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Abbreviations and Acronyms

ACS = acute coronary syndromes
BNP = B-type natriuretic peptide
CAD = coronary artery disease
CHD = coronary heart disease
CI = confidence interval
DM = diabetes mellitus
eGFR = estimated glomerular filtration rate
EMP = endothelium-derived microparticle(s)
HDL = high-density lipoprotein
HR = hazard ratio
hsCRP = high-sensitivity C-reactive protein
LDL = low-density lipoprotein

development of atherothrombogenic complications (4) and associated with future cardiovascular events in high-risk patients (5–7); however, it has not been incorporated into the previous multiple biomarkers strategy. Endothelial dysfunction can be clinically detected by measuring impairment of endothelium-dependent vasodilatation in response to acetylcholine during coronary angiography or by brachial artery flow-mediated vasodilation (5,8). These physiological tests are complex, operator dependent, and provide limited quantitative data (9,10).

Endothelium-derived microparticles (EMP) are small membrane-shed vesicles generated from endothelial cell surfaces in response to cellular activation or injury/apoptosis, and can potentially reflect endothelial dysfunction (11,12). Recently, we reported that CD144-EMP is derived selectively from human endothelial cells (13) and that circulating plasma CD144-EMP levels correlate significantly with coronary endothelial dysfunction and are significantly elevated in patients with type 2 diabetes and atherosclerosis (13). Although EMP are still only used for research purpose and in specialized laboratories because of their elusive nature and difficult assessment due to very small size (14), these findings underscore the potential application of CD144-EMP as a quantitative biomarker of endothelial dysfunction.

We hypothesized that the addition of a quantitative measure of endothelial dysfunction to a multiple biomarkers strategy could improve the prediction of future cardiovascular events. The hypothesis was tested by investigating the utility of plasma CD144-EMP levels for prediction of future cardiovascular events in stable patients at high risk for coronary heart disease (CHD), and examined the usefulness of the modified multiple biomarkers strategy, including endothelial dysfunction assessed by EMP, to predict cardiovascular complications.

Methods

Study patients. In this prospective study, we screened 519 consecutive Japanese patients between May 2003 and August 2007 at Kumamoto University Hospital. Patients with severe valvular heart disease requiring surgical intervention within 1 month, scheduled for coronary revascularization, active infection, or malignant disease were excluded from the study (n = 31). The 488 patients who fulfilled the study criteria were divided into the following 4 groups: low-risk patients who had no or 1 CHD risk factor, patients with multiple risk factors without documented coronary artery disease (CAD), patients with documented CAD at stable condition (stable-CAD), and patients with acute coronary syndromes (ACS) (Fig. 1). Stable-CAD represented patients with angiographically documented organic coronary

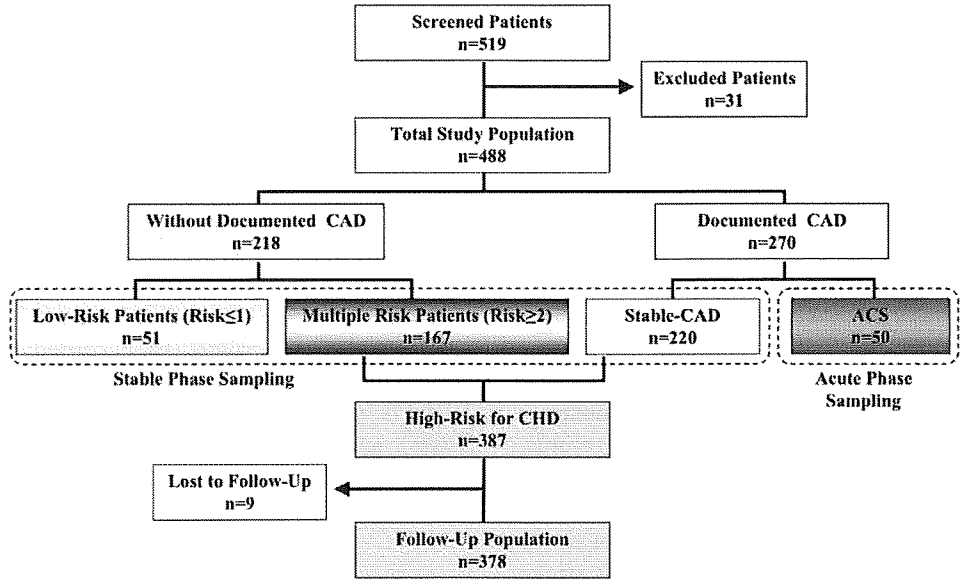

Figure 1 Flow Diagram of Subject Recruitment

Thirty-one patients were excluded for the following reasons: malignant diseases (n = 20), unstable conditions (n = 6), systemic inflammatory disease (n = 3), and active infections (n = 2). ACS = acute coronary syndromes; CAD = coronary artery disease; CHD = coronary heart disease.

stenosis of >50% by quantitative coronary angiography in major coronary arteries. Risk factors for CHD were defined as age ≥65 years (15); current smoking; family history of ischemic heart disease; hypertension (>140/90 mm Hg or taking antihypertensive medication) (16); dyslipidemia (high-density lipoprotein [HDL] cholesterol <40 mg/dl, low-density lipoprotein [LDL] cholesterol ≥140 mg/dl, triglycerides ≥150 mg/dl, or receiving lipid-lowering treatment); diabetes mellitus (DM) (17); body mass index ≥25.0 kg/m^2 (16); hsCRP ≥2.0 mg/l; or chronic kidney disease (estimated glomerular filtration rate [eGFR] <60 ml/min/1.73 m^2). The glomerular filtration rate was estimated using the modified formula of Modification of Diet in Renal Disease study equation, which was proposed by the Japanese Society of Nephrology (18). This study protocol was conducted in accordance with guidelines approved by the ethics committee at our institution.

Measurement of plasma levels of CD144-EMP and blood parameters. Blood samples were withdrawn by venipuncture into vacutainer tubes containing sodium citrate after a 12-h overnight fast for stable patients and on admission to the emergency room for ACS patients, before any mechanical intervention. Fresh plasma was assayed immediately for CD144-EMP by flow cytometry using the method described previously (13,14). We verified plasma levels of CD144-EMP with standard plasma for each sample. Standard plasma were subdivided into 1-use volume and stocked at −80°C. One thawing of stock plasma did not affect CD144-EMP levels. We measured hsCRP by a nephelometry with BN II (Siemens, Berlin, Germany) and BNP by a fluorescence enzyme immunoassay with AIA-21 (Tosoh Bioscience, Tokyo, Japan). Total cholesterol, HDL cholesterol, triglyceride, LDL cholesterol, and creatinine concentrations were determined by routine laboratory methods.

Study protocol. First, we compared plasma levels of CD144-EMP among low-risk patients (CHD risk factor ≤1), multiple risk patients (CHD risk factors ≥2), stable-CAD, and ACS patients. Second, patients with multiple risk factors or stable-CAD were categorized as high-risk patients for CHD and followed up every month at the outpatient department until July 2008 or at end point (Fig. 1). The end point was cardiovascular death, nonfatal myocardial infarction, unstable angina, ischemic stroke, or coronary revascularization to new lesions. Cardiovascular events were documented by phone calls to the patients or their families, followed by a review of medical records, electrocardiogram, ultrasound echocardiogram, and cardiac enzyme data. Cardiovascular death was defined as death due to myocardial infarction, congestive heart failure, or documented sudden cardiac death. Diagnosis of ischemic stroke was made if the patient had clinical and radiological evidence of stroke without intracranial hemorrhage. For subjects experiencing more than 2 acute events, only the first event was considered in the analysis. Revascularization therapy based only on angiographic data, including percutaneous coronary intervention-mediated restenosis, was not counted as a cardiovascular event. We used the previously reported cutoff values of 52.6 pg/ml (19) and 2.0 mg/l (20), and the median levels for BNP, hsCRP, and CD144-EMP, respectively, to divide our follow-up population into 2 groups: the high-level group and low-level group for the particular parameter.

Statistical analysis. Results were expressed as mean ± SD or as frequencies (percentages), while BNP, hsCRP, and CD144-EMP levels were expressed as median and interquartile range. The frequencies of risk factors and medications were compared between 2 groups by using chi-square analysis. Continuous variables were compared between 2 groups by the unpaired t test or Mann-Whitney U test, as appropriate. Data of the 4 groups were compared by 1-way analysis of variance, Kruskal-Wallis test, and chi-square analysis. Survival analysis was performed using the Kaplan-Meier method and assessed with the log-rank test.

The predictive value for cardiovascular events was assessed by Cox proportional hazards regression. The following variables were incorporated first into the univariate model: age, sex, current smoking, hypertension, DM, body mass index, HDL cholesterol, LDL cholesterol, eGFR, BNP, hsCRP, and CD144-EMP. Variables with p values <0.20 were then entered into a forward stepwise multivariate Cox proportional hazards analysis. In this model, we evaluated the effect of the biomarkers, BNP, hsCRP, and CD144-EMP, according to quintile increment in biomarkers levels.

Proportional hazards assumption was confirmed by Schoenfeld's test. Estimates of the C statistic for Cox proportional hazards regression models were calculated (21). The comparison of C statistics after the addition of the biomarkers to the model with Framingham risk was estimated (22). We also examined whether the addition of various combinations of biomarkers improved the discriminatory power of the model.

We assessed the calibration of Cox regression models by the Grønnesby and Borgan (23) calibration test, which compares the number of events that are expected based on estimation from 5 risk score groups. To evaluate whether the global model fit improved after the addition of the biomarkers, we performed likelihood ratio tests.

The statistical analyses were carried out using SPSS version 15.0J for Windows (SPSS Inc., Chicago, Illinois), STATA version 10.0 (StataCorp LP, College Station, Texas), and SAS version 9.1.3 (SAS Institute Inc., Cary, North Carolina). Statistical significance was defined as a value of $p < 0.05$ from 2-sided tests.

Results

Enrollment, classification, and follow-up of patients. We screened 519 patients, but 31 patients were excluded (Fig. 1). Data of the remaining 488 patients were subjected to analysis. In this study population, 387 patients at high

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risk for CHD were followed up, and the data of 378 patients (multiple risk factors, n = 167; stable-CAD, n = 220) were available for analysis of cardiovascular events while 9 patients were lost to follow-up (Fig. 1). The follow-up period was 1 to 62 months (mean 36 months).

Comparison of CD144-EMP levels. All clinical factors except the frequency of current smoking were significantly different among patients with various CHD risk. The plasma levels of CD144-EMP increased significantly with increased coronary risk factors and with complicated clinical manifestations (patients at low-risk: n = 51, median [interquartile range], 0.303 [0.142 to 0.367] × 10^6; multiple risk factors: n = 167, 0.508 [0.387 to 0.681] × 10^6; stable-CAD: n = 220, 0.604 [0.449 to 0.795] × 10^6; ACS: n = 50, 0.983 [0.718 to 1.150] × 10^6/ml, $p < 0.001$) (Fig. 2). LDL cholesterol, eGFR, and hsCRP were higher in ACS than stable-CAD (ACS vs. stable-CAD: LDL cholesterol: 121.2 ± 30.0 mg/dl vs. 110.8 ± 32.7 mg/dl, eGFR: 65.7 ± 20.8 ml/min/1.73 m^2vs. 58.9 ± 21.4 ml/min/1.73 m^2, and hsCRP: 2.2 [0.7 to 7.8] mg/l vs. 1.2 [0.5 to 3.6] mg/l). Moreover, CD144-EMP levels were significantly higher in ACS patients than in stable-CAD patients (Fig. 2).

Baseline clinical features of patients at high risk for CHD. Table 1 summarizes the baseline clinical features of patients at high risk for CHD (multiple risk factors or stable-CAD; follow-up population). The mean age was 66.9 years and 61.4% were men. Plasma levels of CD144-EMP correlated weakly with hsCRP ($r = 0.16$, $p = 0.002$) and did not correlate with BNP ($r = 0.08$, $p = 0.14$). Multivariate logistic regression analysis identified male sex and DM as significant risk factors of high EMP levels (above median) (men: hazard ratio [HR]: 1.685, 95% confidence interval [CI]: 1.076 to 2.639, $p = 0.02$; DM: HR: 1.551, 95% CI: 1.009 to 2.386, $p = 0.046$).

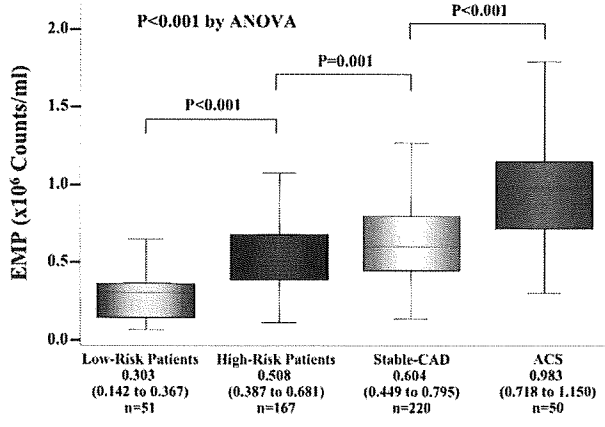

Figure 2 Plasma Levels of CD144-EMP in Patients With Various Cardiovascular Risks

The **line within the box** represents the median value; the **top and bottom lines of the box** represent the 25th and 75th percentiles, respectively; and the **top and bottom vertical lines outside the boxes** represent the 90th and 10th percentiles, respectively. ANOVA = analysis of variance; EMP = endothelium-derived microparticle; other abbreviations as in Figure 1.

Table 1 Baseline Clinical Characteristics of 378 Follow-Up Patients at High Risk for CHD

	All Subjects (n = 378)
Age, yrs	66.9 ± 9.8
Sex, male/female (%/%)	232/146 (61.4/38.6)
Current smoking	68 (18.0)
Hypertension	279 (73.8)
Diabetes mellitus	157 (41.5)
Body mass index, kg/m^2	23.7 ± 3.4
HDL cholesterol, mg/dl	52.2 ± 16.4
LDL cholesterol, mg/dl	114.4 ± 31.4
eGFR, ml/min/1.73 m^2	63.0 ± 20.9
BNP, pg/ml	57.0 (22.7–156.3)
High-sensitivity CRP, mg/l	0.9 (0.4–2.4)
EMP, × 10^6/ml	0.569 (0.427–0.761)
Medications	
Antihypertensive drugs	338 (89.4)
Statins	174 (46.0)

Data are mean ± SD, n (%), or median (interquartile range).
BNP = B-type natriuretic peptide; CHD = coronary heart disease; CRP = C-reactive protein; eGFR = estimated glomerular filtration rate; EMP = endothelium-derived microparticle; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Cardiovascular events and biomarker levels. We recorded 55 cardiovascular events in patients at high risk for CHD during the follow-up period. Patients of the high EMP group developed significantly more cardiovascular events than the low EMP group during the follow-up (Table 2). Specifically, the incidences of cardiovascular death and ACS were significantly higher in the high-EMP group than in the low-EMP group (Table 2). Kaplan-Meier analysis based on high and low levels of biomarkers showed a significantly higher probability of cardiovascular events in the presence of high levels of BNP, hsCRP, and EMP during the follow-up (log-rank test: BNP $p < 0.001$, hsCRP $p < 0.001$, and EMP $p < 0.001$) (Figs. 3A to 3C).

Cox proportional hazard analysis and C statistics for cardiovascular events. Univariate and multivariate Cox proportional hazards analysis for cardiovascular events showed that age, BNP, hsCRP, and CD144-EMP were independent predictors of future cardiovascular events in

Table 2 Cardiovascular Events in Patients With High or Low EMP Levels

	High EMP Group (n = 189)	Low EMP Group (n = 189)	p Value
Total cardiovascular events	41	14	<0.001
Cardiovascular death	14	3	0.01
Acute coronary syndromes	12	3	0.03
Nonfatal myocardial infarction	4	0	0.12
Unstable angina	8	3	0.22
Ischemic stroke	5	5	1.00
Coronary revascularization to new lesions	10	3	0.09

Data are number of patients.
EMP = endothelium-derived microparticle.

patients at high risk for CHD (age: HR: 1.042, 95% CI: 1.007 to 1.080, p = 0.02; BNP: HR: 1.242, 95% CI: 1.004 to 1.536, p = 0.046; hsCRP: HR: 1.468, 95% CI: 1.150 to 1.875, p = 0.002; EMP: HR: 1.345, 95% CI: 1.094 to 1.652, p = 0.005) (Table 3). Framingham risk was not incorporated into multivariate analysis because it was constructed by the same variables in univariate analysis. Framingham risk was confirmed to be a significant factor by univariate analysis in the present study (HR: 1.043, 95% CI: 1.011 to 1.076, p = 0.008). We then estimated the C statistic of Framingham risk alone. Separate incorporation of each biomarker into the Framingham risk model showed that all biomarkers increased the C statistic for prediction of cardiovascular events (C statistics: Framingham risk alone 0.636, Framingham risk + BNP 0.695, Framingham risk + hsCRP 0.696, and Framingham risk + EMP 0.682) (Table 4). Moreover, we examined the additive usefulness of EMP in multiple biomarkers strategy based on Framingham risk and BNP, hsCRP, or both. EMP increased the C statistics in multiple biomarkers strategy (C statistics: Framingham risk + BNP 0.695, Framingham risk + BNP + EMP 0.741; Framingham risk + hsCRP 0.696, Framingham risk + hsCRP + EMP 0.734; and Framingham risk + BNP + hsCRP 0.732, Framingham risk + BNP + hsCRP + EMP 0.763) (Table 4). The p value for the Schoenfeld's tests indicated that proportional hazards assumptions were appropriate (p = 0.70). We also confirmed good calibration for the model in patients at high risk for CHD by Grønnesby and Borgan (23) statistics (p = 0.34). Furthermore, models that included all biomarkers had better global fit than models with only Framingham risk, as evaluated by the likelihood ratio test (p = 0.02).

We examined the effect modification of interaction among all biomarkers and found that there was an interaction term between EMP and hsCRP (p = 0.03).

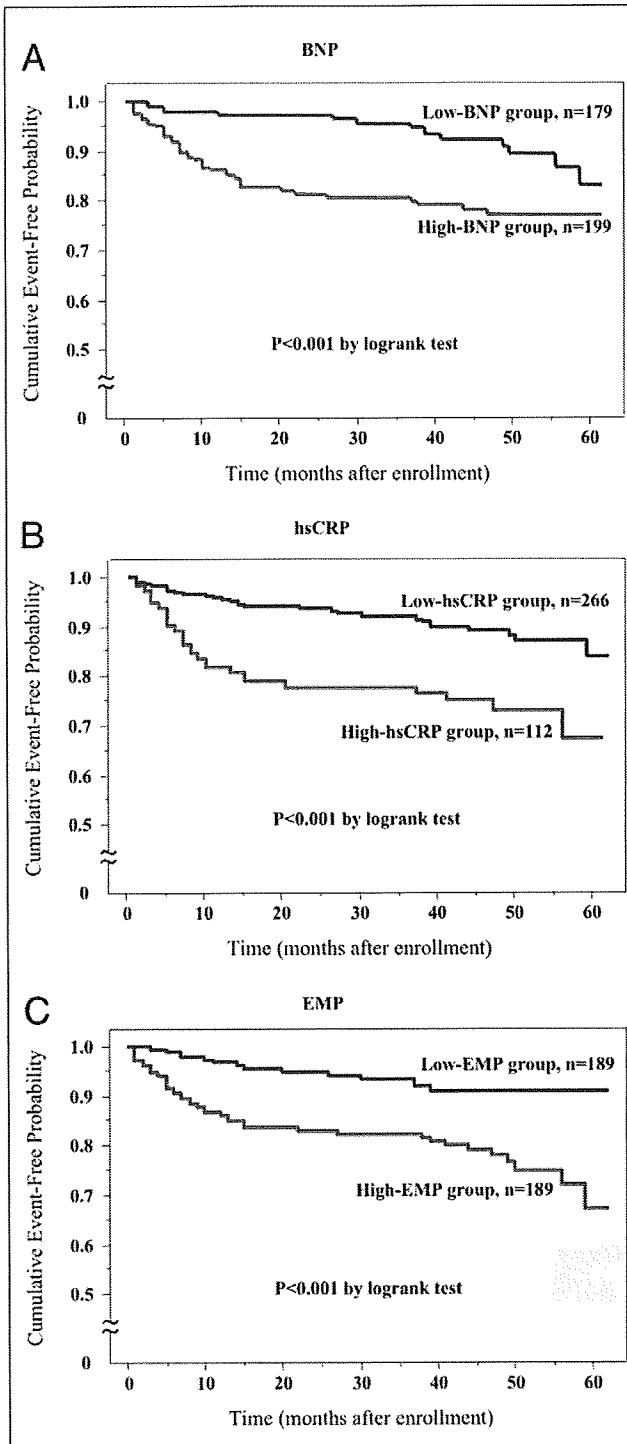

Figure 3 Kaplan-Meier Analysis for the Probability of Cardiovascular Events

(A to C) Based on each cutoff point of B-type natriuretic peptide (BNP), high-sensitivity C-reactive protein (hsCRP), and CD144 endothelium-derived microparticles (EMP). BNP 52.6 pg/ml, hsCRP 2.0 mg/l, and CD144-EMP 0.569 × 10^6/ml.

Discussion

We demonstrated that circulating plasma levels of CD144-EMP in patients at high risk for CHD were independent predictors of future cardiovascular events. We also found that the addition of multiple biomarkers, including endothelial dysfunction, as assessed by CD144-EMP, to the Framingham risk model improved classification of risk, as evidenced by a substantial increase in the C statistics. Thus, quantitative evaluation of cardiovascular risk leading to atherothrombogenic complications from multiple aspects that include endothelial dysfunction can be clinically useful and valuable in patients at high risk for CHD.

Although the mean age of the study population and combination of biomarkers were issues of concern in the study design, the multiple biomarkers strategy, which is based on adding several biomarkers to the prediction model, including the established risk factors, is useful for risk stratification of cardiovascular events (2,3). It has already been demonstrated that BNP and hsCRP are independent predictors in healthy subjects (24,25) and CHD patients (26,27), and are significant biomarkers that improve C statistics for death and cardiovascular events (2,3). Endo-

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Table 3 Univariate and Multivariate Cox Proportional Hazards Analysis for Cardiovascular Events in Follow-Up Patients

	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	p Value	HR (95% CI)	p Value
Age, per yr	1.045 (1.010–1.080)	0.01	1.042 (1.007–1.080)	0.02
Sex (male)	1.498 (0.845–2.655)	0.17	Not selected	—
Current smoking	0.886 (0.433–1.810)	0.74	Not selected	—
Hypertension	0.808 (0.451–1.447)	0.47	0.616 (0.341–1.112)	0.11
Diabetes mellitus	1.967 (1.150–3.364)	0.01	1.597 (0.922–2.766)	0.10
Body mass index, per kg/m^2	0.975 (0.904–1.052)	0.52	Not selected	—
HDL cholesterol, per mg/dl	0.988 (0.971–1.006)	0.19	Not selected	—
LDL cholesterol, per mg/dl	0.997 (0.989–1.006)	0.53	Not selected	—
eGFR, per ml/min/1.73 m^2	0.982 (0.969–0.995)	0.006	Not selected	—
BNP, quintile increment	1.461 (1.190–1.792)	<0.001	1.242 (1.004–1.536)	0.046
High-sensitivity CRP, quintile increment	1.693 (1.335–2.146)	<0.001	1.468 (1.150–1.875)	0.002
EMP, quintile increment	1.469 (1.203–1.792)	<0.001	1.345 (1.094–1.652)	0.005

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

thelial dysfunction has also been recognized as an independent predictor of future cardiovascular events (5–7). Despite the pathophysiological significance of endothelial dysfunction in cardiovascular medicine, one cannot clinically assess coronary endothelial dysfunction because the available method is complex and invasive. It is probably for this reason that endothelial dysfunction was not incorporated into the multiple biomarkers strategy. In addition to the use of coronary reactivity to acetylcholine or brachial artery flow-mediated vasodilation, endothelial dysfunction can be assessed by measuring circulating levels of intercellular adhesion molecule 1, E-selectin (28), and von Willebrand factor (29). Soluble biomarkers offer the advantage of convenience and quantitative assessment; however, there is little evidence at present that such markers can accurately predict future cardiovascular events. Because the aforementioned molecules can be produced from cells other than endothelial cells such as leukocytes (28) and platelets, we need to identify a highly specific soluble biomarker that reflects endothelial dysfunction and can predict the prognosis of CHD patients.

Microparticles are released from various circulating blood cells and have many pathophysiological properties, as procoagulants and messengers (11). Microparticles detected by CD144 antigens (vascular endothelial cadherin), which are endothelial cell-type specific transmembrane adhesion molecules located only on the endothelium, exist in human plasma and are derived selectively from human endothelial cells, and their plasma levels can be a clinically specific marker for endothelial dysfunction (13,30). In the present study, we used the CD144-EMP assay to quantitate endothelial dysfunction. Although the clinical significance of measurement of microparticles has not been established yet, as stated in the preceding text, the method used for measurement of CD144-EMP is more specific, safe, simple, and rapid. Moreover, the fact that plasma levels of CD144-EMP independently predicted future cardiovascular events in the present study indicates that measurement of plasma CD144-EMP levels could be potentially useful for risk assessment of endothelial dysfunction with potential cardiovascular complications.

Table 4 C Statistics for Cox Proportional Hazards Model to Predict Cardiovascular Events in Follow-Up Patients

Risk Factors and Biomarkers	C Statistic	Increment in C Statistic
Framingham risk	0.636	0.046
Framingham risk + EMP	0.682	
Framingham risk + BNP	0.695	0.046
Framingham risk + BNP + EMP	0.741	
Framingham risk + hsCRP	0.696	0.038
Framingham risk + hsCRP + EMP	0.734	
Framingham risk + BNP + hsCRP	0.732	0.031
Framingham risk + BNP + hsCRP + EMP	0.763	

Biomarkers were incorporated as variables of 5 ingredients that were divided by quintiles. hsCRP = high-sensitivity C-reactive protein; other abbreviations as in Table 1.

Endothelial dysfunction is one component of vulnerable plaques and closely associated with the occurrence of ACS (31). Vulnerable plaques are characterized by a thin fibrous cap with a large lipid core and superficial erosion of the luminal endothelium. Severe endothelial dysfunction may predispose to vulnerable endothelium, and the main feature of endothelial vulnerability is probably endothelial erosion. A vulnerable endothelium can promote atherothrombogenic complications through endothelial erosion, but there are no reliable methods for evaluating the risk of endothelial vulnerability, including endothelial erosion (31,32). Therefore, cardiovascular risk stratification that includes evaluation of endothelial dysfunction is a sound approach. Analysis of the risk in different disease pathways is important, and we propose that evaluation of endothelial dysfunction could be an important and clinically useful strategy. Based on the concept of vascular protection, a specific and quantifiable marker that can monitor endothelial dysfunction is neces-

sary, as is the need to design intensive treatment to improve endothelial dysfunction.

A weak correlation between EMP and hsCRP resulted in statistical modification of interaction between EMP and hsCRP. EMP levels correlated to some extent with various inflammatory markers, because inflammatory cytokines can induce the release of EMP, and the latter, in turn, promote endothelial injury, leading to endothelial dysfunction (11).

Study limitations. One limitation of the present study is the relatively small number of patients in a single center. However, this should result in underestimation, stressing the need for further multicenter studies in a larger population to confirm the present results. There is no consensus about measurement of EMP for assessment of endothelial damage and prothrombotic state at this stage, and microparticles are still used only for research purposes. There is a need to standardize the EMP assay for the development and establishment of routine clinical tests, because measurement of CD144-EMP could be potentially useful for the evaluation of endothelial dysfunction. The number of this study population was not estimated by power calculation. It is effective and necessary to have a plan for the number of patients required for a prospective study.

Conclusions

Endothelial dysfunction leading to cardiovascular complications can be assessed quantitatively by measurement of plasma levels of CD144-EMP. Moreover, a multiple biomarkers strategy that includes endothelial dysfunction assessed by CD144-EMP can provide better risk stratification of cardiovascular events and, hence, more thorough clinical assessment of patients who might benefit from more aggressive treatment strategies that improve prognosis.

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REFERENCES

1. Ridker PM, Brown NJ, Vaughan DE, Harrison DG, Mehta JL. Established and emerging plasma biomarkers in the prediction of first atherothrombotic events. Circulation 2004;109:IV6–19.
2. Wang TJ, Gona P, Larson MG, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. N Engl J Med 2006;355:2631–9.
3. Zethelius B, Berglund L, Sundstrom J, et al. Use of multiple biomarkers to improve the prediction of death from cardiovascular causes. N Engl J Med 2008;358:2107–16.
4. Widlansky ME, Gokce N, Keaney JF Jr., Vita JA. The clinical implications of endothelial dysfunction. J Am Coll Cardiol 2003;42:1149–60.
5. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 2000;101:1899–906.
6. Chan SY, Mancini GB, Kuramoto L, Schulzer M, Frohlich J, Ignaszewski A. The prognostic importance of endothelial dysfunction and carotid atheroma burden in patients with coronary artery disease. J Am Coll Cardiol 2003;42:1037–43.
7. Modena MG, Bonetti L, Coppi F, Bursi F, Rossi R. Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. J Am Coll Cardiol 2002;40:505–10.
8. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002;39:257–65.
9. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation 2007;115:1285–95.
10. Kuvin JT, Karas RH. Clinical utility of endothelial function testing: ready for prime time? Circulation 2003;107:3243–7.
11. Boulanger CM, Amabile N, Tedgui A. Circulating microparticles: a potential prognostic marker for atherosclerotic vascular disease. Hypertension 2006;48:180–6.
12. VanWijk MJ, VanBavel E, Sturk A, Nieuwland R. Microparticles in cardiovascular diseases. Cardiovasc Res 2003;59:277–87.
13. Koga H, Sugiyama S, Kugiyama K, et al. Elevated levels of VE-cadherin-positive endothelial microparticles in patients with type 2 diabetes mellitus and coronary artery disease. J Am Coll Cardiol 2005;45:1622–30.
14. Shet AS. Characterizing blood microparticles: technical aspects and challenges. Vasc Health Risk Manag 2008;4:769–74.
15. Grundy SM, Balady GJ, Criqui MH, et al. Primary prevention of coronary heart disease: guidance from Framingham: a statement for healthcare professionals from the AHA Task Force on Risk Reduction. American Heart Association. Circulation 1998;97:1876–87.
16. Pearson TA, Blair SN, Daniels SR, et al. AHA guidelines for primary prevention of cardiovascular disease and stroke: 2002 update: Consensus Panel Guide to Comprehensive Risk Reduction for Adult Patients Without Coronary or Other Atherosclerotic Vascular Diseases. American Heart Association Science Advisory and Coordinating Committee. Circulation 2002;106:388–91.
17. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2003;26 Suppl 1:S5–20.
18. Imai E, Matsuo S, Makino H, et al. Chronic Kidney Disease Japan Cohort (CKD-JAC) study: design and methods. Hypertens Res 2008;31:1101–7.
19. Omland T, Sabatine MS, Jablonski KA, et al. Prognostic value of B-type natriuretic peptides in patients with stable coronary artery disease: the PEACE trial. J Am Coll Cardiol 2007;50:205–14.
20. Ridker PM, Danielson E, Fonseca FA, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 2008;359:2195–207.
21. Pencina MJ, D'Agostino RB. Overall C as a measure of discrimination in survival analysis: model specific population value and confidence interval estimation. Stat Med 2004;23:2109–23.
22. D'Agostino RB, Russell MW, Huse DM, et al. Primary and subsequent coronary risk appraisal: new results from the Framingham study. Am Heart J 2000;139:272–81.
23. Grønnesby JK, Borgan O. A method for checking regression models in survival analysis based on the risk score. Lifetime Data Anal 1996;2:315–28.
24. Koenig W, Sund M, Frohlich M, et al. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg cohort study, 1984 to 1992. Circulation 1999;99:237–42.
25. Wang TJ, Larson MG, Levy D, et al. Plasma natriuretic peptide levels and the risk of cardiovascular events and death. N Engl J Med 2004;350:655–63.
26. Danesh J, Wheeler JG, Hirschfield GM, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med 2004;350:1387–97.

27. Omland T, Richards AM, Wergeland R, Vik-Mo H. B-type natriuretic peptide and long-term survival in patients with stable coronary artery disease. Am J Cardiol 2005;95:24–8.
28. Meigs JB, Hu FB, Rifai N, Manson JE. Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. JAMA 2004;291:1978–86.
29. Boos CJ, Balakrishnan B, Blann AD, Lip GY. The relationship of circulating endothelial cells to plasma indices of endothelial damage/dysfunction and apoptosis in acute coronary syndromes: implications for prognosis. J Thromb Haemost 2008;6:1841–50.
30. Amabile N, Guerin AP, Leroyer A, et al. Circulating endothelial microparticles are associated with vascular dysfunction in patients with end-stage renal failure. J Am Soc Nephrol 2005;16:3381–8.
31. Naghavi M, Libby P, Falk E, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: part I. Circulation 2003;108:1664–72.
32. Sugiyama S, Kugiyama K, Aikawa M, Nakamura S, Ogawa H, Libby P. Hypochlorous acid, a macrophage product, induces endothelial apoptosis and tissue factor expression: involvement of myeloperoxidase-mediated oxidant in plaque erosion and thrombogenesis. Arterioscler Thromb Vasc Biol 2004;24:1309–14.

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Vital sign triage to rule out diabetic ketoacidosis and non-ketotic hyperosmolar syndrome in hyperglycemic patients

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ABSTRACT

Aims: To develop a prediction algorithm to rule out diabetic ketoacidosis (DKA) and non-ketotic hyperosmolar syndrome (NKHS) based on vital signs for early triage of patients with diabetes.

Methods: The subjects were consecutive adult diabetic patients with hyperglycemia (blood glucose ≥250 mg/dl) who presented at an emergency department. Based on a derivation sample (n = 392, 70% of 544 patients at a hospital in Okinawa), recursive partitioning analysis was used to develop a tree-based algorithm. Validation was conducted using the other 30% of the patients in Okinawa (n = 152, internal validation) and patients at a hospital in Tokyo (n = 95, external validation).

Results: Three risk groups for DKA/NKHS were identified: a high-risk group of patients with glucose >400 mg/dl or systolic blood pressure <100 mmHg; a low risk group of patients with glucose ≤400 mg/dl and normal vital signs (systolic blood pressure ≥100 mmHg, pulse ≤90/min, and respiratory rate ≤20/min); and an intermediate risk group. The prevalences of DKA/NKHS were 2% (derivation set), 0% (internal validation set), and 0% (external validation set) in the low risk group, respectively.

Conclusions: Our algorithm may help DKA/NKHS triage and patients with normal vital signs can be initially triaged as low risk for DKA/NKHS.

1. Introduction

Diabetic ketoacidosis (DKA) is an acute life-threatening complication in type 1 and type 2 diabetes [1–3]. Clinical presentations of DKA are sometimes non-specific and mimic other acute diseases [4], but DKA patients usually present with vital sign disturbances due to hemodynamic and metabolic derangement [5,6]. Tachycardia of DKA is a sign of dehydration, hypovolemia, or accompanying conditions associated with DKA such as infection or electrolyte imbalance [6]. Kussmaul's respiration reflects a deep respiratory effort plus tachypnea [7], and this characteristic respiration in DKA patients leads to compensatory respiratory alkalosis for alleviating metabolic acidosis [5]. Blood pressure in DKA

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patients may also be lower than the normal range when hypovolemia is severe or when sepsis is present. Body temperature may be either higher than the normal range due to accompanying infection or lower in some cases with severe dehydration or advanced sepsis [8].

On the other hand, non-ketotic hyperosmolar syndrome (NKHS), which causes a similar hemodynamic derangement (severe hyperosmolar dehydration without significant ketoacidosis), is relatively rare but can have greater mortality than DKA. Both DKA and NKHS are considered as typical states of diabetic emergency and need urgent diagnosis and management.

Since DKA/NKHS may occur when patients with diabetes have vital sign disturbances, initial triage for ruling-out DKA/NKHS based on vital signs can be performed in an emergency department or outpatient clinic [9]. However, a model is required for rapid evaluation of the risk for DKA/NKHS in patients with diabetes and to assure routine clinical examination for acute conditions. Thus, our objective was to develop a triage model to stratify the risk of DKA/NKHS using initial vital signs and blood glucose concentrations based on simple tests such as the fingerstick glucose test in patients with diabetes presenting at an emergency department.

2. Methods

2.1. Subjects

We conducted medical record reviews for the four-year period from January 2000 to December 2003 at Okinawa Chubu Hospital, a 550-bed community teaching hospital serving a population of 400,000 in Okinawa, Japan; and for the two-year period from January 2005 to December 2006 at St. Luke's International Hospital, a 520-bed community teaching hospital serving a population of 300,000 in Tokyo, Japan. The inclusion criteria were (1) consecutive adult (≥18-year-old) patients at the emergency department, (2) clinical history of diabetes, (3) initial blood glucose concentrations of ≥250 mg/dl (≥13.9 mmol/l) using a fingerstick glucometer (Okinawa Chubu Hospital) or a quick blood glucose test (St. Luke's International Hospital), and (4) blood gas analysis performed for evaluation of the presence of DKA. The study was approved by the Institutional Review Boards of the two hospitals.

2.2. Data collection

Data were collected for demographics, initial vital signs, blood glucose and laboratory tests. In the two hospitals, emergency department nurses receive educational sessions for accurate vital sign measurement before serving in triage roles. Upon patient arrival at the emergency department, triage nurses measured and recorded systolic and diastolic blood pressure, pulse, respiratory rate and body temperature, with the pulse and respiratory rates measured over 30 s and doubled. The triage nurses also routinely measured blood glucose using a fingerstick glucometer for patients with a self-declared history of diabetes.

2.3. Criteria for DKA/NKHS

DKA was judged to be present if a patient met all of the following three criteria: (1) blood glucose ≥250 mg/dl (≥13.9 mmol/l), (2) metabolic acidosis with pH ≤7.30, or a serum bicarbonate concentration ≤18 mequiv./l in arterial or venous blood, and (3) positive ketonemia (elevated concentration of serum acetoacetate or β-hydroxybutyrate). These criteria were based on the working classification established by Kitabchi et al. [4,10,11]. NKHS was clinically diagnosed by the presence of severe hyperglycemia and serum hyperosmolarity and absence of ketonemia.

2.4. Statistical analysis

For comparison of demographics, vital signs and blood glucose between DKA/NKHS and non-DKA/NKHS patients, a t-test was used for continuous data and a chi-square test for binary data. To develop the triage model, we used a split-sample strategy for data from Okinawa Chubu Hospital, in which 70% of the patients were randomly sampled and used to derive an algorithm using recursive partitioning analysis based on vital sign variables and blood glucose concentrations, and the remaining 30% were used as an internal validation set [12]. Data from St. Luke's International Hospital were used for external validation of the algorithm.

We used a recursive partitioning analysis to build a prediction algorithm. This technique generates a classification tree with series of binary splits, in which patients are assigned to mutually exclusive subgroups according to a set of predictors. When applied to our group data, each binary split in a tree produced two subgroups, one containing a relatively high proportion of DKA/NKHS patients, and the other, a relatively high proportion of non-DKA/NKHS patients. We then interpreted the combination of these binary splits as a prediction algorithm for classifying patients according to the probability of DKA/NKHS.

Entropy, a measure of impurity of a node, was used as the node splitting criterion to identify the cutoff point for the best separation of the two subgroups. This index selects the partition with the greatest purity by measuring the amount of variance in the proportion of DKA/NKHS patients between each potential pair from the partition. It reaches a minimum value when only one class is present at a subgroup node. The partitioning starts after evaluating each predictor for its potential to separate subgroups and selects the best predictor with the most pure division for the first split. This procedure is then repeated for each of the two subgroups that are generated from the first split, again evaluating all potential cutoff points of each variable to identify the predictor that provides the best separation.

In the recursive partitioning analysis, vital sign data were grouped using clinically reasonable cutoff points: 90, 100, 110 and 120 mmHg for systolic blood pressure; 80, 90, 100 and 110/min for pulse rate; and 20, 22, 24 and 26/min for respiratory rate. Blood glucose concentrations were grouped using cutoff levels of 350, 400, 450 and 500 mg/dl. The model outcome was the prevalence of DKA/NKHS among terminal nodes. Entropy criteria were used for node splitting to reduce node impurity [13]. Several terminal nodes were grouped into three risk levels

(high, intermediate and low) based on the DKA/NKHS prevalence of each node. Since our goal was to generate a triage rule that would not miss DKA/NKHS among diabetic patients, the misclassification cost for labeling DKA/NKHS patients as non-DKA/NKHS was set at 15 times higher than that for labeling non-DKA/NKHS patients as DKA/NKHS. Varying misclassification costs in this manner tends to result in a final model with higher sensitivity [13].

DKA/NKHS prevalence was evaluated within a 95% confidence interval (CI) in each classified group and the characteristics of the final model were assessed by calculating the sensitivity, specificity, positive and negative predictive value, and positive and negative likelihood ratio with 95% CIs. An exact binomial method was used to obtain all 95% CIs. CART v. 5.0 (Salford Systems, San Diego, CA) was used for recursive partitioning analysis and SAS v. 9.1 (SAS Institute, Cary, NC) for general statistics. All p-values were two-sided and $p < 0.05$ was considered to be statistically significant.

3. Results

Data for 544 patients from Okinawa Chubu Hospital were used in derivation of the algorithm or for internal validation. The mean age of the patients was 59 years old and 287 (53%) were male. Among these patients, 134 (24.6%) met the criteria for DKA/NKHS (120 DKA and14 NKHS). The mean age of the DKA/NKHS patients (53 years old) was significantly lower than that (62 years old) of the non-DKA/NKHS patients ($p < 0.001$). The DKA/NKHS patients included a significantly higher percentage of males ($p = 0.001$) and had a higher median glucose concentration (498 vs. 399 mg/dl, $p < 0.001$) compared to the non-DKA/NKHS patients. In the non-DKA/NKHS patients, the common clinical conditions based on diagnostic records by physicians at the emergency department ($n \geq 10$) included respiratory disease ($n = 51$), musculoskeletal disorder ($n = 31$), urologic disease ($n = 30$), vertigo or dizziness ($n = 20$), non-specific hyperglycemia ($n = 20$), non-specific abdominal pain ($n = 18$), soft-tissue infection ($n = 14$), stroke ($n = 13$), coronary artery disease ($n = 12$), heart failure ($n = 11$) and neuropathic pain ($n = 10$).

Data for 95 patients from St. Luke's International Hospital were used for external validation of the algorithm. The mean age of the patients was 69 years old (range, 21–106), 45 (46%) were men, and 17 (18%) met the criteria for DKA/NKHS (12 DKA and 5 NKHS). The mean age of the DKA/NKHS patients (63 years old) did not differ significantly from that (71 years old) of the non-DKA/NKHS patients ($p = 0.15$), and there was no gender difference between the DKA/NKHS and non-DKA/NKHS patients from St. Luke's International Hospital ($p = 0.604$).

Demographics/laboratory data and vital signs of DKA/NKHS and non-DKA/NKHS patients are shown in Tables 1 and 2, respectively. At Okinawa Chubu Hospital, DKA/NKHS patients had higher pulse and respiratory rates and lower systolic and diastolic blood pressure compared to non-DKA/NKHS patients. There was no difference in body temperature between the groups. At St. Luke's International Hospital, DKA/NKHS patients had a significantly greater pulse rate and lower systolic/diastolic blood pressure, but the respiratory rate did not differ between the DKA/NKHS and non-DKA/NKHS groups.

Recursive partitioning analysis generated four classification splits for the high, intermediate and low risk groups

Table 1 – Demographic and laboratory data in patients with and without DKA/NKHS[a].

	DKA/NKHS ($n = 134$)	Non-DKA/NKHS ($n = 410$)	p-Value
Okinawa Chubu Hospital (derivation or internal validation, $N = 544$)			
Age, yr	53 ± 18	62 ± 17	<0.001
Male gender, n (%)	87 (65)	200 (49)	0.001
Glucose, mg/dl	498 ± 163	399 ± 126	<0.001
BUN, mg/dl	44 ± 27	17 ± 10	<0.001
Creatinine, mg/dl	2.7 ± 2.0	1.2 ± 1.1	<0.001
Arterial pH	7.20 ± 0.16	7.41 ± 0.09	<0.001
Arterial pCO_2, mmHg	23 ± 11	37 ± 7.9	<0.001
Arterial bicarbonate, mequiv./l	11 ± 7.7	24 ± 5.2	<0.001
	DKA/NKHS ($n = 17$)	Non-DKA/NKHS ($n = 78$)	p-Value
St. Lukes International Hospital (external validation, $N = 95$)			
Age, yr	63 ± 22	71 ± 14	0.15
Male gender, n (%)	7 (41)	38 (49)	0.57
Glucose, mg/dl	759 ± 287	364 ± 120	<0.001
BUN, mg/dl	39 ± 24	32 ± 20	0.16
Creatinine, mg/dl	1.4 ± 0.63	1.4 ± 1.7	0.86
Arterial pH	7.25 ± 0.11	7.37 ± 0.14	0.002
Arterial pCO_2, mmHg	26 ± 12	40 ± 14	<0.001

DKA, diabetic ketoacidosis; NKHS, non-ketotic hyperosmolar syndrome. BUN, blood urea nitrogen.
[a] Values are shown as mean ± standard deviation when not specified otherwise.

Table 2 – Vital signs between patients with and without DKA/NKHS[a].

	DKA/NKHS (n = 134)	Non-DKA/NKHS (n = 410)	p-Value
Okinawa Chubu Hospital (derivation or internal validation, N = 544)			
SBP, mmHg	116 ± 37	138 ± 29	<0.001
DBP, mmHg	60 ± 27	73 ± 15	<0.001
PR, /min	109 ± 21	92 ± 17	<0.001
RR, /min	27 ± 8	22 ± 4	<0.001
BT, °C	36.7 ± 1.2	36.8 ± 1.6	0.95
	DKA/NKHS (n = 17)	Non-DKA/NKHS (n = 78)	p-Value
St. Lukes International Hospital (external validation, N = 95)			
SBP, mmHg	114 ± 19	139 ± 35	0.003
DBP, mmHg	56 ± 25	73 ± 24	0.011
PR, /min	111 ± 14	99 ± 23	0.01
RR, /min	23 ± 7	21 ± 6	0.285
BT, °C	36.8 ± 0.9	36.9 ± 1.0	0.874

SBP, systolic blood pressure; DBP, diastolic blood pressure; PR, pulse rate; RR, respiratory rate; BT, body temperature; DKA, diabetic ketoacidosis; NKHS, non-ketotic hyperosmolar syndrome.
[a] Values are shown as mean ± standard deviation.

(Fig. 1). The high-risk group included patients with glucose concentrations >400 mg/dl (first split); and of the remaining patients, those with systolic blood pressure <100 mmHg (second split). Among the non-high-risk patients, the intermediate risk group included patients with pulse rate >90/min (third split), and of the remaining patients, those with a respiratory rate >20/min (fourth split). Patients not meeting any of these criteria were placed in the low risk group.

The prevalence of DKA/NKHS in the low, intermediate and high-risk groups is shown in Table 2. In the low risk group, there were two patients with DKA/NKHS [2%; 95% confidence interval (CI), 0–10%] in the derivation set, none in the internal validation set (0%; 95% CI, 0–8%), and none in the external validation set (0%; 95% CI, 0–21%). In contrast, in the high-risk group, there were 75 patients with DKA/NKHS (50%; 95% CI, 42–59%) in the derivation set, 21 in the internal validation set (51%; 95% CI, 35–67%), and 16 in the external validation set (36%; 95% CI, 22–52%).

Classification characteristics for diabetic ketoacidosis between high and intermediate-low risk groups and between high-intermediate and low risk groups are shown in Table 3. Using the cutoff point for high vs. intermediate-low risk gave specificities of 75% (derivation), 83% (internal validation), and 64% (external validation), whereas the cutoff point for high-intermediate vs. low risk gave sensitivities of 98% (derivation) and 100% (both internal and external validation). Overall, the relatively low specificity was a trade-off for high sensitivity in this model.

The model misclassified two patients with DKA/NKHS in the derivation set into the low risk group. These patients were

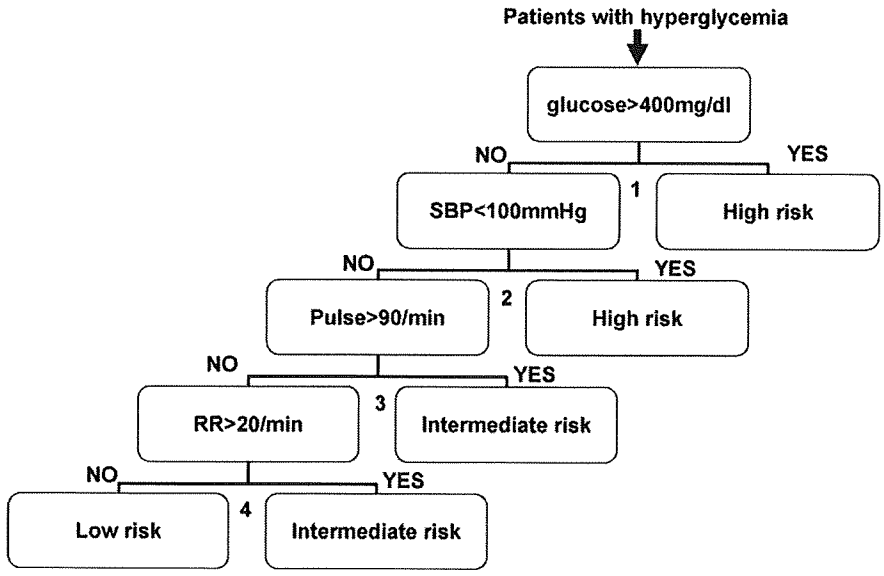

Fig. 1 – Triage algorithm for classifying diabetic patients into three different risk groups. Abbreviations: SBP, systolic blood pressure; RR, respiratory rate. The numbers indicate the classification splits.

Table 3 – Prevalence of DKA/NKHS in risk groups in each population set.

Risk group	No. of patients, *n*	Patients with DKA/NKHS, *n*	Prevalence of DKA/NKHS, %	(95% CI)
Derivation set (*N* = 392)				
High risk	149	75	50	(42–59)
Intermediate risk	174	23	13	(9–19)
Low risk	69	2	2	(0–10)
Internal validation set (*N* = 152)				
High risk	41	21	51	(35–67)
Intermediate risk	77	13	16	(9–27)
Low risk	34	0	0	(0–8)
External validation set (*N* = 95)				
High risk	44	16	36	(22–52)
Intermediate risk	35	1	3	(0–15)
Low risk	16	0	0	(0–21)

CI, confidence interval; DKA, diabetic ketoacidosis; NKHS, non-ketotic hyperosmolar syndrome.

Table 4 – Classification characteristics of the algorithm for DKA/NKHS in each population set.

Characteristic	High-risk group vs. intermediate-low risk group	High-intermediate risk group vs. low risk group
Derivation set (*N* = 392)		
Sensitivity, % (95% CI)	75 (65–83)	98 (93–100)
Specificity, % (95% CI)	75 (69–80)	23 (18–28)
Internal validation set (*N* = 152)		
Sensitivity, % (95% CI)	62 (44–79)	100 (92–100)
Specificity, % (95% CI)	83 (75–89)	29 (21–38)
External validation set (*N* = 95)		
Sensitivity, % (95% CI)	94 (71–100)	100 (80–100)
Specificity, % (95% CI)	64 (52–75)	21 (12–31)

CI, confidence interval; DKA, diabetic ketoacidosis; NKHS, non-ketotic hyperosmolar syndrome.

a 69-year-old woman with schizophrenia and blood pressure 140/70 mmHg, pulse rate 84/min, and respiratory rate 20/min; and a 62-year-old woman with hip fracture and blood pressure 110/60 mmHg, pulse rate 72/min, and respiratory rate 20/min. Therefore, these patients had respiratory rates close to the cutoff point between the low and intermediate risk groups. The model did not misclassify any DKA/NKHS patients into the low risk group in the internal and external validation sets (Table 4).

4. Discussion

The results show that our model could be helpful for DKA/NKHS triage in an emergency department. Based on common data such as vital signs and blood glucose, the model classifies diabetic patients into three risk groups for DKA/NKHS. Patients with diabetes can be triaged safely as low risk when they have blood glucose ≤400 mg/dl and normal vital signs, whereas patients in the high-risk group have about a 50% probability for DKA/NKHS. Our risk-averse model seems to be clinically reasonable, since it is important to avoid false negative triage in assessment of DKA/NKHS, which is potentially life-threatening and requires early detection and aggressive treatment [9,14].

Our study followed mostly standard methodology for developing clinical prediction rules [19]. We defined the outcome clearly and used common clinical data so that a future prospective study can be performed to determine the generalizability of the model. The model is also clinically reasonable since it is compatible with the pathophysiology of DKA/NKHS. Furthermore, the classification algorithm has a simple structure and is easy for physicians and emergency department staff to use, as illustrated in Fig. 1. Physicians have long recognized the importance of vital sign observations and vital sign measurement has proven to be useful for detecting serious diseases during triage in emergency departments [15,16]. However, these studies have focused on acute general conditions, rather than individual diseases, and our study may be the first to provide evidence on the important role of vital sign measurement for DKA/NKHS triage.

In diabetic patients with suspected DKA, blood gas analysis is the test of choice [17,18], since DKA can be excluded based on a normal pH and normal bicarbonate concentration. However, use of our simple triage algorithm may reduce the immediate need for blood gas testing for low risk patients with diabetes. Our algorithm may also have roles outside hospital emergency departments. Depending on the local health care structure, private outpatient clinics may not have technical equipment for blood gas testing, but glucose analyzer kits are usually available in these clinics. The triage algorithm may help to decide whether to transfer diabetic patients to an emergency department for suspected DKA/NKHS. Additionally, emergency medical technicians can be educated in the use

of the triage algorithm to determine the severity of hyperglycemia, as well as the risk of DKA/NKHS.

There is a debate about the reliability of noninvasive vital sign measurements, since the reproducibility of these measurements in a clinical setting by trained observers can show significant inter-observer variability [20]. Thus, this inherent variability should be recognized through careful interpretation of vital signs, especially for respiratory rate measurement. However, another recent study showed improved reliability of vital signs when measured by well-trained nurses in a triage booth in an emergency department [21]. Thus, vital sign measurements by well-trained nurses appear to be reliable. Future studies may be needed for analyzing reproducibility of vital signs measurements in patients with DKA/NKHS.

There may be a selection bias in this study, since we excluded patients who did not undergo blood gas testing based on the decision of an emergency physician. However, the vital signs of these patients were usually within or close to normal limits due to mild conditions, which led the physician to judge them unlikely to have DKA/NKHS, especially for patients with mild hyperglycemia of about 250–300 mg/dl [22,23]. Thus, we suspect that most of these patients would have fallen into the low risk category in our model and that none would have developed DKA/NKHS. Therefore, exclusion of these patients should not change the results significantly. Another limitation in this study is that we could not collect data for co-morbidity of diabetes and precipitating factors for DKA/NKHS. The presence of these factors in patients with diabetes can lead to the greater risk for DKA/NKHS. Future studies are needed for incorporating these factors for developing a prediction model for DKA/NKHS.

In summary, we developed a simple and sensitive triage algorithm for classification of patients with diabetes into risk-stratified groups for DKA/NKHS. Our study reinforces the important medical tradition of evaluating vital signs. To effectively triage patients with diabetes at risk for DKA/NKHS, the algorithm should be used in combination with individualized clinical judgments by health care teams.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

[1] K. Ellemann, J.N. Soerensen, L. Pedersen, B. Edsberg, O.O. Andersen, Epidemiology and treatment of diabetic ketoacidosis in a community population, Diabetes Care 7 (1984) 528–532.
[2] O. Pinhas-Hamiel, L.M. Dolan, P.S. Zeitler, Diabetic ketoacidosis among obese African-American adolescents with NIDDM, Diabetes Care 20 (1997) 484–486.
[3] J. Valabhji, M. Watson, J. Cox, C. Poulter, C. Elwig, R.S. Elkeles, Type 2 diabetes presenting as diabetic ketoacidosis in adolescence, Diabet. Med. 20 (2003) 416–417.
[4] A.E. Kitabchi, B.M. Wall, Diabetic ketoacidosis, Med. Clin. North Am. 79 (1995) 9–37.
[5] J.Y. Guh, Y.H. Lai, L.K. Yu, S.J. Shin, J.H. Tsai, Evaluation of ventilatory responses in severe acidemia in diabetic ketoacidosis, Am. J. Nephrol. 17 (1997) 36–41.
[6] M.R. Burge, N. Garcia, C.R. Qualls, D.S. Schade, Differential effects of fasting and dehydration in the pathogenesis of diabetic ketoacidosis, Metabolism 50 (2001) 171–177.
[7] A. Minagar, W.J. Weiner, Adolf Kussmaul and his respiratory sign, J. Med. Biogr. 9 (2001) 81–183.
[8] J.M. Guerin, P. Meyer, J.M. Segrestaa, Hypothermia in diabetic ketoacidosis, Diabetes Care 10 (1987) 801–802.
[9] D.S. Bell, J. Alele, Diabetic ketoacidosis. Why early detection and aggressive treatment are crucial, Postgrad. Med. 101 (1997) 193–198, 203–204.
[10] A.E. Kitabchi, G.E. Umpierrez, M.B. Murphy, E.J. Barrett, R.A. Kreisberg, J.I. Malone, et al., Management of hyperglycemic crises in patients with diabetes, Diabetes Care 24 (2001) 131–153.
[11] A.E. Kitabchi, G.E. Umpierrez, M.B. Murphy, E.J. Barrett, R.A. Kreisberg, J.I. Malone, et al., Hyperglycemic crises in diabetes, Diabetes Care 27 (Suppl. 1) (2004) S94–102.
[12] L. Breiman, J. Friedman, R. Olshen, C. Stone, Classification and Regression Trees, Chapman & Hall CRC Press LLC, Boca Raton, FL, 1984.
[13] H. Zhang, B. Singer, Recursive Partitioning in the Health Sciences, Springer-Verlag, New York, 1999.
[14] R.D. Hardern, N.D. Quinn, Emergency management of diabetic ketoacidosis in adults, Emerg. Med. J. 20 (2003) 210–213.
[15] R.D. Waldrop, D.E. Harper, C. Mandry, Prospective assessment of triage in an urban emergency department, South Med. J. 90 (1997) 1208–1212.
[16] R.J. Cooper, D.L. Schriger, H.L. Flaherty, E.J. Lin, K.A. Hubbell, Effect of vital signs on triage decisions, Ann. Emerg. Med. 39 (2002) 223–232.
[17] Y. Gokel, S. Paydas, Z. Koseoglu, N. Alparslan, G. Seydaoglu, Comparison of blood gas and acid–base measurements in arterial and venous blood samples in patients with uremic acidosis and diabetic ketoacidosis in the emergency room, Am. J. Nephrol. 20 (2000) 319–323.
[18] Z. Hassan, D.M. Subramonyam, S. Thakore, Venous blood gas in adult patients with diabetic ketoacidosis, Emerg. Med. J. 20 (2003) 363–364.
[19] A. Laupacis, N. Sekar, I.G. Stiell, Clinical prediction rules. A review and suggested modifications of methodological standards, JAMA 277 (1997) 488–494.
[20] Z.V. Edmonds, W.R. Mower, L.M. Lovato, R. Lomeli, The reliability of vital sign measurements, Ann. Emerg. Med. 39 (2002) 233–237.
[21] A. Worster, L. Elliott, T.J. Bose, E. Chemeris, Reliability of vital signs measured at triage, Eur. J. Emerg. Med. 10 (2003) 108–110.
[22] M.F. Delaney, A. Zisman, W.M. Kettyle, Diabetic ketoacidosis and hyperglycemic hyperosmolar nonketotic syndrome, Endocrinol. Metab. Clin. North Am. 29 (2000) 683–705, V.
[23] J.L. Chiasson, N. Aris-Jilwan, R. Belanger, S. Bertrand, H. Beauregard, J.M. Ekoé, et al., Diagnosis and treatment of diabetic ketoacidosis and the hyperglycemic hyperosmolar state, CMAJ 168 (2003) 859–866.

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HbA1c to Detect Diabetes Mellitus in Healthy Adults: When Should We Re-check?

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Objective: To evaluate the optimal interval for re-checking HbA1c levels below diagnostic threshold of 6.5% for healthy adults.

Research Design and Methods: Retrospective cohort study. Participants were 16,313 apparently healthy Japanese adults not taking glucose-lowering medications at baseline. Annual HbA1c measures from 2005 to 2008 at the Center for Preventive Medicine, a community teaching hospital in Japan, estimated cumulative incidence of diabetes.

Results: Mean age (SD) of participants was 49.7 (12.3) years and 53% were male. Mean (SD) of HbA1c at baseline were 5.4 % (0.5). At three years, for those with HbA1c at baseline of less than 5.0 %, 5.0-5.4%, 5.5-5.9%, and 6.0-6.4%, cumulative incidence (95%CI) was 0.05% (0.001- 0.3), 0.05% (0.01 – 0.11), 1.2% (0.9 – 1.6), and 20% (18 – 23), respectively.

Conclusions: In those with an $HbA1_C$ under 6.0%, rescreening at intervals shorter than three years identifies few individuals (~1% or less) with an $HbA1_C \geq 6.5\%$.

Since fasting and post glucose challenge levels to predict risk of diabetic retinopathy, blood glucose levels have been the international standard for diagnosis (1). Recently a shift to haemoglobin A1c (HbA1c) for diagnosis has been proposed because HbA1c integrates longer term glucose levels and has better the pre-analytic stability (2).

The proposed diagnostic threshold of 6.5% was based on retinopathy risk at different levels of HbA1c (2). However, optimal frequency for repeating HbA1c has not been determined (3).

We used a large, longitudinal data set to explore the value of repeating HbA1c at different intervals to identify subjects who might progress above the threshold (≥6.5%).

METHODS

Study Participants. Between January and December 2005, we consecutively enrolled all adults (>20 years old) attending the Center for Preventive Medicine at St. Luke's International Hospital in Tokyo, Japan for the health check program. The program promotes early detection of chronic diseases and disease risk factors. We excluded people who took glucose-lowering medications at baseline

Data collection. We extracted data from records of people undergoing annual health checks from January 2005 to December, 2008. We excluded those without health check in year 1, 2 or 3. St. Luke's International Hospital Institutional Review Board approved the study.

Measurements. The annual health check collected demographic information and medical history with an initial evaluation (vital signs and laboratory data). Laboratory data included HbA1c, fasting plasma glucose (FPG), and lipids (total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol. Venous blood was drawn after an overnight fast and analyzed at the Central Laboratory at St. Luke's International Hospital. HbA1c assays were performed by an Automated Glycohemoglobin Analyzer HLC-723G (Tosoh, Tokyo, Japan) with a coefficient of variation (CV) of <1.34% and certified by the National Glycohemoglobin Standardization Program (4). We classified as possible diabetes persons with either a single measured HbA1c≥6.5% (5, 6) or self-reported

commencement of glucose-lowering treatment. As a sensitivity analysis, we used FPG≥126 mg/dl as one of the diagnostic criteria.

Statistical methods. Analyzes used SPSS software 15.0J (SPSS Japan, Tokyo, Japan), except 95% confidence intervals (CI) which were based on an exact binominal (7) using Stata version 10 (STATA Corp, College Station, TX).

RESULTS

From January 2005 to July 2008, 16,313 people of the enrolled population of 39,284 underwent annual checks. Mean age (SD) of participants was 49.7 (12.3) years; 53% were male. The mean (SD) body mass index was 22.5 (3.2) kg/m^2; fasting plasma glucose was 99.2 (12.7) mg/dl; HbA1c at baseline was 5.4 (0.5) %; total, LDL cholesterol, and HDL cholesterol level at baseline were 204.3 (33.8) mg/dl, 117.6 (29.7) mg/dl, and 62.4 (15.8) mg/dl, respectively; and systolic blood pressure 119 (18) mmHg and diastolic blood pressure 73 (11) mmHg. The trends of mean HbA1c levels for the entire cohort from 2005 to 2008 slightly increased over the three years (0.05% per year). The demographic characteristics of nonparticipants and participants were similar.

At 3 years the cumulative incidence of diabetes was 3.2 % (95%CI: 3.0 – 3.4). However, this varies greatly by initial level of HbA1c. At three years, for those with HbA1c of less than 5.0 %, 5.0-5.4%, 5.5-5.9%, and 6.0-6.4% at baseline, cumulative incidence (95%CI) was 0.05% (0.001- 0.3%), 0.05% (0.01 – 0.11%), 1.2% (0.9 – 1.6%), and 20% (18 – 23%), respectively and adding FPG≥126 mg/dl to the diagnostic criteria showed the similar results (Figure). Logistic regression suggested that only BMI (Odds Ratio 1.14/kg/m^2) and FPG (Odds Ratio 1.06/mg/dl) added to the baseline HbA1c; age, gender, SBP, and LDL were non-significant. The average coefficient of variation (CV) of $HbA1_C$ stratified by baseline HbA1c was 2.7% and did not differ among these subgroups.

DISCUSSION

This study confirms that the rise in HbA1c in a non-diabetic population is slow. Participants who are well under the diagnostic threshold of HbA1c of 6.5% are unlikely to exceed this within several years of follow-up.

Much of the increased detection of diabetes in those with a higher baseline HbA1c was at one year, and may be attributable to measurement error and short term variation in HbA1c. The CV (including within subject variation) varies between 2 and 5 % (8); a CV of 5% would mean a 95% measurement interval of a single HbA1c in this range would be +/- 0.6%. This degree of variation would lead to some individuals having sequential tests from just below to just above 6.5%. Although the variation could occur at all time points, this is much less likely in the 5.0-5.9% range.

Our findings echo the slow rise of HbA1c found in trials with diabetic patients. For example, in the UKPDS study the patients on diet alone had a rise of less than 0.2% per year (9). Our non-diabetic cohort had an even lower average change in HbA1c of 0.05% per year.

This study has several limitations. First, the follow-up is incomplete as not all participants came back ever year. This could be addressed by other analysis, such as a linear mixed model. However, any bias would be likely to favour those developing diabetes to re-attend. Second, a few participants (1.1%) began taking glucose-lowering drugs, but this is unlikely to make a large difference to our conclusions. Third, our data are from one institution in Tokyo, Japan, might not generalize to other populations. For example, adult mean BMI levels of 22-23 kg/m^2 are found in Africa and Asia, while levels of 25-27 kg/m^2 are prevalent across North America

and Europe and then BMI level could be related to the cumulative incidence of diabetes. Finally, although the ADA criteria recommend a repeat HbA1c test to confirm the diagnosis of type 2 diabetes (2), our study included only a single measurement of HbA1c.

In conclusion, for the purpose of detecting new cases of diabetes, in those with an initial HbA1c under 6.0%, rescreening at intervals shorter than three years identifies few individuals (~1% or less) with an HbA1c $\geq$ 6.5%. At $HbA1_C \geq 6\%$, rescreening even at a 1- year interval would be reasonable strategy to identify disease.

***Author Contributions*:** O.T wrote manuscript, AF wrote manuscript, PG wrote and reviewed manuscript and, TS and FT contributed discussion.

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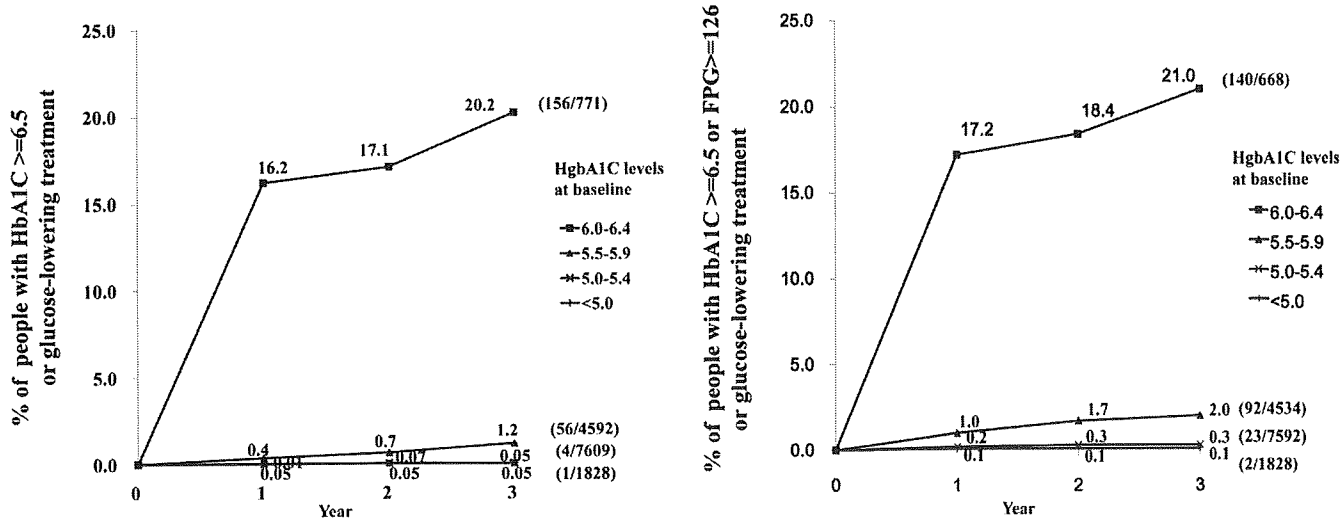
Conflict of Interest: None

REFERENCES

1. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*. 1997;20(7):1183-97.
2. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes Care*. 2009;32(7):1327-34.
3. Kahn R, Alperin P, Eddy D, Borch-Johnsen K, Buse J, Feigelman J, Gregg E, Holman RR, Kirkman MS, Stern M, Tuomilehto J, Wareham NJ. Age at initiation and frequency of screening to detect type 2 diabetes: a cost-effectiveness analysis. *Lancet*. 2010;375(9723):1365-74.
4. Terreni A, Paleari R, Caldini A, Ognibene A, Mosca A, Messeri G. Evaluation of the analytic performances of the new HPLC system HLC-723 G7 for the measurement of hemoglobin A1c. *Clin Biochem*. 2003;36(8):607-10.
5. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2010;33 Suppl 1:S62-9.
6. Bloomgarden ZT. A1C: recommendations, debates, and questions. *Diabetes Care*. 2009;32(12):e141-7.
7. Clopper SJ, Pearson ES. The use of confidence of fiducial limits illustrated in the case of the binominal. *Biometrika*. 1932;26:404-13.
8. Schwartz KL, Monsur JC, Bartoces MG, West PA, Neale AV. Correlation of same-visit HbA1c test with laboratory-based measurements: a MetroNet study. *BMC Fam Pract*. 2005;6:28.
9. United Kingdom Prospective Diabetes Study (UKPDS): Relative efficacy of randomly allocated diet, sulphonylurea, insulin, or metformin in patients with newly diagnosed non-insulin dependent diabetes followed for three years. *BMJ*. 1995;310(6972):83-8.

Legends

Figure: Percent of patients at annual re-checks with HbA1C above 6.5% or FPG above 126 mg/dl (by Baseline HbA1c)



Abbreviations: HbA1c, haemoglobin A1c, FPG, fasting plasma glucose

HEART

Lipid re-screening: what is the best measure and interval?

Osamu Takahashi, Paul P Glasziou, Rafael Perera, et al.

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Lipid re-screening: what is the best measure and interval?

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ABSTRACT

Objectives To estimate the long-term true change variation ('signal') and short-term within-person variation ('noise') of the different lipid measures and evaluate the best measure and the optimal interval for lipid re-screening.

Design Retrospective cohort study from 2005 to 2008.

Setting A medical health check-up programme at a centre for preventive medicine in a teaching hospital in Tokyo, Japan.

Participants 15 810 apparently healthy Japanese adults not taking cholesterol-lowering drugs at baseline, with a mean body mass index of 22.5 kg/m^2 (SD 3.2).

Main outcome measures Annual measurement of the serum total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and calculation of the ratio of TC/HDL and LDL/HDL. Measurement of the ratio of long-term true change variation ('signal') to the short-term within-person variation ('noise') for each measure.

Results At baseline, participants (53% male) with a mean age of 49 years (range 21–92) and a mean TC level of 5.3 mmol/l (SD 0.9 mmol/l) had annual check-ups over 4 years. Short-term within-person variations of TC, LDL, HDL, TC/HDL, and LDL/HDL were 0.12 (coefficient of variation (CV) 6.4%), 0.08 (CV 9.4%), 0.02 (CV 8.0%) mmol^2/l^2, 0.08 (CV 7.9%) and 0.05 (CV 10.6%), respectively. The ratio of signal-to-noise at 3 years was largest for TC/HDL (1.6), followed by LDL/HDL (1.5), LDL (0.99), TC (0.8) and HDL (0.7), suggesting that cholesterol ratios are more sensitive re-screening measures.

Conclusion The signal-to-noise ratios of standard single lipid measures (TC, LDL and HDL) are weak over 3 years and decisions based on these measures are potentially misleading. The ratios, TC/HDL and LDL/HDL, seem to be better measures for monitoring assessments. The lipid re-screening interval should be >3 years for those not taking cholesterol-lowering drugs.

INTRODUCTION

Dyslipidaemia is common in industrialised countries,[1] including Japan,[2] and is an important modifiable risk factor for coronary heart disease (CHD) and cardiovascular disease (CVD).[3–5] Large randomised controlled studies and meta-analysis studies have established that cholesterol-lowering treatment reduces CHD morbidity and mortality,[3 6–8] Thus, cholesterol level screening is important for adults to identify those at risk of CVD and is widely available.[9 10]

Screening the cholesterol level includes interpretation of the initial level and also that of a series of sequential levels over time,[11] in which we should consider the variations of both short-term within-person and long-term among individuals in the population.[12] Lipid guidelines for primary prevention of CVD and CHD recommend a targeting level for lipid level and how to interpret initial measurements; however, they rarely specify subsequent monitoring in primary prevention, and guidelines vary in their recommendation. In the United Kingdom, the National Institute for Clinical Excellence[13 14] for the primary prevention of CVD recommends that people aged 40–74 years without a history of CVD should have total cholesterol (TC) and high-density lipoprotein cholesterol (HDL) measured; however, the optimal interval for these measurements is not provided.

On the other hand, in the United States, the National Cholesterol Education Program in Adults[3] recommends that all adults aged ≥20 years have a fasting lipoprotein profile that includes TC, low-density lipoprotein cholesterol (LDL), HDL and triglycerides once every 5 years. Moreover, the United States Preventive Services Task Force[15] suggests that doctors routinely screen men aged ≥35 years and women aged ≥45 years for lipid disorders. Again, however, the optimal re-screening interval is unclear. Even when guidelines suggest screening intervals, the basis for these suggestions is not provided and no guidelines consider within-person and long-term variation in their re-screening strategies.

Detecting dyslipidaemia and intervening early can reduce the impact but it also has some important drawbacks such as inconvenience and cost; in addition, false-positive results may be obtained that can lead to inappropriate action.[11 16] A good monitoring test should correlate with the final clinical outcomes but also differentiate changes in the condition (signal) from the background of measurement variability (noise).[12] Considering such variations could avoid leading to inappropriate intervention or minimise the harm.[12 17–19] Thus, in choosing test options, the signal-to-noise (S/N) ratio may be a key factor in determining the value of the test for monitoring.[16]

We, therefore, aimed to estimate the variation in long-term true changes in the different lipid profile measures and the short-term within-person variations and thus estimate the S/N ratio in cholesterol screening. Since several previous studies have suggested that lipid ratios (TC/HDL and LDL/HDL) have greater independent predictive values for CHD than serum TC or LDL levels,[20–22] we compared S/N ratio among different types of cholesterol measures, including