Serum Vascular Adhesion Protein-1 Predicts 10-Year Cardiovascular and Cancer Mortality in Individuals With Type 2 Diabetes

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OBJECTIVE—Vascular adhesion protein-1 (VAP-1) participates in inflammation and catalyzes the breakdown of amines to produce aldehyde, hydrogen peroxide, and ammonia. Serum VAP-1 correlates positively with both acute hyperglycemia and diabetes. We conducted a cohort study to evaluate whether serum VAP-1 predicts 10-year survival in type 2 diabetic patients.

RESEARCH DESIGN AND METHODS—Between July 1996 and June 2003, we enrolled 661 type 2 diabetic subjects at National Taiwan University Hospital. Serum VAP-1 in the samples obtained at enrollment was measured by time-resolved immunofluorometric assay. The vital status of all subjects was ascertained by linking their data with computerized death certificates in Taiwan.

RESULTS—The medium follow-up period was 10.4 years. Subjects with serum VAP-1 in the highest tertile had a hazard ratio (HR) of 2.19 (95% CI 1.17–4.11) for all-cause mortality adjusted for age, sex, smoking, history of cardiovascular disease, obesity, hypertension, hemoglobin A_{1c}, diabetes duration, total cholesterol, use of statins, abnormal ankle-brachial index, estimated glomerular filtration rate (eGFR), and proteinuria. The adjusted HRs for logarithmically transformed serum VAP-1 were 5.83 (95% CI 1.17–28.97) for cardiovascular mortality, 6.32 (95% CI 1.25–32.00) for mortality from cardiovascular and diabetic causes, and 17.24 (95% CI 4.57–65.07) for cancer mortality. There were four variables, including age, serum VAP-1, proteinuria, and eGFR, which could enhance mortality prediction significantly.

CONCLUSIONS—Serum VAP-1 can predict 10-year all-cause mortality, cardiovascular mortality, and cancer mortality independently in type 2 diabetic subjects. Serum VAP-1 is a novel biomarker that improves risk prediction over and above established risk factors. ***Diabetes*** **60:993–999, 2011**

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Vascular adhesion protein-1 (VAP-1), discovered by Salmi and Jalkanen (1), is a dual-function protein. Endothelial VAP-1 can act as an adhesion molecule (2) and is involved in leukocyte rolling, adhesion, and transmigration, which are central steps during leukocyte extravasation to sites of inflammation, such as atherosclerotic lesions (3). Another function of VAP-1 is as an enzyme, semicarbazide-sensitive amine oxidase (SSAO). SSAO can catalyze the breakdown of primary amines to produce aldehyde, hydrogen peroxide, and ammonia (4). Hydrogen peroxide is a source of oxidative stress and can contribute to the development of atherosclerotic lesions. Along with aldehyde and glucose, hydrogen peroxide can modify various proteins to generate advanced glycated end products (AGEs), another important factor in the development of atherosclerosis (3). Therefore, as an adhesion molecule and an enzyme, SSAO/VAP-1 can participate in the development of atherosclerosis. Indeed, mice overexpressing VAP-1 in the endothelium have shown increased concentrations of serum AGEs, enhanced leukocyte binding, upregulation of hepatic redox-sensitive proteins, and accelerated atherosclerosis (5).

VAP-1 has a circulating form, which retains its enzymatic function. Although there are two other proteins with SSAO activity, circulating VAP-1, the product of the *AOC3* gene, has been shown to be the main source of SSAO in human serum (6). Serum VAP-1 originates from many tissues. In tissue-specific transgenic mice models, mice overexpressing human *VAP-1* in the endothelium, adipocyte, and smooth muscle have human VAP-1 in serum (7,8), suggesting that these tissues are the sources of serum VAP-1. In addition, in five subjects with hepatic diseases, the concentration of VAP-1 in the hepatic blood was shown to be slightly higher than that in the portal blood, suggesting that the liver may be another source of serum VAP-1 (9), at least in subjects with hepatic diseases. Cell culture studies have shown that human adipose tissue explants and 3T3-L1 adipocytes can secrete VAP-1 into the culture medium, which is inhibited by treatment with a metalloprotease inhibitor (10,11). These experiments suggested that, at least in part, serum VAP-1 comes from the shedding of VAP-1 from the surface of adipocytes through the effect of a metalloprotease. However, the way in which serum VAP-1 is cleared from circulation remains unknown. We have reported that subjects with chronic kidney disease have higher serum VAP-1 (12), suggesting a possibility that serum VAP-1 may be excreted by the kidneys.

Recently, we have shown that serum VAP-1 is elevated in acute and chronic hyperglycemia and in patients with diabetes (13). We also found that serum VAP-1 is associated positively with albuminuria and is elevated in subjects with chronic kidney disease (12); both are risk factors for atherosclerosis. We have also noted that the change in serum VAP-1 after glucose challenge was correlated with systemic oxidative stress, AGEs, and carotid intima-medial thickness, which is an index for atherosclerosis (14). Our results indicated that serum VAP-1 may be a good predictor for cardiovascular mortality. Therefore, we explored this issue in subjects with type 2 diabetes in this prospective cohort study.

RESEARCH DESIGN AND METHODS

Subjects. We performed a prospective cohort study (15). Between July 1996 and June 2003, subjects with type 2 diabetes who were regularly followed up at outpatient clinics at the Division of Endocrinology and Metabolism, National Taiwan University Hospital, Taipei, Taiwan, were invited consecutively to participate in the study. Written informed consent was obtained from each subject, and the study protocol was reviewed and approved by the institutional review board.

Each subject was interviewed and underwent a physical examination by physicians. The history of cardiovascular disease was confirmed from the admission records. Venous blood sampling was performed after overnight fasting for the determination of plasma glucose, hemoglobin A_{1c}, serum total cholesterol, triglyceride, and creatinine by using an automatic analyzer (Toshiba TBA 120FR, Toshiba Medical Systems, Tokyo, Japan). The estimated glomerular filtration rate (GFR) was calculated with the MDRD formula (16). Serum samples were stored at −80°C in a refrigerator before the measurement of VAP-1. Spot urine samples were collected to determine the presence of proteinuria by performing reflectance colorimetry (Arkray AX4280, Kyoto, Japan). The ankle-brachial index (ABI) was measured as the ratio of the systolic blood pressure of the posterior tibial artery or dorsalis pedis and the brachial artery. An ABI greater than 1.3 or less than 0.9 was defined as abnormal (17).

Patients were followed up until 31 December 2008. Vital status, date of death, and cause of death of all subjects were obtained from the computerized death certificates maintained by the Department of Health, Executive Yuan in Taiwan (18). Cause of death was defined by the ICD9 (cardiovascular death, 390–459 and 785; diabetes, 250; malignancy, 140–239 and 288).

Measurement of serum VAP-1. Serum VAP-1 and its SSAO activity are quite stable. When stored properly at −70°C, it has been shown to remain intact after two years (19). Serum VAP-1 was measured by time-resolved immunofluorometric assay as stated previously (12–14). Briefly, the assay used a biotin-conjugated monoclonal anti-human VAP-1 antibody (Biotie Therapies, Finland) as a capturer on a streptavidin-coated microtiter plate. Detection of bound serum VAP-1 was performed using a different europium-conjugated anti-human VAP-1 antibody (Biotie Therapies). The time-resolved fluorescence was measured using a fluorometer (Victor^2 Multilabel Counter, PerkinElmer Finland Oy) at 615 nm. Serum VAP-1 concentration was quantified on the basis of a reference sample of highly purified human serum VAP-1 (Biovian). The R^2 of the standard curves was 0.997–1.000. The intraassay coefficients of variation were 3.7, 5.2, and 8.9% for quality control samples with concentrations 1,000, 500, and 100 ng/mL, respectively. The interbatch coefficients of variation from quality control samples were 4.4–10.2%.

Statistical analysis. Categorical variables were reported as the percentage of patients in the subgroup. The distributions of continuous variables were examined by the Shapiro-Wilk test. Continuous variables distributed normally were presented as means and SD. Continuous variables with skewed distribution were analyzed after logarithmic transformation and were presented as medians (interquartile ranges). The Student t tests, χ^2 tests, and ANOVA were used to identify the differences in clinical characteristics between survivors and nonsurvivors and among subgroups by serum VAP-1 tertile. Survival in subgroups was estimated by the Kaplan-Meier method and was tested by log-rank test. Multivariate Cox proportional hazards models were applied to estimate the hazard ratios (HRs) of predictors for all-cause and disease-specific mortality. Variables significantly associated with survival time in univariate Cox proportional hazards models and clinically important variables were included in multivariate analyses. Because only 418 subjects (63.2%) had data on serum creatinine, estimated GFR was added last to the statistical models. A proportional hazards assumption was evaluated by log-log plots, observed versus expected plots, and was tested for goodness of fit based on Schoenfeld residuals and scaled Schoenfeld residuals. The concordance statistics, which are similar to the area under the receiver-operating characteristic (ROC) curve, and area under the ROC curve, were used to assess the ability of the statistical model to predict the mortality or survival of the patients during the 10-year follow-up. They ranged from 0.5 (no predictive ability) to 1 (perfect predictive ability). A two-tailed P value below 0.05 was considered significant. Stata/SE 9.0 for Windows (StataCorp LP, TX) was used for statistical analyses.

RESULTS

We included 661 subjects with type 2 diabetes (327 men and 334 women), with mean age 61.9 ± 9.8 years and a medium follow-up period of 10.4 (interquartile range, 7.0–11.4) years. The correlation coefficients between serum VAP-1 and fasting plasma glucose, postprandial plasma glucose, and HbA_{1c} were 0.20, 0.27, and 0.32, respectively (all $P < 0.0001$).

During follow-up, 160 subjects died, including 59 (36.9%) from malignancy, 30 (18.8%) from diabetes, 27 (16.9%) from cardiovascular diseases, 12 (7.5%) from kidney diseases, nine (5.6%) from infectious diseases, eight (5.0%) from gastrointestinal or liver diseases, five (3.1%) from traumatic injuries, and others. At baseline, these subjects were older, had longer duration of diabetes, higher HbA_{1c}, and lower estimated GFR (Table 1). A higher percentage of these subjects had a smoking habit, history of cardiovascular disease, hypertension, proteinuria, and abnormal ABI. Their serum VAP-1 levels were higher, and more subjects had serum VAP-1 in the highest tertile.

People with serum VAP-1 in the highest tertile were older, had higher mortality, longer duration of diabetes, higher fasting plasma glucose, higher postprandial plasma glucose, higher HbA_{1c}, and lower estimated GFR (Table 2). There was a higher percentage of women and subjects with proteinuria and fewer smokers in this tertile.

Kaplan-Meier survival curves showed that during 10.4 years of follow-up, subjects with serum VAP-1 in the highest tertile had a lower rate of survival than subjects in the other tertiles (Fig. 1). In Table 3, the HRs of all-cause mortality are shown to be significantly higher in subjects with serum VAP-1 in the highest tertile, after adjusting for age, sex, smoking, history of cardiovascular disease, BMI category, hypertension, HbA_{1c}, diabetes duration, total cholesterol, use of statins, abnormal ABI, estimated GFR, and/or proteinuria in different models. Serum VAP-1 significantly predicted all-cause mortality in different subgroups divided by age (at 65 years), sex, smoking status, history of cardiovascular disease, BMI category (at 24 and 27 kg/m^2), hypertension, HbA_{1c} (at 7.4%), diabetes duration (at 9 years), plasma total cholesterol (at 200 mg/dL), use of statins, ABI category, estimated GFR (at 77 mL/min per 1.73 m^2), and proteinuria, adjusted for age, sex, smoking, history of cardiovascular disease, BMI category, hypertension, HbA_{1c}, diabetes duration, total cholesterol, statins, and ABI, apart from the stratification variable. Further adjustment for estimated GFR and proteinuria showed similar results, except in overweight subjects, those without hypertension, and in subjects with plasma total cholesterol greater than 200 mg/dL (all P = 0.05–0.10).

The results of disease-specific mortality are shown in Table 4. Serum VAP-1 predicted 10-year cardiovascular mortality independent of age, sex, smoking, and history of cardiovascular disease. There were 30 subjects whose underlying cause of death was diabetes. According to the rules defined by the World Health Organization (20), diabetes is likely to be selected as the underlying cause of death in subjects who die directly from cardiovascular diseases. Therefore, we analyzed predictors for cardiovascular

TABLE 1
Baseline characteristics of survivors and nonsurvivors with type 2 diabetes

	At follow-up		
	Alive	Dead	*P*
N (%)	501 (75.8)	160 (24.2)	
Age (years)	60.2 ± 9.3	67.4 ± 9.1	<0.0001
Men (%)	246 (49.1)	81 (50.6)	0.7
Smoking (%)	79 (15.8)	34 (21.3)	0.022
History of cardiovascular disease (%)	50 (10.0)	26 (16.3)	0.03
SBP (mmHg)	134 ± 16	137 ± 18	0.065
DBP (mmHg)	79 ± 9	79 ± 10	0.6
Hypertension drugs (%)	152 (30.3)	70 (43.8)	0.002
Hypertension (%)	285 (56.9)	113 (70.6)	0.002
Duration of diabetes (years)	7.0 (3.0–14.0)	12.0 (7.0–18.0)	<0.0001
Fasting plasma glucose, mmol/L (mg/dL)	8.27 ± 2.55 (149 ± 46)	8.44 ± 2.55 (152 ± 46)	0.6
Postprandial plasma glucose, mmol/L (mg/dL)	11.82 ± 4.11 (213 ± 74)	12.71 ± 4.39 (229 ± 79)	0.054
HbA_{1c} (%)	7.6 ± 1.4	7.9 ± 1.5	0.036
Total cholesterol, mmol/L (mg/dL)	5.20 ± 0.96 (201 ± 37)	5.22 ± 1.22 (202 ± 47)	0.7
Statins (%)	19 (3.8)	6 (3.8)	1.0
Triglyceride, mmol/L (mg/dL)	1.52 (1.06–2.17) 135 (94–192)	1.51 (1.10–2.34) 134 (97–207)	0.2
BMI (kg/m^2)	24.65 ± 3.27	24.54 ± 3.25	0.7
24–47 (%)	165 (32.9)	44 (27.5)	0.3
≥27 (%)	102 (20.4)	41 (25.6)	
Creatinine, μmol/L (mg/dL)	80 (71–88) 0.9 (0.8–1.0)	97 (71–123) 1.1 (0.8–1.4)	<0.0001
Estimated GFR (mL/min per 1.73 m^2)	81 (22)	63 (24)	<0.0001
Proteinuria (%)	50 (10.0)	61 (38.1)	<0.001
ABI <0.9 or >1.3 (%)	29 (5.8)	24 (15)	<0.001
Serum VAP-1 (ng/mL)	681 (581–799)	786 (636–967)	<0.0001
By tertile			<0.001
Middle, 630–780 (%)	176 (35.1)	39 (24.3)	
Highest, ≥ 780 (%)	140 (27.9)	82 (51.2)	

Means ± SD or medians (interquartile ranges) are shown. DBP, diastolic blood pressure; SBP, systolic blood pressure.

or diabetes-related mortality. As shown in models 2 and 3, serum VAP-1 significantly predicted cardiovascular and diabetes-related mortality adjusted for history of cardiovascular disease, age, sex, smoking, BMI category, hypertension, HbA_{1c}, diabetes duration, total cholesterol, use of statins, and/or estimated GFR. Fifty-nine subjects died of cancer, including colon cancer (n = 26), hepatobiliary cancer (n = 13), lymphoma or leukemia (n = 5), lung cancer (n = 4), urinary tract cancer (n = 4), and others (n = 7). Of interest, serum VAP-1 predicted cancer-related mortality independently (model 4). More specifically, serum VAP-1 independently predicted colon cancer-related mortality (HR 12.19 for logarithmically transformed serum VAP-1, 95% CI 2.37–62.7, P = 0.003, adjusted for age, sex, smoking, and BMI category).

One might argue that the predictors may not explain early mortality well, especially cancer mortality. Therefore, we analyzed the results after excluding subjects who died within the first three years of follow-up. Serum VAP-1, as well, can predict all-cause mortality (HR of highest serum VAP-1 tertile 2.33, 95% CI 1.13–4.81, $P < 0.05$), diabetes-related (HR of ln serum VAP-1 10.96, 95% CI 1.77–67.97, $P < 0.05$), cardiovascular- and diabetes-related (HR of ln serum VAP-1 10.88, 95% CI 1.77–67.04, $P < 0.05$), and cancer mortality (HR of ln serum VAP-1 13.51, 95% CI 2.79–65.38, $P < 0.01$) in adjusted models. However, serum VAP-1 did not significantly predict cardiovascular mortality (HR of ln serum VAP-1 3.62, 95% CI 0.64–20.43, $P > 0.05$), which may reflect the small number of cardiovascular mortality.

Table 5 shows the incremental predictive ability of different variables for all-cause mortality. Using serum VAP-1 alone can distinguish 63% of all pairs of subjects (one died and one survived), with an area under the ROC curve (AUC) of 0.64. In model 1, the increment in concordance statistics and AUC were 0.11 and 0.12 for serum VAP-1 and 0.01 and 0.01 for history of cardiovascular disease, respectively. In model 2, only serum VAP-1, age, and smoking increased the concordance statistics, whereas serum VAP-1, age, smoking, BMI category, hypertension, HbA_{1c}, diabetes duration, total cholesterol, use of statins, and abnormal ABI increased the AUC. In model 3, only four variables increased the concordance statistics, including serum VAP-1, age, estimated GFR, and proteinuria. Smoking increased the AUC in model 3.

DISCUSSION

In this study, we found that serum VAP-1 can independently predict 10-year all-cause mortality, cardiovascular mortality, and cancer-related mortality in subjects with type 2 diabetes and in most subgroups. The improved predictive ability of serum VAP-1 was comparable with that of age, smoking, serum creatinine, and proteinuria.

Recently, inflammation has been found to be an important cause of atherosclerosis (3). Circulating leukocytes, especially monocytes, are recruited to blood vessels, where they transmigrate and are activated. Various chemokines, cytokines, and enzymes are secreted, which may modify low-density lipoprotein, propagate inflammation, and result in atherosclerosis. Endothelial VAP-1 can participate in inflammation by binding granulocytes, lymphocytes, and monocytes, with the aid of SSAO activity (2). In subjects with acute myocardial infarction, VAP-1 can mediate

TABLE 2
Baseline characteristics by serum VAP-1 tertile in people with type 2 diabetes

Serum VAP-1 tertile (ng/mL)	<630	630–780	≥780	*P*
N	224	215	222	
Survivors (%)	185 (82.3)	176 (81.9)	140 (63.1)*†	<0.001
Age (years)	60.1 ± 9.6	61.8 ± 9.5	63.7 ± 9.9*†	0.0005
Men (%)	132 (58.9)	102 (47.4)*	93 (41.9)*	0.001
Smoking (%)	48 (21.4)	35 (16.3)	30 (13.5)*	0.079
History of cardiovascular disease (%)	31 (13.8)	18 (8.4)	27 (12.2)	0.2
SBP (mmHg)	134 ± 16	134 ± 14	135 ± 19	0.9
DBP (mmHg)	79 ± 9	78 ± 9	79 ± 9	0.6
Hypertension drugs (%)	68 (30.4)	74 (34.4)	80 (36.0)	0.4
Hypertension (%)	133 (59.4)	130 (60.5)	135 (60.8)	0.9
Duration of diabetes (years)	5.0 (2.0–12.0)	9.0 (4.0–13.0)*	11.0 (6.0–18.0)*†	<0.0001
Fasting plasma glucose (mmol/L, mg/dL)	7.71 ± 2.00 (139 ± 36)	8.27 ± 2.39* (149 ± 43)	8.99 ± 3.05*† (162 ± 55)	<0.0001
Postprandial plasma glucose (mmol/L, mg/dL)	11.21 ± 3.55 (202 ± 64)	11.60 ± 3.50 (209 ± 63)	13.32 ± 5.00*† (240 ± 90)	<0.0001
HbA_{1c} (%)	7.1 ± 1.2	7.8 ± 1.3*	8.2 ± 1.6*†	<0.0001
Total cholesterol (mmol/L, mg/dL)	5.09 ± 1.01 (197 ± 39)	5.30 ± 0.98 (205 ± 38)	5.22 ± 1.11 (202 ± 43)	0.14
Statins (%)	4 (1.8)	13 (6.0)	8 (3.6)	0.065
Triglyceride (mmol/L, mg/dL)	1.52 (1.13–2.25) 134.5 (100–199.5)	1.63 (1.16–2.22) 144 (103–197)	1.45 (0.99–2.11) 128 (88–187)	0.4
BMI (kg/m^2)	24.65 ± 3.00	24.79 ± 3.22	24.43 ± 3.55	0.5
24–47 (%)	74 (33.0)	71 (33.3)	64 (28.8)	0.6
≥27 (%)	42 (18.8)	51 (23.7)	50 (22.5)	
Creatinine (μmol/L, mg/dL)	80 (71–97) 0.9 (0.8–1.1)	80 (71–88) 0.9 (0.8–1.0)	80 (71–115)*† 0.9 (0.8–1.3)	0.005
Estimated GFR (mL/min per 1.73 m^2)	82 (23)	79 (22)	71 (25)*†	0.0001
Proteinuria (%)	23 (10.6)	26 (12.4)	62 (28.2)*†	<0.001
ABI <0.9 or >1.3 (%)	14 (6.3)	17 (7.9)	22 (9.9)	0.4

Means ± SD or medians (interquartile ranges) are shown. *$P < 0.05$ vs. first tertile (serum VAP-1 < 630 ng/mL); †$P < 0.05$ vs. second tertile (serum VAP-1 630–780 ng/mL).

leukocyte binding to endothelia in the infarcted areas (21). Because serum VAP-1 originates from various tissues, it may serve as a measure of systemic inflammation. On the other hand, serum VAP-1 retains its SSAO activity (6). End products of SSAO can modify various proteins and generate AGEs (4). Moreover, end products of SSAO can also propagate inflammation by upregulating the expression and facilitating the release of selectins in endothelium (22). Both functions of SSAO end products contribute to the development of atherosclerosis. Therefore, because

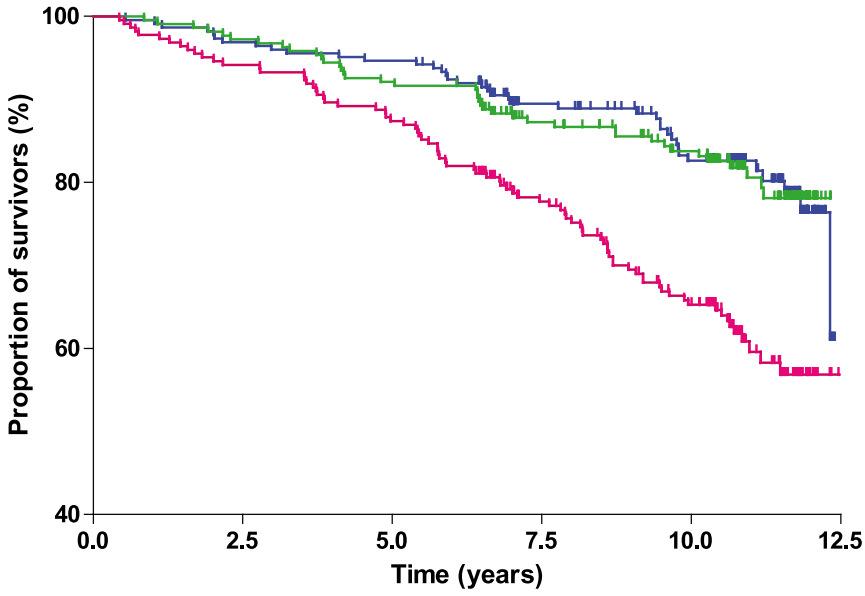

FIG. 1. Kaplan-Meier survival curves by tertile of serum VAP-1 levels. *P* = 0.0001 among subgroups by tertile. Blue line, subjects with serum VAP-1 in the first tertile; green line, subjects with serum VAP-1 in the second tertile; red line, subjects with serum VAP-1 in the third tertile.

TABLE 3
HRs (95% CI) of 10-year all-cause mortality in people with type 2 diabetes

	Model			
	1	2	3	4
VAP-1 tertile (ng/mL)				
<630	1.00	1.00	1.00	1.00
630–780	0.96 (0.61–1.52)	0.86 (0.54–1.38)	0.87 (0.54–1.39)	0.89 (0.45–1.77)
≥780	2.03* (1.37–3.02)	1.94† (1.25–3.00)	2.00† (1.29–3.09)	2.19‡ (1.17–4.11)
P for trend	<0.001	0.003	0.002	0.008
Age (years)	1.07* (1.05–1.09)	1.07* (1.05–1.09)	1.07* (1.05–1.09)	1.06* (1.03–1.09)
Female sex	0.93 (0.67–1.30)	0.91 (0.64–1.28)	0.91 (0.64–1.28)	0.93 (0.59–1.48)
Smoking	1.89† (1.26–2.85)	1.95† (1.29–2.95)	1.88† (1.24–2.85)	2.25† (1.34–3.79)
History of cardiovascular disease		1.49 (0.95–2.31)	1.48 (0.95–2.30)	1.30 (0.76–2.23)
BMI (kg/m^2)				
<24		1.00	1.00	1.00
24–27		0.86 (0.59–1.26)	0.86 (0.59–1.26)	0.60 (0.36–1.02)
≥27		1.35 (0.90–2.01)	1.35 (0.90–2.01)	0.95 (0.56–1.60)
P for trend		0.2	0.2	0.5
Hypertension		1.33 (0.94–1.90)	1.30 (0.91–1.85)	1.16 (0.72–1.87)
HbA_{1c} (%)		1.09 (0.97–1.22)	1.08 (0.96–1.21)	1.12 (0.97–1.29)
Diabetes duration (years)		1.00 (0.98–1.02)	1.00 (0.98–1.02)	0.99 (0.96–1.01)
Total cholesterol (mg/dL)		1.00 (1.00–1.00)	1.00 (1.00–1.00)	1.00 (0.99–1.01)
Statins		0.88 (0.38–2.04)	0.82 (0.35–1.91)	0.71 (0.28–1.84)
ABI <0.9 or >1.3			1.46 (0.91–2.32)	1.01 (0.55–1.85)
Estimated GFR (mL/min per 1.73 m^2)				0.99§ (0.98–1.00)
Proteinuria				2.38† (1.44–3.92)

*$P < 0.001$; †$P < 0.01$; ‡$P < 0.05$; §$P = 0.060$.

serum VAP-1 can serve as a measure of systemic inflammation and a source of SSAO end products, it may be a good biomarker for cardiovascular diseases. Indeed, in this report, we found that serum VAP-1 can predict 10-year cardiovascular mortality in subjects with type 2 diabetes independent of the established cardiovascular risk factors. In support of our findings, plasma SSAO has been reported to predict all-cause mortality in subjects with chronic heart failure, with most subjects (134/195, 68.7%) dying from cardiovascular diseases or sudden death (23).

Subjects with diabetes are associated with an increased risk of the development of and mortality associated with various cancers, including colorectal cancer (24), hepatocellular carcinoma (25), and others. Hyperinsulinemia, increased bioavailability of insulin-like growth factors-1, and hypoadiponectinemia may be part of the causes of oncogenesis and tumor progression in patients with diabetes (26,27). Surprisingly, we found that serum VAP-1 predicted cancer-related mortality in subjects with type 2 diabetes. Because serum VAP-1 was higher in subjects with diabetes (13), VAP-1/SSAO may serve as a link between diabetes and cancer. Studies in a knock-out mice model indicated that VAP-1 may play a role in angiogenesis, recruitment of myeloid cells, and the growth of melanoma and lymphoma

TABLE 4
HRs (95% CI) of 10-year disease-specific mortality in people with type 2 diabetes

	Cardiovascular	Diabetes	Cardiovascular + diabetes	Cancer
Ln VAP-1 (ng/mL)	5.83‡ (1.17–28.97)	9.71† (2.02–46.67)	6.32‡ (1.25–32.00)	17.24* (4.57–65.07)
History of cardiovascular disease	1.43 (0.49–4.21)	1.64 (0.61–4.40)	1.97 (0.80–4.86)	
Age (years)	1.09* (1.04–1.14)	1.11* (1.06–1.16)	1.11* (1.06–1.16)	1.04‡ (1.00–1.08)
Female sex	1.34 (0.59–3.05)	0.81 (0.37–1.80)	0.90 (0.41–1.97)	0.81 (0.41–1.61)
Smoking	1.14 (0.32–4.02)	3.92† (1.62–9.50)	4.05† (1.74–9.45)	2.41‡ (1.05–5.55)
BMI (kg/m^2)				
<24		1.00	1.00	1.00
24–27		1.00 (0.41–2.42)	0.72 (0.31–1.69)	0.88 (0.39–1.95)
≥27		2.48‡ (1.00–6.13)	1.71 (0.67–4.31)	1.23 (0.56–2.71)
P for trend		0.088	0.5	0.7
Hypertension			0.78 (0.38–1.60)	
HbA_{1c} (%)			1.31‡ (1.06–1.64)	
Diabetes duration (years)			0.99 (0.95–1.03)	
Total cholesterol (mg/dL)			1.00 (0.99–1.01)	
Statins			0.93 (0.20–4.32)	
Estimated GFR (mL/min per 1.73 m^2)			1.00 (0.98–1.02)	0.98‡ (0.97–0.997)

Cause of death: cardiovascular (27 [16.9% of total death]), diabetes (30 [18.8%]), cardiovascular + diabetes (57 [35.6%]), and cancer (59 [36.9%]). *$P < 0.001$; †$P < 0.01$; ‡$P < 0.05$.

TABLE 5
The concordance statistics and AUC without indicated variables in models predicting 10-year all-cause mortality for people with type 2 diabetes

	Model 1		Model 2		Model 3	
	Concordance statistics	AUC	Concordance statistics	AUC	Concordance statistics	AUC
Full model	0.64	0.65 (0.60–0.70)	0.73	0.78 (0.73–0.82)	0.78	0.84 (0.79–0.88)
Variable deleted from models						
Ln serum VAP-1 (ng/mL)	0.53 (0.11)	0.53 (0.12)	0.71 (0.02)	0.75 (0.03)	0.75 (0.03)	0.82 (0.02)
History of cardiovascular disease	0.63 (0.01)	0.64 (0.01)	0.73 (0)	0.78 (0)	0.78 (0)	0.84 (0)
Age (years)			0.68 (0.05)	0.72 (0.06)	0.76 (0.02)	0.82 (0.02)
Female sex			0.73 (0)	0.78 (0)	0.78 (0)	0.84 (0)
Smoking			0.72 (0.01)	0.76 (0.02)	0.78 (0)	0.83 (0.01)
BMI category (kg/m^2)			0.73 (0)	0.77 (0.01)	0.78 (0)	0.84 (0)
Hypertension			0.73 (0)	0.77 (0.01)	0.78 (0)	0.84 (0)
HbA_{1c} (%)			0.73 (0)	0.77 (0.01)	0.78 (0)	0.84 (0)
Diabetes duration (years)			0.73 (0)	0.77 (0.01)	0.78 (0)	0.84 (0)
Total cholesterol (mg/dL)			0.73 (0)	0.78 (0)	0.78 (0)	0.84 (0)
Statins			0.73 (0)	0.77 (0.01)	0.78 (0)	0.84 (0)
ABI <0.9 or >1.3			0.73 (0)	0.77 (0.01)	0.78 (0)	0.84 (0)
Estimated GFR (mL/min per 1.73 m^2)					0.77 (0.01)	0.81 (0.03)
Proteinuria					0.76 (0.02)	0.82 (0.02)

Model 1, Ln serum VAP-1 and history of cardiovascular disease; model 2, Ln serum VAP-1, history of cardiovascular disease, age, sex, smoking, BMI category, hypertension, HbA_{1c}, diabetes duration, total cholesterol, statins, and ABI; model 3, model 2 plus estimated GFR, and proteinuria. Differences of concordance statistics or area under the ROC between reduced and full models are shown in parentheses.

(28). Colorectal cancer patients have been shown to have higher serum VAP-1 than healthy subjects (29). Serum SSAO activity has been correlated with the angiogenic vascular endothelial growth factor in subjects with lung cancer (30). Serum SSAO activity has also been shown to be higher in prostate cancer subjects with bone metastases than subjects without metastases (31). Taken together, these data strongly support our observations and suggest a role for VAP-1/SSAO in cancer growth and metastasis. However, VAP-1 may participate in tumor surveillance. VAP-1 has been shown to mediate the adhesion of tumor-infiltrating lymphocytes to head and neck squamous cell carcinoma and hepatocellular carcinoma and to kill cancer cells (32,33). Subjects with advanced colorectal cancer have been shown to have low serum VAP-1 (29). In human melanoma, a higher expression of VAP-1 in intratumoral microvessels was associated with better five-year survival, although there was only borderline statistical significance and the results were not adjusted for other confounders, such as the presence of metastasis or not (34). To sum up, although VAP-1/SSAO may have opposing roles in cancer development and progression, our results favored the hypothesis that there was a positive effect of VAP-1 on angiogenesis and metastases in subjects with type 2 diabetes. Whether there is any tumor-specific effect of VAP-1 on tumor surveillance remains to be further investigated.

The strength of this study is in the 100% follow-up rate after 10 years, with accurate records of the vital status of a homogenous population of Han Chinese. In addition, the highly sensitive time-resolved immunofluorometric assay for measuring serum VAP-1 enabled us to differentiate subtle differences in circulating VAP-1 concentrations. However, our study had some limitations. First, serum VAP-1 was measured only once at baseline, which may limit the value of serum VAP-1 over time for the prediction of outcomes. Second, generalization of the findings to other populations may be limited because all the subjects in the current study were Han Chinese. Third, determination of proteinuria by reflectance colorimetry is relatively insensitive and may not accurately detect kidney dysfunction.

In conclusion, we showed for the first time that serum VAP-1 can independently predict 10-year all-cause mortality, cardiovascular mortality, and cancer-related mortality in subjects with type 2 diabetes. Our results showed that serum VAP-1 is a novel biomarker and improves risk prediction over and above the established risk factors for cardiovascular and cancer mortality in subjects with type 2 diabetes. Our data also indicated the potential of using VAP-1/SSAO inhibitors or antibodies, which are currently being tested for their ability to treat autoimmune diseases and to treat or prevent cardiovascular diseases and cancer in high-risk individuals with type 2 diabetes.

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