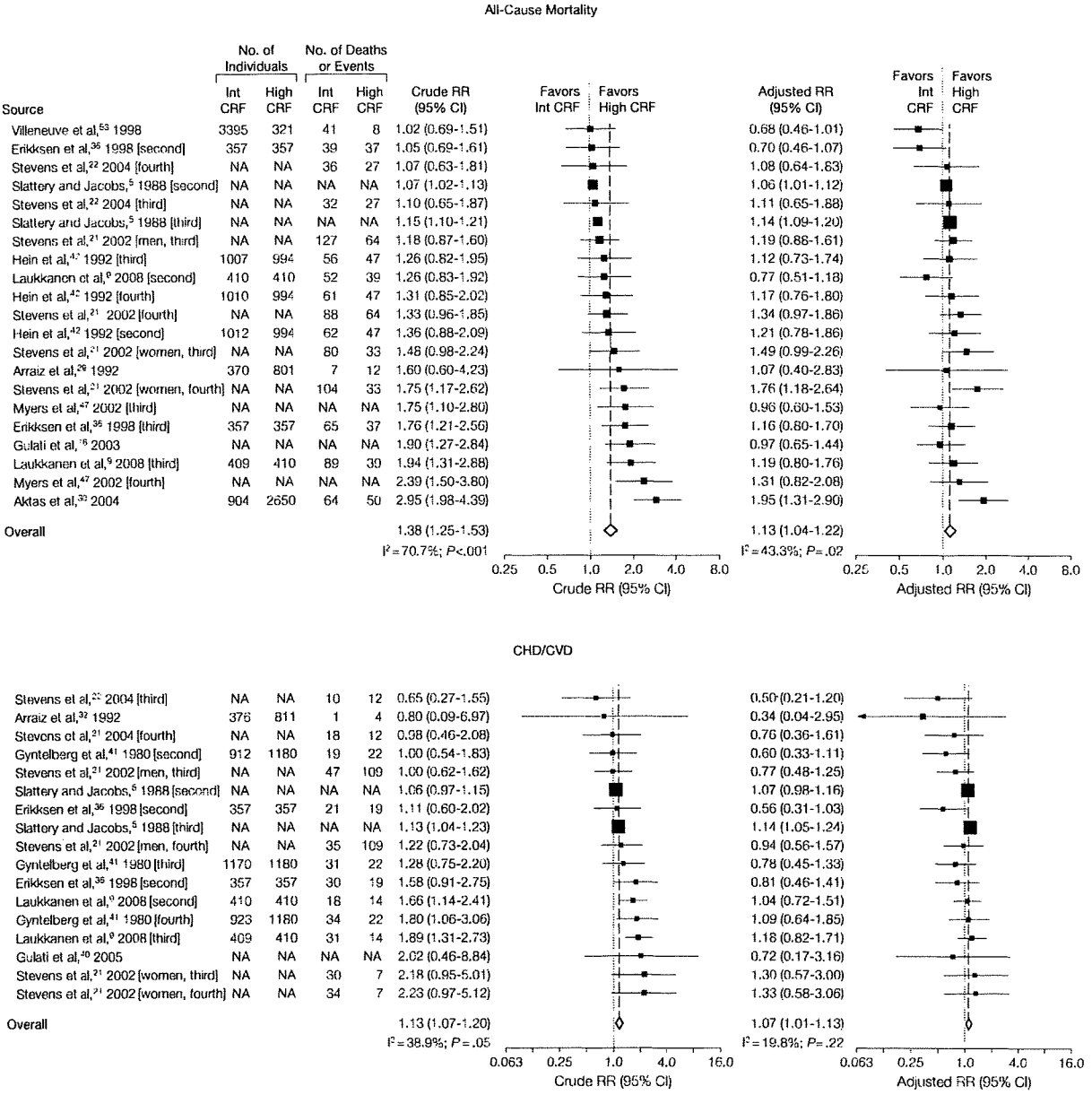
Figure 4. Meta-analysis of All-Cause Mortality and CHD/CVD for Individuals With Low vs Intermediate CRF



CHD indicates coronary heart disease; CI, confidence interval; CRF, cardiorespiratory fitness; CVD, cardiovascular disease; Int, intermediate; MET, metabolic equivalent; NA, not available; RR, risk ratio. Low and intermediate CRF categories were defined as less than 7.9 METs and 7.9 to 10.8 METs of maximal aerobic capacity, respectively, under the assumption that all participants were 50-year-old men. Crude and adjusted RR indicate RRs before and after adjustment for study heterogeneity among the subgroups, respectively. The words first, second, third, and fourth in brackets represent comparisons between the lowest CRF category and the highest, second, third, or fourth CRF category in the relevant study.

would need to complete stage I (1.7 mph at gradient 10°) and stage II (2.5 mph at gradient 12°), respectively, of the standard Bruce protocol, which is one of the most commonly used treadmill tests in clinical settings.[14] If the CRF level is expressed in terms of walking speed, men around 50 years of age must be capable of continuous walking at a speed of 4 mph and women must continuously walk at 3 mph for prevention of CHD,[17] with the assumption that the anaerobic threshold is 50%

Figure 5. Meta-analysis of All-Cause Mortality and CHD/CVD for Individuals With Intermediate vs High CRF



CHD indicates coronary heart disease; CI, confidence interval; CRF, cardiorespiratory fitness; CVD, cardiovascular disease; Int, intermediate; MET, metabolic equivalent; NA, not available; RR, risk ratio. Intermediate and high CRF categories were defined as 7.9 to 10.8 METs and 10.9 METs or more of maximal aerobic capacity, respectively, under the assumption that all participants were 50-year-old men. Crude and adjusted RR indicate RRs before and after adjustment for study heterogeneity among the subgroups, respectively. The words second, third, and fourth in brackets represent comparisons between the second, third, or fourth highest CRF category and the highest CRF category in the relevant study.

to 60% of MAC.[62] It is possible that consideration of low CRF as a major coronary risk factor could be put into practical use in the clinical setting through identification of low exercise tolerance by exercise stress testing or in daily life by the speed at which a person can walk before experiencing exhaustion.

Some cross-sectional population studies have suggested that higher aerobic fitness is associated with more favorable coronary or cardiovascular risk factor profiles[63,64]; therefore, the association between CRF and the risk of all-cause mortality and CHD/CVD could potentially be explained by residual confounding by established risk factors. Our sensitivity analyses indicated that a weaker association was observed between a 1-MET higher level of MAC and risk reduction of CHD/CVD, but not all-cause mortality, in studies with adjustment for smoking or more comprehensive risk factors. This finding suggests that better CRF is independently associated with longevity, while the inverse association between CRF and risk of CHD/CVD is explained partly by established coronary risk factors.

Limitations of this meta-analysis must be considered. First, a possible misclassification bias might affect our results. Misclassification bias could occur in transforming the reported CRF data into MET units. However, all of the prediction equations used in our analyses for estimating MAC have already been validated and are commonly used. Another possible misclassification bias is due to the fact that the definitions of low, intermediate, and high CRF were fundamentally based on study-specific CRF classifications, which varied from study to study but were not based on a standard cutoff. Fortunately, we could assign every exposure in each study to 1 of the 3 categories, which did not overlap with few exceptions, although MAC values in each category are approximately 1 MET smaller than those based on a general standard (eg, data from the National Health and Nutrition Examination Survey[65]). Therefore, the possibility of misclassification bias due to those 2 reasons should be limited. Second, Begg or Egger tests suggested publication bias. However, trim and fill analyses to incorporate potentially existing negative studies did not change the general result, although the risk estimates were moderately attenuated. Nevertheless, this possibility was not fully excluded by this analysis.

Based on the findings of our meta-analysis, we suggest for future research (1) further development of a CHD prediction algorithm (eg, Framingham Scores[66]) that would consider both CRF and the classical coronary risk factors to allow physicians to use CRF as a major risk factor in clinical settings; (2) cost-effectiveness of exercise testing for assessing CRF from the viewpoint of primary prevention of all-cause mortality and CHD; and (3) a clinical trial to determine whether an intervention that improves CRF by exercise reduces the risk of all-cause mortality and CHD.

In conclusion, better CRF was associated with lower risk of all-cause mortality and CHD/CVD. A 1-MET higher level of MAC was associated with a 13% and 15% risk reduction of all-cause mortality and CHD/CVD, respectively. The minimal MAC value for substantial risk reduction in persons aged 50 (SD, 10) years was estimated to be 8 (SD, 1) METs for men and 6 (SD, 1) METs for women. We suggest that CRF, which can be readily assessed by an exercise stress test, could be useful for prediction of CHD/CVD and all-cause mortality risk in a primary care medical practice.

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Acquisition of data: Kodama, Yachi, Sugawara, Totsuka.
Analysis and interpretation of data: Kodama, Tanaka, Asumi, Shimano, Ohashi, Yamada, Sone.
Drafting of the manuscript: Kodama, Maki, Sone.
Critical revision of the manuscript for important intellectual content: Kodama, Saito, Tanaka, Yachi, Asumi, Sugawara, Totsuka, Shimano, Ohashi, Yamada, Sone.
Statistical analysis: Kodama, Saito, Tanaka, Ohashi, Sone.
Obtained funding: Sone.
Administrative, technical, or material support: Kodama, Saito, Tanaka, Maki, Yachi, Asumi, Sugawara, Totsuka, Shimano, Ohashi, Sone.
Study supervision: Yamada, Sone.
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Role of the Sponsors: The research organizations providing funding support did not have any role in the design and conduct of the study, in the correction, management, analysis, and interpretation of the data, or in the preparation, review, or approval of the manuscript.
Additional Information: The eTable is available at http://www.jama.com.

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Original article

Serum beta2-microglobulin concentration as a novel marker to distinguish levels of risk in acute heart failure patients

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Summary
Background: Recently, serum beta2-microglobulin, an endogenous marker for renal function, has been shown to be an independent predictor of mortality in older adults. However, the prognostic role of beta2-microglobulin in heart failure has not been elucidated.
Methods: We prospectively evaluated serum beta2-microglobulin and creatinine concentrations, creatinine-based renal parameters (estimated glomerular filtration rate and creatinine clearance), and echocardiographic data in 131 patients with acute heart failure and creatinine concentrations $\leq$3.0 mg/dL admitted to our hospitals.
Results: During 2.3 $\pm$ 1.3 years, 42 patients died of cardiovascular causes and 12 died of non-cardiac causes. Cardiovascular events were observed in 63 patients: 53 were readmitted due to worsening heart failure, 5 readmitted for cerebral embolism, and 5 died from sudden cardiac death. According to multivariate stepwise Cox proportional hazard analysis, higher baseline serum beta2-microglobulin concentrations ($X^2 = 16$, $p < 0.0001$), previous congestive heart failure ($X^2 = 11$, $p < 0.001$), presence of chronic obstructive pulmonary disease ($X^2 = 8$, $p < 0.01$), and lower diastolic blood pressure ($X^2 = 6$, $p < 0.05$) were independent predictors of increased cardiovascular events. Also, higher baseline serum beta2-microglobulin ($X^2 = 20$, $p < 0.0001$), lower systolic blood pressure ($X^2 = 11$, $p < 0.001$), higher relative left ventricular wall thickness

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($X^2 = 6$, $p < 0.05$), and lower body mass index ($X^2 = 5$, $p < 0.05$) were independent predictors of increased cardiac mortality. The adjusted hazard ratio for cardiovascular events increased with baseline serum beta2-microglobulin above 2.1 mg/L: 2.9 with beta2-microglobulin of 2.2–2.6 mg/L (95%CI 1.2–6.9, $p < 0.05$), 2.9 with beta2-microglobulin of 2.7–3.9 mg/L (95%CI 1.2–7.2, $p < 0.05$), and 4.7 with beta2-microglobulin of ≥4.0 mg/L (95%CI 2.0–11, $p < 0.001$).
Conclusions: Higher baseline serum beta2-microglobulin concentration could be a promising risk marker in acute heart failure patients with creatinine ≤3.0 mg/dL.

Introduction

Recent studies have underscored the importance of renal function parameters, namely estimated glomerular filtration rate (eGFR) [1,2], creatinine clearance [3,4], and cystatin-C [5–7], as strong predictors of mortality and cardiac events in heart failure. Plasma levels of two low molecular weight proteins, cystatin-C and beta2-microglobulin, are indicators of GFR [8,9]. A recent study [10] showed serum beta2-microglobulin to be an independent predictor of total mortality in a general population of older adults, and a better predictor than cystatin-C. However, the prognostic role of serum beta2-microglobulin in patients with heart failure has not been elucidated.

In this prospective study, we evaluated serum beta2-microglobulin as a predictor for long-term outcome in acute heart failure. We also compared the ability of beta2-microglobulin to determine risk in acute heart failure patients with creatinine-based renal parameters.

Methods

Study patients

Between January 2000 and January 2005, 131 consecutive congestive heart failure patients (77 ± 11 years of age) admitted to Kasai City Hospital or Kanzaki General Municipal Hospital, or followed up in the outpatient clinics, were examined. Among the 131 patients, 7 who rejected admission [New York Heart Association (NYHA) functional class II/III: 3/4] were recruited in outpatient clinics. The study was approved by the appropriate institutional review board, and all patients provided informed consent.

Congestive heart failure was confirmed according to Framingham criteria [11], which include nocturnal dyspnea, orthopnea, dyspnea on exertion, edema, and pulmonary rales due to pulmonary congestion confirmed by chest radiography. We excluded patients with acute coronary syndrome, right ventricular failure, critical aortic or mitral stenosis, and severe renal dysfunction (i.e. plasma creatinine concentration >3.0 mg/dL) [1]. Patients with plasma B-type natriuretic peptide (BNP) concentrations <50 pg/mL were excluded due to a low probability of acute heart failure [12,13]. Hypertension, ischemic heart disease, diabetes, and chronic obstructive pulmonary disease (COPD) were defined as previously described [13]. In the present study, 4 patients had previously undergone surgery for cancer, and 7 had concurrent malignancies (total 11 patients: 3 with stomach cancer, 1 with colon cancer, 1 lung cancer, 1 malignant lymphoma, and others), and 1 patient had rheumatoid arthritis.

Echocardiograph study

Baseline echocardiography was performed on each of all 131 patients using a Toshiba Sonolayer SSH 160A or SSA 350A (Toshiba Medical, Tokyo, Japan) or Hitachi ultrasound scanner EUB 6000 (Hitachi Medical, Tokyo, Japan). Left ventricular (LV) end-diastolic dimension, the diastolic thickness of the ventricular septum and the posterior LV wall, LV mass index, and relative LV wall thickness [(septal thickness + posterior LV wall thickness)/LV end-diastolic dimension] were determined as previously described [14,15]. In patients with sinus rhythm, early-to-atrial transmitral peak velocity ratio was measured as previously described [16]. The systolic pressure gradient between the right atrium and right ventricle, which presumes systolic pulmonary arterial pressure, was calculated from the velocity of tricuspid regurgitation. Pulmonary hypertension was defined as a ≥30 mm Hg systolic pressure gradient between the right atrium and ventricle. LV ejection fraction (EF) was determined by Teichholz's formula in patients without significant regional wall motion abnormalities. In patients with regional wall motion abnormalities, we assessed LVEF with the modified Simpson's rule [17] or by visual estimation [18].

Laboratory measurements

Plasma BNP concentration was run fresh and determined by an immunoradiometric assay using an antibody to human BNP (Shionogi, Osaka, Japan) [19]. We measured serum blood urea nitrogen and creatinine (the enzyme method) concentration with routine commercial enzyme assay kits. We used a latex agglutination photometric immunoassay to determine serum concentrations of beta2-microglobulin (normal range 1.0–1.9 mg/L, Eiken Chemicals, Tokyo, Japan). We estimated GFR (mL/min/1.73 m^2) with the abbreviated modification of diet in renal disease (MDRD) equation modified by a Japanese coefficient [20]: $eGFR = 0.741 \times 175 \times [\text{serum creatinine in mg/dL}]^{-1.154} \times [\text{age in years}]^{-0.203}$. For women, the calculated values were multiplied by 0.742. We estimated creatinine clearance (mL/min) with the Cockroft–Gault equation: creatinine clearance = ([140 − age in years] × body weight in kg)/(72 × serum creatinine in mg/dL). For women, the calculated values were multiplied by 0.85.

We obtained all of the data for each patient on the same day ($n = 131$). For 104/131 (79%) patients, the laboratory measurements were obtained within 3 days of echocardiography. Also, for 98/124 (79%) hospitalized patients, the laboratory measurements were taken within the 3rd hospital day. The laboratory data from the remaining 26

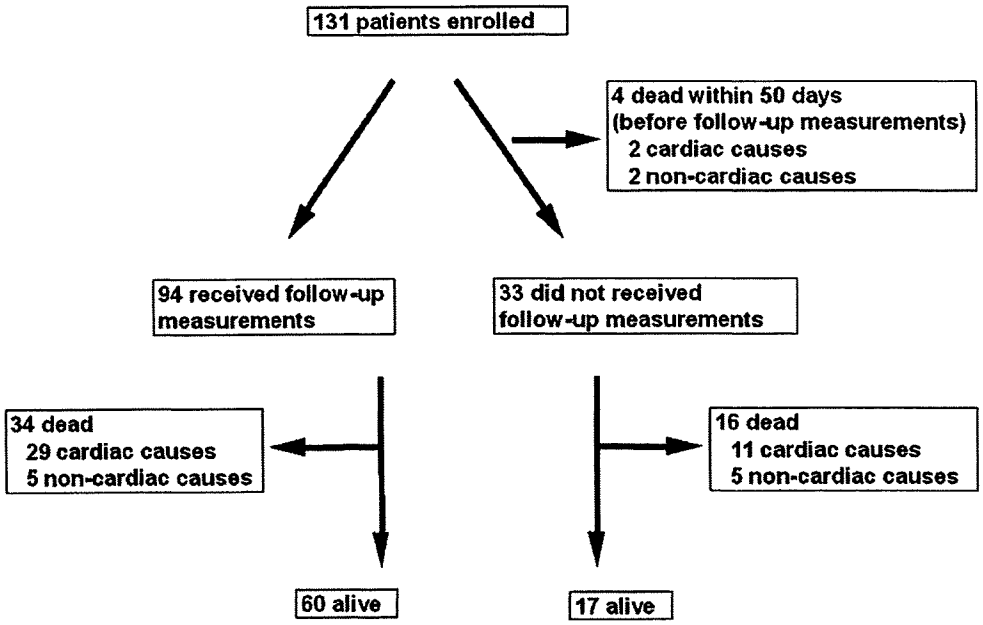

Figure 1 Outcome after treatment of 131 patients with acute heart failure.

patients were taken at the median 5th (4–16th) hospital day.

Endpoints and follow-up

The endpoints were (1) cardiovascular events, defined as sudden cardiac death and hospital admission due to heart failure or cardiovascular complications, and (2) cardiac death, defined as death from cardiovascular causes.

Patients were prospectively followed up for an average of 2.3 ± 1.3 years. For 94/131 (72%) patients, a second set of echocardiography and laboratory measurements was obtained an average of 7 ± 7 weeks after treatment (Fig. 1).

Statistical analysis

We compared data from heart failure patients with cardiac events to event-free patients during follow-up. Group averages are expressed as means ± standard deviation (SD), unless otherwise specified. BNP concentration was not normally distributed, and is expressed as median and interquartile range. Group comparisons of BNP values were performed with non-parametric tests. p-Values < 0.05 were considered significant. The associations of the variables with cardiac events or mortality were determined with a Cox proportional hazard analysis. Only variables with a p-value < 0.05 in the univariate Cox analysis were included in a multivariate stepwise Cox analysis. Forward stepwise selection manner, with both values for inclusion and elimination set at $p = 0.05$, was used to create a final reduced model for predicting outcomes. For cardiac events, 20 significant variables were entered into the multivariate analysis: baseline variables (age, diastolic blood pressure, blood urea nitrogen, creatinine, beta2-microglobulin, eGFR, creatinine clearance, ischemic heart disease, previous myocardial infarction, previous congestive heart failure, COPD, and cancer) and post-treatment variables (NYHA class, pulmonary hypertension, BNP, blood urea nitrogen, creatinine, beta2-microglobulin, eGFR, and creatinine clearance). The proportional hazards assumption was checked with a log minus log plot.

The association of each variable with baseline serum beta2-microglobulin concentration was evaluated with a univariate linear regression analysis. Variables with a p-value < 0.05 were entered into a multivariate stepwise regression.

We evaluated the association of quartiles of renal parameters as predictors of cardiac events. We determined the unadjusted and multivariate-adjusted risk for quartiles 2 through 4 compared with quartile 1. Adjusted cardiac event-free survival curves were constructed for the quartiles of serum beta2-microglobulin after adjustment for all covariates. Data with missing data points were excluded from analysis. Data was analyzed using SPSS 11.0 software (SPSS, Chicago, IL, USA).

Results

Long-term outcome, baseline characteristics and post-treatment data in heart failure patients

During the 2.3 ± 1.3 years after initiation of the study, cardiovascular events were observed in 63/131 (48%) patients: 53 (40%) patients were readmitted due to worsening heart failure, 5 (4%) readmitted for cerebral embolism, and 5 (4%) died from sudden cardiac death.

During follow-up, 54/131 (41%) patients died: 42 (32%) died of cardiovascular causes (33 from worsening heart failure, 2 from worsening cerebral embolism, and 7 from sudden

cardiac death). Namely 2 patients died suddenly after discharge from hospital readmission due to worsening heart failure; 12 (9%) died of noncardiac causes (7 from cancer, 4 from pneumonia, and 1 phlegmon) (Fig. 1).

Comparisons of the baseline characteristics of patients with cardiac events and event-free patients are presented in Table 1. Baseline characteristics associated with cardiac events were advanced age, lower diastolic blood pressure, reduced body surface area, higher blood urea nitrogen, creatinine, and beta2-microglobulin, lower eGFR and creatinine clearance, ischemic heart disease, previous myocardial infarction, previous congestive heart failure, and less use of vasodilators at baseline.

After treatment, the NYHA functional class (I/II/III/IV post-treatment: 1/47/1/1 in event-free patients and 2/35/7/0 in patients with cardiac events) and BNP concentration [median (25th, 75th percentiles) post-treatment: 200 (80, 284) pg/mL in event-free patients and 255 (155, 498) pg/mL in patients with cardiac events] decreased in both groups of patients ($p < 0.0001$). Serum beta2-microglobulin concentration increased ($p < 0.05$) in both groups (2.9 ± 1.4 mg/L in event-free patients and 4.4 ± 2.3 mg/L in patients with cardiac events).

Predictors for long-term outcome

All the variables listed in Table 1 at baseline and after treatment were analyzed using univariate Cox proportional hazard analysis. Among the variables, 20 (see Methods section) and 19 (not shown) were significant predictors of cardiac events and cardiac mortality, respectively.

As shown in Table 2, at baseline, serum beta2-microglobulin concentration was the most powerful predictor of cardiac events and cardiac mortality, as expressed by a chi-square value. A 1.58 mg/L (1 SD) increase in serum beta2-microglobulin was associated with a 1.5-fold increased risk of cardiac events. Previous congestive heart failure, presence of COPD and lower diastolic blood pressure were also independently associated with an increased risk of cardiac events. Likewise, lower systolic blood pressure, higher relative LV wall thickness, and lower body mass index were also independently associated with an increased risk of cardiac mortality.

In several types of malignancies [21,22], it was reported that adverse prognosis is associated with higher serum beta2-microglobulin concentration. Even if 11 patients with previous or concurrent malignancies were excluded from the analysis, serum beta2-microglobulin was still a strong and independent predictor of cardiac events and cardiac mortality (middle section of Table 2). In the multivariate Cox analysis including both baseline and post-treatment variables, baseline serum beta2-microglobulin was again a strong predictor of outcome (bottom section in Table 2). Higher BNP concentration post-treatment also independently contributed to increased cardiac mortality.

Variables associated with baseline serum beta2-microglobulin concentration

Elevated serum beta2-microglobulin has been reported in patients with a variety of non-renal illnesses, including solid malignancies, leukemia, and rheumatic diseases [23,24]. To clarify the effects on serum beta2-microglobulin, all the parameters listed in Table 1 were evaluated by linear regression (Table 3). According to a multivariate stepwise linear regression analysis, age, sex, and serum creatinine were significant independent determinants of baseline serum beta2-microglobulin concentrations. According to the standardized coefficient, serum creatinine had a greater impact on serum beta2-microglobulin than age or sex. The square of the multivariate correlation coefficient (R^2) indicated that these three parameters accounted for 73% of serum beta2-microglobulin concentration.

Quartiles of renal parameters and cardiac-event risk

Next we compared the ability of beta2-microglobulin to distinguish cardiac-event risk with other renal parameters. As shown in Table 4, after multivariate adjustment, the 4th quartile ($\geq$4.0 mg/L) of baseline beta2-microglobulin was associated with a 4.7-fold increased cardiac-event risk compared to the 1st quartile ($\leq$2.1 mg/L). Although the 4th quartiles of baseline creatinine and creatinine clearance were associated with a significantly greater adjusted event risk than the 1st quartile, besides the highest quartile, the 2nd and 3rd quartiles of beta2-microglobulin were also related to an increased risk for cardiac events. Adjusted cardiac event-free survival curves according to the quartile of beta2-microglobulin are shown in Fig. 2.

Discussion

In the present study, we showed that a higher baseline concentration of serum beta2-microglobulin was the most powerful predictor of cardiac events and cardiac mortality in acute heart failure patients with creatinine $\leq$3.0 mg/dL. Furthermore, we demonstrated that baseline serum beta2-microglobulin concentration had a superior ability to distinguish cardiac-event risk in acute heart failure patients compared with creatinine-based renal parameters. The association of baseline concentration of serum beta2-microglobulin with cardiovascular events or cardiac mortality was still obtained when patients with cancer were excluded from analysis.

Risk factors for prognosis

In addition to beta2-microglobulin, several other risk factors for outcome were identified in this study (Table 2). Prognostic values of previous heart failure [25], COPD [26], systolic [7,27] and diastolic blood pressure [25], and body mass index [5,25,27] in heart failure were confirmed previously. Post-treatment BNP levels [28,29] also have been reported to be strong predictors of mortality in heart failure.

Serum beta2-microglobulin as a predictor of long-term outcome

Widely distributed in nucleated cells in the body and shed into the circulation, beta2-microglobulin is filtered

Table 1 Patient baseline characteristics by cardiac events during follow-up.

	Events (−) ($n=68$)	Events (+) ($n=63$)	p-Value
Age (years)	74 ± 12	81 ± 9	0.002
Male	28 (41%)	25 (40%)	0.9
NYHA class II/III/IV	7/35/26	5/36/22	0.9
SBP (mm Hg)	142 ± 27	133 ± 26	0.06
DBP (mm Hg)	80 ± 14	73 ± 15	0.006
Heart rate (beats/min)	78 ± 17	81 ± 20	0.5
Body surface area (m^2)	1.54 ± 0.24	1.46 ± 0.18	0.04
Body mass index (kg/m^2)	23 ± 5	22 ± 3	0.07
Echocardiograph data			
LVEDD (mm)	54 ± 10	52 ± 9	0.3
LVEF (%)	51 ± 18	50 ± 16	0.6
LV mass index (g/m^2)	150 ± 52	152 ± 48	0.9
Septal thickness (mm)	10.8 ± 2.8	10.9±2.5	0.9
Posterior wall thickness (mm)	10.6 ± 2.1	10.9 ± 1.9	0.4
Relative LV wall thickness	0.41 ± 0.11	0.44 ± 0.12	0.2
Left atrial diameter (mm)	50 ± 10	50 ± 10	1.0
E/A[a]	1.1 ± 0.7	1.1 ± 0.7	1.0
Pulmonary hypertension	31(47%)	34 (54%)	0.4
Laboratory data			
BNP (pg/mL)[b]	423 (208, 940)	552 (328, 1040)	0.15
BUN (mg/dL)	21 ± 10	25 ± 13	0.03
Creatinine (mg/dL)	0.9 ± 0.4	1.1 ± 0.5	0.01
Beta2-microglobulin (mg/L)	2.8 ± 1.3	3.6 ± 1.7	0.002
eGFR ($mL/min/1.73\,m^2$)[c]	61 ± 23	50 ± 22	0.006
Creatinine clearance (mL/min)[c]	62 ± 38	43 ± 22	0.0009
Medical history			
Hypertension	40 (59%)	31 (49%)	0.3
Ischemic heart disease	13 (19%)	26 (41%)	0.006
Previous myocardial infarction	10 (15%)	21 (33%)	0.01
Previous CHF	17 (25%)	33 (52%)	0.001
Diabetes mellitus	16 (24%)	15 (24%)	1.0
COPD	4 (6%)	6 (10%)	0.4
Valvular heart disease	32 (47%)	32 (51%)	0.7
Atrial fibrillation	30 (44%)	34 (54%)	0.3
Cancer[d]	4 (6%)	7 (11%)	0.3
Use of medication at baseline			
ACE-inhibitors or ARB	28%	33%	0.5
Beta-blockers	15%	19%	0.5
Digitalis	16%	29%	0.09
Diuretics	50%	65%	0.08
Vasodilators	29%	14%	0.04
Nitrates	40%	37%	0.7
Use of medication after treatment[e]			
ACE-inhibitors or ARB	82%	82%	1.0
Beta-blockers	68%	55%	0.2
Digitalis	32%	36%	0.7
Diuretics	82%	89%	0.4
Vasodilators	20%	7%	0.07
Nitrates	28%	46%	0.08

Data are expressed as mean ± SD, number and/or percent of patients, or median (25th, 75th) percentiles. ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blockers; BNP, B-type natriuretic peptide; BUN, blood urea nitrogen; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; DBP, diastolic blood pressure; E/A, early-to-atrial transmitral peak velocity ratio; EDD, end-diastolic dimension; EF, ejection fraction; eGFR, estimated glomerular filtration rate; LV, left ventricular; NYHA, New York Heart Association; SBP, systolic blood pressure.

[a] Data from patients with atrial fibrillation were excluded.

[b] Median and (25th, 75th percentiles) are shown.

[c] eGFR was calculated by the abbreviated modification of diet in renal disease (MDRD) equation modified by a Japanese coefficient; creatinine clearance was by the Cockroft–Gault equation (see Methods section).

[d] Patients who had previously undergone surgery for cancer ($n=4$) or had concurrent malignancies ($n=7$) at baseline.

[e] Data of medication use after 7 ± 7 weeks from 94 patients for whom follow-up measurements were available.

Table 2 Multivariate stepwise Cox proportional hazard analysis of risk factors for prognosis.

Variable	Cardiac events		Cardiac mortality	
	X^2	HR (95%CI)	X^2	HR (95%CI)
Models including only baseline variables ($n=130$)				
Beta2-microglobulin (per 1.58 mg/L increase)[a]	16	1.5 (1.2–1.8)****	20	1.8 (1.4–2.4)****
Previous CHF	11	2.4 (1.4–4.0)***		
COPD	8	3.9 (1.6–9.7)**		
Diastolic blood pressure (per 15 mm Hg increase)[a]	6	0.7 (0.5–0.9)*		
Systolic blood pressure (per 27 mm Hg increase)[a]			11	0.6 (0.4–0.8)***
Relative LV wall thickness (per 0.116 increase)[a]			6	1.5 (1.1–2.0)*
Body mass index (per 4.2 kg/m^2 increase)[a]			5	0.6 (0.4–0.9)*
Models excluding patients with previous or concurrent malignancies ($n=119$)				
Beta2-microglobulin (per 1.58 mg/L increase)[a]	16	1.5 (1.2–1.9)****	10	1.7 (1.2–2.4)**
Previous CHF	11	2.5 (1.4–4.3)**		
COPD	9	4.1 (1.6–10)**		
Diastolic blood pressure (per 15 mm Hg increase)[a]	5	0.7 (0.5–1.0)*		
Systolic blood pressure (per 27 mm Hg increase)[a]			11	0.5 (0.3–0.8)**
Body mass index (per 4.2 kg/m^2 increase)[a]			6	0.6 (0.3–0.9)*
Blood urea nitrogen (per 12 mg/dL increase)[a]			5	1.5 (1.0–2.0)*
Models including both baseline and post-treatment variables ($n=84$–91)				
Previous CHF	11	3.2 (1.6–6.5)***		
Baseline beta2-microglobulin (per 1.58 mg/L increase)[a]	9	1.5 (1.2–2.0)**	20	1.9 (1.4–2.8)****
Cancer	6	5.0 (1.4–17)*		
COPD	5	4.1 (1.2–14)*		
Age (per 11 years increase)[a]	4	1.6 (1.0–2.5)*		
Baseline diastolic blood pressure (per 15 mm Hg increase)[a]			10	0.5 (0.3–0.8)**
Post-treatment BNP (per 125 pg/mL increase)[b]			6	1.1 (1.0–1.5)*

Beta2-microglobulin and BNP were modeled as continuous variables. BNP, B-type natriuretic peptide; CHF, congestive heart failure; CI, confidence interval; COPD, chronic obstructive pulmonary disease; HR, hazard ratio; LV, left ventricular; X^2, chi-square value.

[a] Per 1 SD unit.

[b] Per 1 median absolute deviation (MAD) unit (as BNP concentration was not normally distributed).

* $p<0.05$ and ≥ 0.01.

** $p<0.01$ and ≥ 0.001.

*** $p<0.001$ and ≥ 0.0001.

**** $p<0.0001$.

Table 3 Baseline variables associated with baseline serum beta2-microglobulin (β2-MG) concentration (mg/L) based on univariate and multivariate ($n=130$, $R^2=0.73$) analyses.

Variable	β2-MG (univariate)		β2-MG (multivariate)		
	r (ρ)	p-Value	Coefficient[a]	Standardized coefficient[b]	p-Value
Age (years)	0.4	<0.0001	0.023	0.16	0.002
Male	−0.2	0.009	−0.56	−0.18	0.001
SBP	0.2	0.01			0.8
BUN	0.6	<0.0001			0.6
Creatinine (mg/dL)	0.8	<0.0001	2.83	0.78	<0.001
eGFR	−0.7	<0.0001			0.5
Creatinine clearance	−0.5	<0.0001			0.3
Use of diuretics	0.3	0.003			0.13

R^2, square of the multivariate correlation coefficient. ρ was used for non-parametric variables. BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure.

[a] Linear regression coefficient.

[b] The standardized coefficient describes the unit-independent contribution of the independent variable to the model.

Table 4 Quartiles of baseline renal parameters and cardiac-event risk in patients with acute heart failure.

	1st Quartile	2nd Quartile	3rd Quartile	4th Quartile
Beta2-microglobulin				
Range (mg/L)	≤2.1	2.2–2.6	2.7–3.9	≥4.0
n	35	31	32	33
1-year event rate (%)[a]	8	23	23	31
Unadjusted HR (95%CI)	1.0	2.9 (1.3–6.6)*	3.6 (1.6–8.1)**	4.0 (1.8–8.9)***
Adjusted HR (95%CI)[a]	1.0	2.9 (1.2–6.9)*	2.9 (1.2–7.2)*	4.7 (2.0–11)***
Creatinine				
Range in women (mg/dL)	≤0.67	0.68–0.84	0.85–1.21	≥1.22
Range in men (mg/dL)	≤0.75	0.76–0.88	0.89–1.10	≥1.11
n	32	34	33	32
1-year event rate (%)[a]	9	12	35	31
Unadjusted HR (95%CI)	1.0	0.7 (0.3–1.6)	1.4 (0.7–2.9)	1.9 (0.9–3.7)
Adjusted HR (95%CI)[a]	1.0	0.8 (0.4–1.8)	2.1 (1.0–4.5)	2.3 (1.0–5.1)*
Estimated GFR				
Range (mL/min/1.73 m^2)	≥70.5	54.3–70.4	38–54.2	≤37
n	32	33	33	33
1-year event rate (%)[a]	9	20	33	25
Unadjusted HR (95%CI)	1.0	1.0 (0.4–2.1)	1.7 (0.8–3.5)	2.2 (1.1–4.4)*
Adjusted HR (95%CI)[a]	1.0	1.1 (0.5–2.4)	2.0 (0.9–4.4)	2.1 (1.0–4.8)
Creatinine clearance				
Range (mL/min)	≥66.2	48.7–66.1	30–48.6	<30
n	32	33	33	33
1-year event rate (%)[a]	6	21	21	43
Unadjusted HR (95%CI)	1.0	2.2 (0.9–5.2)	2.4 (1.0–5.7)*	4.8 (2.2–11)***
Adjusted HR (95%CI)[a]	1.0	2.3 (0.9–5.7)	2.4 (0.9–6.6)	5.1 (1.7–15)**

GFR, glomerular filtration rate; HR, hazard ratio.

[a] Adjusted for all baseline covariates besides renal parameters with $p < 0.05$ in univariate Cox analysis (age, diastolic blood pressure, ischemic heart disease, previous congestive heart failure, chronic obstructive pulmonary disease, and cancer).

* $p < 0.05$ and ≥ 0.01.

** $p < 0.01$ and ≥ 0.001.

*** $p < 0.001$.

by glomeruli and reabsorbed by the proximal tubular cells where it is metabolized. Its plasma concentration increases with renal dysfunction, and may be a surrogate marker of GFR [8,9,24,30]. Indeed, as shown in Table 3, beta2-microglobulin concentration strongly correlated with eGFR and creatinine clearance, and was determined by creatinine, age, and sex, in accordance with previous studies [8,9,23]. Although eGFR and creatinine clearance values

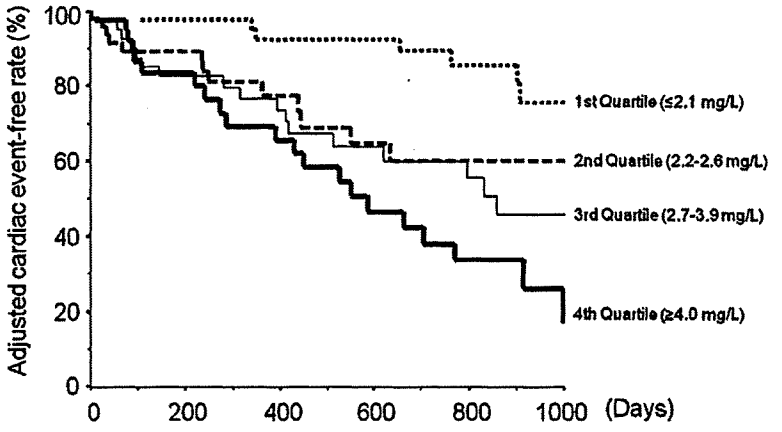

Figure 2 Adjusted cardiac event-free survival curves in acute heart failure patients according to baseline serum beta2-microglobulin concentrations. The data were adjusted for differences in baseline variables, including age, diastolic blood pressure, ischemic heart disease, previous congestive heart failure, chronic obstructive pulmonary disease, and cancer.

were also based on creatinine concentration, age, and sex (see Methods section), serum beta2-microglobulin was a better prognostic marker for acute heart failure patients than these creatinine-based measures (Table 4).

First, recent reports [31,32] showed that GFR could not be estimated accurately with the abbreviated MDRD or Cockroft–Gault equations in persons with normal serum creatinine concentrations, as the correlation between measured GFR and estimated GFR was weak ($R^2 \leq 0.40$). The low body mass index observed in our patients (Table 1) suggested the possibility of malnourishment. Therefore, methods based on creatinine (mean 1.0 ± 0.4 mg/dL in the present study) may overestimate actual GFR, while serum beta2-microglobulin would not be affected by reduced muscle mass [8].

Second, serum creatinine concentration, age, and sex accounted for 73% of serum beta2-microglobulin concentration ($R^2 = 0.73$, Table 3). Regarding the remaining 27% of beta2-microglobulin, several diseases, including malignancies and inflammatory diseases, show an increased production of beta2-microglobulin, resulting in increased serum concentration despite normal GFR [23,24]. Of note, a recent study [10] demonstrated a significant ($p < 0.001$) association of serum beta2-microglobulin concentration with serum high-sensitivity C-reactive protein levels in a healthy, older cohort, suggesting that serum beta2-microglobulin reflects low-grade inflammation. Therefore, serum beta2-microglobulin concentration may also reflect factors other than renal function that are related to mortality.

Limitations

Although the prognostic value of beta2-microglobulin in this study was statistically highly significant, the number of patients was small. Further studies in a large number of patients are needed to confirm the prognostic role of serum beta2-microglobulin in acute heart failure.

Conclusions

Higher baseline serum beta2-microglobulin concentration could be a promising risk marker in acute heart failure patients with creatinine ≤ 3.0 mg/dL.

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COMMENTARY

Pathophysiology of cognitive dysfunction in older people with type 2 diabetes: vascular changes or neurodegeneration?

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Abstract

Recent studies have revealed that type 2 diabetes mellitus (T2DM) is a risk factor for cognitive dysfunction or dementia, especially those related to Alzheimer's disease (AD). Basic research suggests that insulin accelerates Alzheimer-related pathology through its effects on the amyloid beta (Aβ). Several pathological studies with autopsy samples have demonstrated, however, that dementia subjects with diabetes have less AD-related neuropathology than subjects without diabetes. We and others have reported that small vessel diseases affect cognitive function in older diabetics. Asymptomatic ischemic lesions in T2DM subjects may lower the threshold for the development of dementia and this may explain the inconsistency between the basic research and clinicopathological studies. Longitudinal follow-up of T2DM subjects without overt dementia using both amyloid imaging and magnetic resonance imaging may elucidate these issues. Following up until the development of overt dementia would make it possible to compare both amyloid load and ischemic lesions before and after the development of dementia. Moreover, amyloid imaging in non-demented older people with or without insulin resistance would verify the role of insulin in the processing and deposition of Aβ. Vascular risk factors may represent a therapeutic target, while neurodegenerative pathologies have not yet been amenable to treatment. It remains to be investigated whether medical interventions on vascular risk factors have protective effects against the development and progress of dementia.

Keywords: *Alzheimer's disease, ischemic lesions, dementia, insulin, Aβ*

The prevalence of type 2 diabetes mellitus (T2DM) increases with age, and dementia also increases its incidence in later life. Therefore, the coincidence of T2DM and dementia increases with ageing. Moreover, recent studies have indicated that older people with T2DM have a higher risk of cognitive dysfunction or dementia [1]. There is ample evidence that T2DM is related not only to vascular dementia but also to clinical diagnosis of Alzheimer's disease (AD)-type dementia [2]. The precise mechanisms underlying T2DM-related cognitive dysfunction or development of dementia, especially AD-type dementia, remain to be elucidated, although several hypothetical mechanisms have been proposed. High glucose concentration, a major pathological characteristic of diabetes, may have toxic effects on neurons in the brain through osmotic insults and oxidative stress, and the maintenance of chronic high glucose also leads to the enhanced formation of advanced glycation endoproducts, which have potentially toxic effects on neurons. Diabetes is associated with an increased release of inflammatory cytokines, and the excess inflammation may be neurotoxic.

T2DM, especially in conjunction with obesity, is characterised by insulin resistance and/or hyperinsulinaemia. Insulin resistance is defined as an inadequate response by insulin target tissues, such as skeletal muscle, liver and adipose tissue, to the physiological effects of circulating insulin, and often is accompanied by raised insulin levels. Basic research suggests that insulin accelerates AD-related pathology through its effects on the amyloid beta (Aβ) metabolism and tau phosphorylation [2]. Insulin reportedly raises Aβ concentrations in plasma in AD subjects, and these effects may contribute to the risk of AD in T2DM. The desensitization of insulin receptors, insulin resistance, reduces the synthesis of several proteins, including insulin-degrading enzyme (IDE). IDE degrades Aβ as well as insulin, and reduced amounts of IDE may result in greater

amyloid deposition. Less insulin signalling may also induce increased activity of glycogen synthase kinase-3β, which leads to the enhanced phosphorylation of tau protein and the formation of neurofibrillary tangles (NFTs). Several pathological studies with autopsy samples have demonstrated, however, that dementia subjects with diabetes have less AD-related neuropathology than subjects without diabetes. One study demonstrated that diabetics show significantly less AD-associated neuropathology [3], while another failed to show any relationship between diabetes and AD-associated neuropathology [4]. Why does this disparity exist between clinicopathological data and the implications of basic research?

AD has been thought to be a neurodegenerative disorder, which can be sharply distinguished from vascular dementia. Recent studies, however, suggest that the distinction between AD and vascular dementia may not be tenable. There is now substantial and growing evidence that vascular disorders and/or impaired cerebral perfusion contribute to the development of sporadic AD. For example, cerebrovascular pathology including stroke seems to play an important role in the eventual development of the clinical symptoms of AD [5].

On cerebral magnetic resonance imaging (MRI), white matter hyperintensities and lacunae, both of which are frequently observed in the elderly, are generally viewed as evidence of small vessel disease in the brain (white matter lesions and lacunae). These lesions are frequently concomitant with Alzheimer-related neuropathology (senile plaques and NFTs) and contributes to cognitive impairment in AD subjects [6]. We previously reported that small vessel diseases affect cognitive function in older diabetics who have not developed either overt dementia or symptomatic stroke [7, 8]. The number of asymptomatic infarcts and the extent of white matter lesions in the brain detected with MRI were found to be associated with the scores of several cognitive functional tests, especially the digit symbol substitution test, a neurocognitive test that primarily reflects declines in perceptual speed. We also reported that an inflammatory cytokine, tumour necrosis factor-α, which is a risk factor for atherosclerosis, is related to cognitive dysfunction in older nondemented diabetics [9]. A recent study demonstrated that T2DM subjects with clinical diagnosis of dementia have less Alzheimer-related pathology but more ischaemic lesions [10]. This supports the hypothesis that small vessel disease lowers the threshold for the development of dementia. That is, if subjects have the same level of cognitive dysfunction, those with a combination of two types of pathologies have fewer pathological changes in each of their pathologies than those with a single pathology which is severe enough to cause the cognitive dysfunction. Therefore, these pathological reports do not necessarily refute the possibility that DM accelerates the development of Alzheimer-related neuropathology in the patients with clinical diagnosis of dementia. Arvanitakis *et al.* demonstrated in 2004 that T2DM increases the incidence of AD by clinical diagnosis [11], but T2DM ameliorated perceptual speed but not global cognition. A previous study [12] and our own studies [7, 8] showed that cerebral ischaemic lesions are preferentially associated with a lower measure of perceptual speed. These results also suggest that small vessel disease contributes to cognitive decline in these populations.

Hypertension is often accompanied by diabetes, and several longitudinal studies appear to support the notion that hypertension predisposes to cognitive decline and the development of dementia [13]. Vascular alterations induced by high blood pressure may contribute to cognitive dysfunction. Hypertension is also associated with cerebrovascular disease including lacunar brain infarcts and white matter lesions, which may contribute to cognitive impairment in diabetics.

Recently, amyloid imaging technology with positron emission tomography, which visualises Aβ depositions in the human brain, has been developed and is now widely available [14] although some limitations of resolution and specificity still exist. This technology can be used to investigate the relative contributions of ischaemic and neurodegenerative changes to the increasing development of dementia in T2DM subjects. Longitudinal follow-up of T2DM subjects without overt dementia using both amyloid imaging and MRI may help to elucidate these issues, especially with higher field MRI with some potential for the imaging small vessel diseases [15] as well as diffusion tensor imaging method [16]. Following up until the development of overt dementia would make it possible to compare both amyloid load and ischaemic lesions before and after the development of dementia. Moreover, amyloid imaging in non-demented older people with or without insulin resistance would verify the hypothesis that insulin plays a role in the processing and deposition of Aβ. These investigations are important considering the future availability of disease-modifying therapeutics such as Aβ vaccination and inhibitors for Aβ secretions.

At present, vascular risk factors may represent a therapeutic target, while neurodegenerative pathologies have not yet been amenable to treatment. Vascular risk factors including diabetes and hypertension are reportedly associated with the progression of lacunae and white matter lesions [17]; however, the beneficial effects on cognitive function of pharmaceutical interventions with antidiabetics and antihypertensives are less clear in terms of the inhibition of the progress of lacunae and white matter lesions. It remains to be investigated whether medical interventions on vascular risk factors have protective effects against the development and progress of dementia. If such protective effects do exist, the underlying mechanism of the therapeutic effects should be interesting, whether it relies on the inhibition of the development of vascular lesions or in that of the neurodegenerative process.

With the current increase in the older population, T2DM-associated cognitive dysfunction and dementia are an increasingly larger problem. A greater understanding of the relevant pathophysiology and the establishment of better therapeutic interventions are urgent issues.