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The Hoorn Study

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Abstract—Increased levels of von Willebrand factor (vWf) and C-reactive protein (CRP) predict cardiovascular mortality in selected populations. It is uncertain whether vWf and CRP predict mortality in a general population and whether vWf and CRP predict mortality through similar pathways. This study investigated the association of vWf and CRP with cardiovascular and all-cause mortality among diabetic and nondiabetic subjects. An age-, sex-, and glucose tolerance–stratified sample (n=631) of a population-based cohort aged 50 to 75 years was followed prospectively for 5 years. After 5 years of follow-up, 58 subjects had died (24 of cardiovascular causes). vWf (>1.56 IU/mL) and CRP (>2.84 mg/L) levels in the upper tertile were associated with, respectively, a 3- and 2-fold increase in cardiovascular mortality after adjustment for age, sex, and glucose tolerance status. Analyses in nondiabetic and diabetic subjects separately gave similar results. After further adjustment for hypertension, levels of HDL cholesterol and triglyceride, smoking habits, ischemic heart disease, and peripheral arterial disease, the relative risks (RRs) were 3.0 (95% CI 1.2 to 7.9) for vWf and 1.4 (95% CI 0.6 to 3.5) for CRP. When both vWf and CRP were included in the latter multivariate analysis, the RRs were 3.0 (95% CI 1.1 to 7.9) for vWf and 1.3 (95% CI 0.5 to 3.4) for CRP. The association between vWf and risk of cardiovascular mortality was independent of blood group (O versus non-O) and, moreover, similar among subjects with different blood groups. Repeating the analyses for all-cause mortality gave similar results for CRP. For vWf, the RR was 2.0 (95% CI 1.1 to 3.5) after adjustment for all other risk factors. Increased levels of vWf are independently associated with cardiovascular and all-cause mortality in both diabetic and nondiabetic subjects. The association between increased levels of CRP and cardiovascular mortality was partly explained by other risk factors. Mutual adjustment of vWf and CRP did not markedly change the results, favoring the hypothesis that vWf and CRP predict mortality through different pathways. **(*Arterioscler Thromb Vasc Biol*. 1999;19:3071-3078.)**

Key Words: von Willebrand factor ■ C-reactive protein ■ cardiovascular mortality ■ non–insulin-dependent diabetes mellitus ■ acute phase reactant

Accumulating evidence indicates that endothelial dysfunction and chronic low-grade inflammation play a pivotal role in the pathogenesis of atherothrombotic disease.[1–3]

Increased levels of von Willebrand factor (vWf) have been proposed to reflect generalized endothelial dysfunction.[4,5] Indeed, subjects with peripheral,[6] cerebral,[7] and coronary artery atherosclerotic disease[8] have increased levels of vWf compared with control subjects. Furthermore, high levels of vWf have been shown to predict cardiovascular mortality in patients recently presenting with cardiovascular disease.[7–11] However, 2 population-based studies showed no significant association of high vWf levels with cardiovascular mortality.[12,13]

C-reactive protein (CRP), an acute-phase reactant, is a marker of inflammation. In healthy subjects, its concentration is generally low, rising 5-fold to >100-fold in acute illness. Slightly increased, but conventionally normal, CRP levels may reflect a chronic low-grade inflammatory state and have been found to be an independent predictor of cardiovascular mortality among subjects at high risk of atherothrombotic events,[8,14–16] as well as among healthy subjects.[17–19]

Levels of both vWf[20,21] and CRP[22] are increased in non–insulin-dependent diabetes mellitus (NIDDM) compared with levels in control subjects. NIDDM is associated with a 2- to 4-fold increased cardiovascular mortality,[23] but there are few prospective data for vWf and cardiovascular disease in NIDDM[21,24] and none for CRP.

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We performed a prospective population-based cohort study among nondiabetic and diabetic subjects to investigate the relation between vWf and CRP concentrations on one hand and cardiovascular and all-cause mortality on the other. A further aim of the present study was to investigate whether vWf and CRP affect the risk of mortality through similar pathways. We reasoned that if vWf and CRP confer mutually independent excess risks of mortality, this would argue in favor of the idea that vWf and CRP affect mortality risk through substantially different pathways.

Methods

General Study Design

The Hoorn study is a population-based cohort study of disturbances of glucose tolerance in a white population aged 50 to 75 years conducted from 1989 to 1992 (n=2484 subjects; response rate 71%).[25] An extensive investigation was performed in an age-, sex-, and glucose tolerance–stratified random sample (n=631, response rate 89%).[25–27]

From these subjects, we obtained an ankle-brachial blood pressure index (n=631) and a resting ECG (n=625). Subjects were classified as having (1) peripheral arterial disease (PAD) when they had an ankle-brachial pressure index <0.9 in either leg and/or when they had undergone a peripheral arterial bypass or amputation,[28] (2) ischemic heart disease (IHD) when they had an ECG with a Minnesota code 1.1 to 1.3, 4.1 to 4.3, 5.1 to 5.3, or 7.1 and/or had undergone coronary bypass surgery or angioplasty, and (3) cerebrovascular disease when they had evidence of a past transient ischemic attack or stroke according to the World Health Organization (WHO) cardiovascular questionnaire.[29]

vWf and CRP

Concentrations of vWf and CRP were assessed in deep frozen (−70°C) heparin plasma samples. No plasma samples were available for 21 subjects. vWf antigen levels were estimated in duplicate by ELISA, essentially as described,[30] with the use of polyclonal antibodies from Dako (Glostrup, Denmark), and they were expressed as percentage of vWf detected in pooled citrated plasma of healthy volunteers. According to the 4th International Standard for vWf in plasma (NIBSC code 97/586), the pooled citrated plasma contained 1.03 IU/mL of vWf antigen. CRP levels were measured in duplicate with an enzyme immune assay that used rabbit antibodies against CRP (Dako) as both the capture and tagging antibody, with a sensitivity of 0.2 mg/L.[31] CRP standard serum (Behring Diagnostics GmbH) was used for calibration. In 11 subjects, the CRP level was undetectable and therefore set at 0.2 mg/L.

Other Measurements

Data on blood pressure, weight, height, body mass index, glycated hemoglobin, fasting specific plasma insulin, serum total cholesterol, HDL cholesterol, and triglyceride levels were obtained.[25] ABO blood groups were determined by standard agglutination techniques using commercial test erythrocytes. LDL cholesterol was calculated by the Friedewald formula,[32] except when the triglyceride level was >4.55 mmol/L (n=23). Hypertension was defined as diastolic pressure ≥95 mm Hg, systolic pressure ≥160 mm Hg, and/or the use of antihypertensive drugs.[33] Current smoking was defined as currently smoking cigarettes and/or cigars.

Follow-Up

Data on the vital status of the subjects on April 1, 1997, were collected from the mortality register of the municipality of Hoorn. Of 49 subjects who moved out of town, information on vital status was obtained from the new local municipalities. For each subject, we determined whether or not death had occurred in the first 5 years of follow-up and, if so, the date at which death occurred. For all subjects who had died, the cause of death was classified according to the 9th edition of the International Classification of Diseases.[26] Cardiovascular mortality was defined as codes 390 to 459, cancer mortality, as codes 140 to 240. Information on cause of death could not be obtained for 6 (10%) of the deceased subjects.

All participants gave informed consent for the present study, which was approved by the local ethics committee.

Statistical Analyses

All analyses were performed with SPSS 7.5 for Windows 95. Survival over 5 years of follow-up was calculated by Kaplan-Meier curves for different groups, and differences were tested by the log rank test. Differences between groups in continuous variables that had a normal distribution were tested by Student *t* tests; in continuous variables that had a skewed distribution, by Mann-Whitney tests; and in percentage of subjects with versus without the presence of dichotomous variables, by χ^2 tests. The correlation between vWf and log-transformed CRP was assessed with the Pearson correlation coefficient. Predictors of 5-year cardiovascular and all-cause mortality were determined by Cox proportional hazards multiple regression analysis (in all cases, because of the stratification procedure) with adjustment for age, sex, and glucose tolerance status. Results are described as relative risks (RRs [hazard ratios]) with 95% CIs.

Potential risk factors measured on a continuous scale were used as such in the regression models, except for HDL cholesterol and body mass index, because the association of these variables with all-cause mortality was nonlinear. Therefore, a low HDL cholesterol level was defined as a level <0.9 mmol/L[34]; obesity, as a body mass index >27 kg/m^2 for men and >26 kg/m^2 for women.[35] Levels of fasting insulin and triglyceride were log-transformed because of a better fit of the regression model. Subjects were grouped according to levels of vWf and CRP. Subjects with levels in the highest tertile were compared with those with levels in the 2 lower tertiles. vWf and CRP were thus entered into the regression models as dichotomized variables, ie, upper tertile versus lower tertiles. (The lowest and middle tertiles were taken together because preliminary analyses showed that the RRs of mortality for these 2 lower tertiles of vWf and CRP were similar, whereas the RR for the upper tertile of vWf and CRP was increased.) To evaluate a possible effect-modifying role of potential risk factors with regard to cardiovascular or all-cause mortality, Cox regression analyses were performed with the risk factor of interest, vWf (or CRP), their product term, age, sex, and glucose tolerance status in the model. A significant RR for the product term was considered effect modification by that risk factor. To assess whether the associations of vWf and CRP with mortality were independent, regression analyses were primarily adjusted for all risk factors that were statistically significant in initial analyses, secondarily adjusted for the presence of cardiovascular morbidity, and finally adjusted for other potential risk factors of interest that showed no significant association in the initial analyses.

To investigate whether vWf and CRP affected risk of mortality through similar pathways, regression analyses were performed that included both vWf and CRP as independent variables. A 2-sided probability value of $P<0.05$ was considered statistically significant.

Results

Figures 1 and 2 (insets) show the distribution of vWf and CRP. The ranges of vWf and CRP levels were 0.24 to 3.89 IU/mL and 0.2 to 35.2 mg/L, respectively. Thirty-three (5.4%) of the subjects had a CRP level >10.0 mg/L. Table 1 shows the baseline characteristics of the study population. Levels of vWf and CRP in the upper tertile compared with the lower tertiles were significantly associated with higher age, higher levels of fasting glucose, glycated hemoglobin, and insulin, and higher body mass index, systolic blood pressure, and prevalence of NIDDM, hypertension, and PAD. CRP levels in the upper tertile were, in addition, significantly associated with higher levels of triglycerides, higher waist-to-hip ratio, and a higher prevalence of women, current smoking status, and IHD compared with CRP levels in the lower tertiles (data not shown).

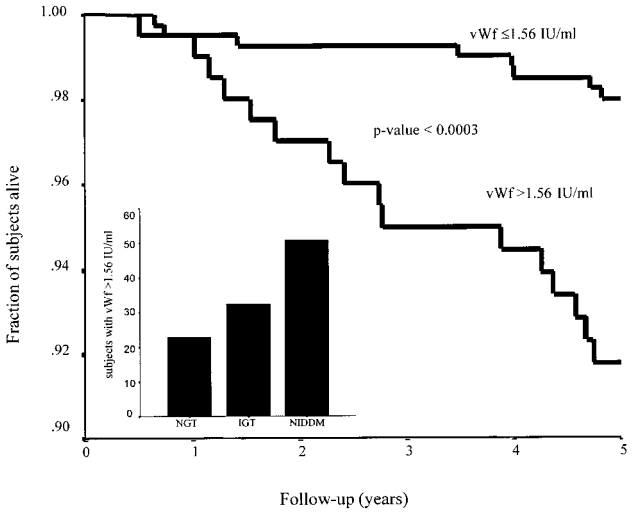

Figure 1. Cardiovascular survival (Kaplan-Meier method) according to plasma vWf levels in the upper tertile (>1.56 IU/mL) vs the lower 2 tertiles. Inset shows percentage of vWf >1.56 IU/mL per glucose tolerance status.

After 5 years of follow-up, 58 of the 631 subjects had died, of whom 24 (41%) had died of cardiovascular disease. Subjects who died had higher levels of vWf (mean±SD) and CRP (median interquartile range) compared with those that survived: 1.76±0.72 versus 1.33±0.68 IU/mL and 3.33 (1.36 to 6.88) versus 1.68 (0.79 to 3.35) mg/L, respectively. Table 1 shows RRs of mortality associated with potential risk factors.

von Willebrand Factor

In the entire group, vWf levels in the upper tertile were associated with an ≈4-fold increased risk of cardiovascular mortality (Figure 1) and, after adjustment for age, sex, and glucose tolerance status, with an ≈3-fold increased risk (Table 2). After further adjustment for hypertension, current smoking, low level of HDL cholesterol, level of triglyceride, IHD, and PAD, the RR associated with vWf was 3.04 (Table 2). Subgroup analyses in nondiabetic and diabetic subjects showed that vWf was associated with a 4-fold and 2-fold increased risk of cardiovascular death, respectively, after correction for age, sex, and impaired glucose tolerance (Table 2). Further adjustment increased the RR associated with vWf to 11.83 among nondiabetic subjects, whereas the RR among diabetic subjects remained 2.51 (Table 2).

The RR of all-cause mortality associated with vWf in the upper tertile was 2.03, which was not affected by adjustment (Table 2).

To investigate whether the RR of vWf was similar among different risk groups, we performed analyses with interaction terms added (see Methods). Impaired glucose tolerance, NIDDM, current smoking, levels of triglyceride and total cholesterol, body mass index, IHD, and PAD showed no significant interaction (for interaction term, $P>0.2$). The RRs of cardiovascular mortality associated with vWf in the upper tertile among women, in subjects >65 years of age, and in the presence of hypertension or low levels of HDL cholesterol were 12.3 ($P=0.03$), 6.5 ($P=0.07$), 5.0 ($P=0.12$), and 4.5 ($P=0.10$) times higher, respectively, than when these factors were absent (data not shown).

The hypothesis has been advanced that the ABO blood group could be the explanation for the association between vWf level and cardiovascular mortality, because blood groups are associated with both cardiovascular disease and levels of vWf.[36] The prevalences of blood groups O, A, B, and AB were 45%, 39%, 11%, and 6%. After adjustment for age, sex, and glucose tolerance status, blood group non-O was associated with a 2-fold increased cardiovascular mortality compared with blood group O (RR 2.08 [95% CI 0.85 to 5.07] among all subjects). Additional adjustment for risk factors mentioned in Table 2 did not materially change this result (data not shown). The levels of vWf were significantly lower in blood group O compared with blood group non-O (mean±SD 1.16±0.58 and 1.49±0.72 IU/mL, respectively; $P<0.05$). The RRs of cardiovascular mortality associated with vWf level and blood group non-O were not importantly affected by mutual adjustment (eg, for model 1 in Table 2, RRs 3.02 [1.22 to 7.53] and 1.77 [0.72 to 4.38], respectively). Further adjustment for risk factors mentioned in Table 2 gave similar results (data not shown). Analyses performed among blood group O and blood group non-O separately gave similar results (eg, for model 1 in Table 2, RRs 2.01 [0.42 to 9.51] and 3.28 [1.05 to 10.25] among all subjects, respectively).

C-Reactive Protein

In the entire group, CRP levels in the upper tertile were associated with an ≈3-fold increased risk of cardiovascular mortality (Figure 2) and, after adjustment for age, sex, and glucose tolerance status, with an ≈2-fold increased risk (Table 3). After further adjustment for hypertension, current smoking, low level of HDL cholesterol, triglyceride level, IHD, and PAD, the RR associated with CRP was 1.41 (Table 3). The RRs of cardiovascular mortality associated with CRP in the upper tertile were similar among nondiabetic and diabetic subjects (≈2-fold). After further adjustment, the RR of CRP decreased to 0.83 among nondiabetic and to 1.34 among diabetic subjects (Table 3).

CRP was a significant predictor of all-cause mortality after adjustment for age, sex, and glucose tolerance status in the

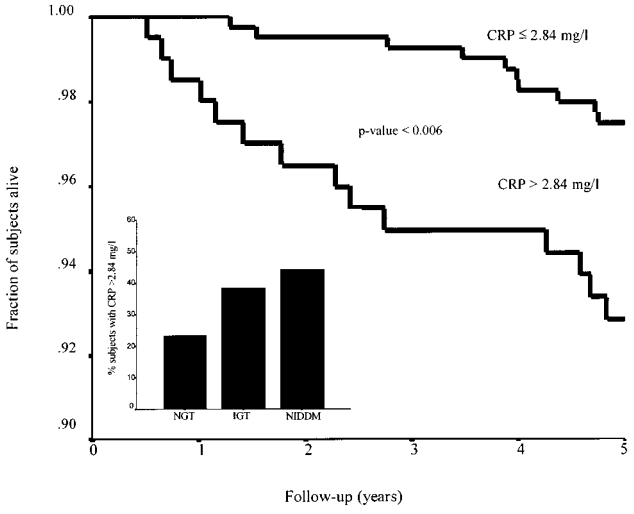

Figure 2. Cardiovascular survival (Kaplan-Meier method) according to plasma CRP levels in the upper tertile (>2.84 mg/L) vs the lower 2 tertiles. Inset shows percentage of CRP >2.84 mg/L per glucose tolerance status.

TABLE 1. Baseline characteristics and RR of 5-Year Cardiovascular and All-Cause Mortality Associated With Potential Risk Factors

Risk Factor	Baseline Characteristics for All Subjects (n=631)	Mortality According to Indicated Change of Risk Factors		
		Change of Risk Factor	Cardiovascular Mortality, RR (95% CI)	All-Cause Mortality, RR (95% CI)
Male, %	48	Yes vs no	1.62 (0.72–3.65)	1.73 (1.03–2.92)
Age, y	64±7	Per 5-year increase	1.66 (1.15–2.39)	1.63 (1.29–2.06)
HbA1c, % of hemoglobin	5.9±1.3	Per 1% of hemoglobin increase	1.08 (0.84–1.38)	1.15 (0.98–1.34)
Fasting insulin, pmol/L	84 (63–119)	Per 10% pmol/L increase*	0.99 (0.90–1.08)	1.02 (0.96–1.08)
Impaired glucose tolerance, %	27	Yes vs no	0.50 (0.10–2.47)	1.21 (0.55–2.67)
NIDDM, %	27	Yes vs no	4.19 (1.63–10.76)	3.72 (1.99–6.99)
Body mass index, kg/m^2	27.2±4.0	Yes vs no†‡	1.61 (0.61–4.21)	1.39 (0.76–2.51)
Total cholesterol, mmol/L	6.6±1.2	Per 1.0 mmol/L increase	1.30 (0.95–1.79)	1.14 (0.92–1.40)
LDL cholesterol, mmol/L	4.5±1.1	Per 1.0 mmol/L increase	1.31 (0.90–1.90)	1.09 (0.85–1.40)
HDL cholesterol, mmol/L	1.3±0.4	Yes vs no§	4.00 (1.66–9.65)	2.38 (1.28–4.43)
Triglycerides, mmol/L	1.6 (1.2–2.2)	Per 10% mmol/L increase*	1.10 (1.02–1.18)	1.07 (1.01–1.12)
Hypertension, %	39	Yes vs no	3.36 (1.29–8.76)	1.56 (0.91–2.69)
Current smokers, %	28	Yes vs no	2.35 (0.99–5.54)	2.21 (1.28–3.82)
IHD, %	15	Yes vs no‖	3.14 (1.36–7.24)	2.05 (1.16–3.64)
Peripheral arterial disease, %	11	Yes vs no¶	3.75 (1.62–8.68)	2.06 (1.14–3.71)
Stroke, %	5	Yes vs no#	Not applicable**	0.35 (0.05–2.51)

Baseline values are percentages, mean±SD, or median (interquartile range). Mortality values are RR with 95% CIs obtained with Cox regression analyses of 5-year cardiovascular and all-cause mortality associated with continuous or dichotomous variables after adjustment for age, sex, impaired glucose tolerance, and NIDDM, except when this was the variable under consideration. HbA1c indicates glycated hemoglobin.

*Log-transformed.

†>27 vs ≤27 kg/m^2 for males and >26 vs ≤26 kg/m^2 for females.

‡Associations with waist-to-hip ratio were weaker.

§<0.9 vs ≥0.9 mmol/L.

‖Minnesota code 1.1–1.3, 4.1–4.3, 5.1–5.3, or 7.1 on the ECG, coronary bypass operation, and/or angioplasty.

¶Ankle-brachial pressure index <0.90 and/or peripheral arterial bypass or amputation.

#Stroke or transient ischemic attack according to the WHO questionnaire.

**No cardiovascular deaths among subjects with previous stroke.

entire group (Table 3). After adjustment for other risk factors, the RR decreased from 1.88 to 1.33 (Table 3).

Impaired glucose tolerance, NIDDM, hypertension, current smoking, low levels of HDL cholesterol, levels of triglyceride and total cholesterol, body mass index, IHD, and PAD showed no significant interaction (for interaction term, $P>0.2$). The RRs of cardiovascular mortality associated with CRP in the upper tertile among women and subjects older than 65 years were, respectively, 3.6 ($P=0.18$) and 6.5 ($P=0.13$) times higher than those among men and subjects aged <65 years (data not shown).

Additional Analyses

The correlation between vWf and log-transformed CRP in the entire group was 0.10 ($P=0.014$). The RRs of cardiovascular mortality associated with vWf and CRP in their respective upper tertiles were not importantly affected by mutual adjustment (Table 4). Further adjustment also did not materially affect the results (Table 4). vWf and CRP showed no mutual interaction (for interaction term, $P=0.99$).

Twenty-six (45%) of the subjects died of cancer. After adjustment for age, sex, and glucose tolerance status, both vWf and CRP were not significantly associated with risk of cancer mortality (RRs 1.50 [0.67 to 3.34] and 1.23 [0.54 to 2.82], respectively).

Because the RRs of mortality were similar in the lower tertiles of either vWf or CRP and increased in the upper tertile only, we further investigated whether there was a threshold value of vWf and CRP for predicting mortality by changing the definitions of a "high" vWf and CRP concentration. When vWf was dichotomized as with 1.22 IU/mL (median) or 1.74 IU/mL (highest quartile) as cutoffs, the risk estimates for cardiovascular mortality were slightly lower (eg, for model 1 in Table 2, RRs 2.36 [0.93 to 5.99] and 2.63 [1.16 to 5.98] compared with 2.80 [1.18 to 6.66], respectively). When CRP was dichotomized with 2.11 mg/L (used in Reference 18), 3.20 mg/L (used in Reference 17), or 3.60 mg/L (used in Reference 15) as cutoffs, the RR of cardiovascular mortality was lower (eg, for model 1 in Table 3, RRs 1.35 [0.56 to 3.22], 1.93 [0.85 to 4.41], and 1.58 [0.68 to 3.64] compared with 2.23 [0.95 to 5.21], respectively). Exclusion of subjects with vWf level <0.50 IU/mL (n=39) or of subjects with CRP level >10.0 mg/L gave similar results (data not shown). Analyses with a shorter follow-up duration showed higher RRs of cardiovascular mortality associated with vWf and CRP levels in their respective upper tertiles. For example, after adjustment for age, sex, and glucose tolerance status, the RRs for vWf and CRP in the first 3 years of follow-up were 4.02 (1.09 to 14.93) and 4.46 (1.14 to 17.41); for 3 to 5 years of follow-up, the values were 1.86 (0.55 to 6.37) and 1.15

TABLE 2. Relative Risk of 5-Year Cardiovascular and All-Cause Mortality Associated With Presence of vWF Highest Tertile (>1.56 IU/mL) After Adjustment for Potentially Confounding Risk Factors

		RR (95% CI)			
		Cardiovascular Mortality (n=24)			All-Cause Mortality (n=58)
Model	Added Variables	All Subjects* (n=610)	Nondiabetic Subjects† (n=441)	Diabetic subjects (n=169)	All Subjects* (n=610)
1	Age, sex, impaired glucose tolerance, and NIDDM	2.80 (1.18–6.66)	4.10 (0.96–17.54)	2.30 (0.80–6.64)	2.03 (1.19–3.47)
2	Model 1 plus hypertension, current smoking, low HDL cholesterol level,‡ and low triglyceride level§	3.08 (1.20–7.91)	10.96 (1.57–76.71)	2.50 (0.77–8.15)	2.03 (1.16–3.55)
3	Model 2 plus IHD‖ and peripheral arterial disease¶	3.04 (1.16–7.94)	11.83 (1.59–87.87)	2.51 (0.75–8.41)	2.04 (1.16–3.61)
4	Model 3 plus obesity# and cholesterol level	2.91 (1.11–7.63)	...	...	1.98 (1.12–3.52)

Values are RR (95% CI) of 5-year mortality associated with vWF concentration obtained with Cox multiple regression analyses. Models are as follows: model 1, stratification variables; model 2, as model 1, plus all risk factors significantly associated with cardiovascular mortality (shown in Table 3, left column); model 3, as model 2, plus cardiovascular morbidity significantly associated with mortality (shown in Table 3, left column); and model 4, as model 3, plus major risk factors that were nonsignificant (Table 3).

*No plasma samples available for 21 subjects. †RRs of mortality of vWf among subjects with normal and impaired glucose tolerance were similar; these categories were therefore pooled in the analyses. ‡HDL cholesterol level <0.9 mmol/L. §Log-transformed triglyceride levels. ‖Minnosota code 1.1–1.3, 4.1–4.3, 5.1–5.3, or 7.1 on ECG coronary bypass operation, and/or angioplasty. ¶Ankle-brachial pressure index <0.90 and/or peripheral arterial bypass or amputation. #Body mass index >27.0 vs ≤27.0 kg/m^2 for men and >26.0 vs ≤26.0 kg/m^2 for women.

(0.32 to 4.10), respectively. The difference between these risk estimates (the first 3 years compared with 3 to 5 years of follow-up) was not significant ($P=0.40$ and $P=0.15$, respectively).

Discussion

This prospective study showed that higher levels of vWf are associated with 5-year cardiovascular and all-cause mortality in both diabetic and nondiabetic subjects. This association is independent of conventional risk factors and blood groups. The level of CRP is a predictor of all-cause mortality, which, however, is not independent of other risk factors. Mutual adjustment of vWf and CRP did not markedly affect the RRs of mortality. These results, together with the weak correlation between vWf and CRP at baseline ($r=0.10$), argue in favor of the idea that vWf and CRP predict mortality through different pathways.

Our finding that vWf is an independent predictor of cardiovascular mortality is in line with results of some[7–11] but not all studies.[11–13,37–39] To the best of our knowledge, this is the first study that provides evidence for an independent association of vWf with cardiovascular mortality in the general population. The precise mechanism by which vWf increases cardiovascular risk is unclear. It has been suggested that vWf is a marker of generalized endothelial dysfunction,[4,5] which is an important feature of atherothrombotic disease.[1–3] Alternatively, it has been hypothesized that vWf, as an acute-phase reactant,[40] reflects endothelial activation and stimulation (without necessarily implying endothelial dysfunction) and, as such, is a marker of more severe disease in general. Accordingly, the ECAT study (Juhan-Vague et al[41]) showed that among subjects with angina pectoris, the independent RR of cardiovascular mortality associated with vWf disappeared after adjustment for variables related to inflammation, ie, CRP and/or fibrinogen. In other words, the risk predicted by vWf was explained by the risk predicted by CRP and/or fibrinogen. In contrast, we found that mutual adjustment of vWf and CRP did not materially change the

TABLE 3. Relative Risk of 5-Year Cardiovascular and All-Cause Mortality Associated With Presence of CRP in Highest Tertile (>2.84 mg/L) After Adjustment for Potentially Confounding Risk Factors

		RR (95% CI)			
		Cardiovascular Mortality (n=24)			All-Cause Mortality (n=58)
Model	Added Variables	All Subjects* (n=610)	Nondiabetic Subjects† (n=441)	Diabetic Subjects (n=169)	All Subjects* (n=610)
1	Age, sex, impaired glucose tolerance, and NIDDM	2.23 (0.95–5.21)	1.73 (0.40–7.45)	2.39 (0.80–7.13)	1.88 (1.10–3.24)
2	Model 1 plus hypertension, current smoking, low HDL cholesterol level,‡ and low triglyceride level§	1.93 (0.81–4.63)	0.85 (0.17–4.25)	1.97 (0.62–6.27)	1.67 (0.96–2.89)
3	Model 2 plus IHD‖ and peripheral arterial disease¶	1.41 (0.57–3.50)	0.83 (0.17–4.09)	1.34 (0.41–4.43)	1.39 (0.79–2.46)
4	Model 3 plus obesity# and cholesterol level	1.32 (0.52–3.35)	...	...	1.33 (0.75–2.37)

Values are RR (95% CI) of 5-year mortality associated with CRP concentration obtained with Cox multiple regression analyses. Models are as described in Table 2.

*No plasma samples available for 21 subjects. †RRs of mortality of CRP among subjects with normal and impaired glucose tolerance were similar; these categories were therefore pooled in the analyses. ‡HDL cholesterol level <0.9 mmol/L. §Log-transformed triglyceride levels. ‖Minnosota code 1.1–1.3, 4.1–4.3, 5.1–5.3, or 7.1 on ECG, coronary bypass operation, and/or angioplasty. ¶Ankle-brachial pressure index <0.9 and/or peripheral arterial bypass or amputation. #Body mass index >27.0 vs ≤27.0 kg/m^2 for men and >26.0 vs ≤26.0 kg/m^2 for women.

TABLE 4. Relative Risk of 5-Year Cardiovascular and All-Cause Mortality Associated With Plasma Concentration of vWf and CRP in Upper Tertile After Mutual Adjustment

		RR (95% CI)			
		Cardiovascular Mortality		All-Cause Mortality	
Model	Added Variables	vWf in Upper Tertile	CRP in Upper Tertile	vWf in Upper Tertile	CRP in Upper Tertile
1	Age, sex, impaired glucose tolerance, NIDDM, and vWf or CRP*	2.64 (1.10–6.31)	2.04 (0.87–4.79)	1.95 (1.14–3.35)	1.79 (1.04–3.09)
2	Model 1 plus hypertension, current smoking, low HDL cholesterol level,† low triglyceride level,‡ and vWf or CRP*	2.97 (1.15–7.66)	1.82 (0.75–4.41)	2.00 (1.14–3.50)	1.63 (0.94–2.83)
3	Model 2 plus IHD,§ peripheral arterial disease,‖ and vWf or CRP*	3.00 (1.14–7.87)	1.32 (0.52–3.35)	2.03 (1.15–3.60)	1.37 (0.77–2.42)

Values are RR (95% CI) of 5-year mortality associated with vWf and CRP concentration in the upper tertile (>1.56 IU/mL and >2.84 mg/L, respectively) vs the lower tertiles obtained with Cox multiple regression analyses. Models are as follows: model 1, stratification variables; model 2, as model 1, plus all risk factors significantly associated with mortality (shown in Table 3, left column); model 3, as model 2, plus cardiovascular morbidity significantly associated with mortality (shown in Table 3, left column).

*With the variable added that was not already in the model. †HDL cholesterol level <0.9 mmol/L. ‡Log-transformed triglyceride levels. §Minnosota code 1.1–1.3, 4.1–4.3, 5.1–5.3, or 7.1 on ECG, coronary bypass operation, and/or angioplasty. ‖Ankle-brachial pressure index <0.9 and/or peripheral arterial bypass or amputation.

risk estimates, suggesting different mechanisms through which vWf and CRP predict mortality. Finally, vWf plays an important role not only in platelet adhesion and aggregation but also in coagulation[42,43] and may thus enhance the risk of thrombogenesis. Accordingly, the increase of vWf within 48 hours after a myocardial infarction was a predictor of the rate of restenotic cardiovascular events within 14 days, whereas the increase of CRP, as a marker of acute-phase response, was not.[44] Our finding that the RR of mortality associated with vWf is strongest in the first few years of follow-up is compatible with the view that vWf is related more to atherothrombotic than to atherosclerotic disease. In sum, our data are consistent with the concept that a high vWf level, in the general diabetic and nondiabetic population, is a marker of generalized endothelial dysfunction and/or a prothrombotic state but not with the view that a high vWf level reflects an acute-phase response.

Slightly increased levels of CRP have been found to be associated with increased cardiovascular risk in subjects with angina pectoris[8,14,15] and in healthy subjects.[17–19] It has been hypothesized that a chronic low-grade bacterial infection, which can cause raised CRP levels within the normal range and is associated with coronary heart disease,[45,46] is the mechanism through which CRP predicts cardiovascular mortality.[18,19] On the other hand, CRP itself has bioactive properties that may counterregulate the inflammatory response.[46–48] Nevertheless, these anti-inflammatory properties have been found only in studies using concentrations of CRP above the conventional normal range and may thus be less important for slightly increased levels of CRP. We found that high CRP levels were associated with a 2-fold increased risk of cardiovascular mortality. This result is in partial agreement with previous studies among apparently healthy subjects.[16–19] First, in contrast to previous studies,[17–19] we showed that the association of CRP with cardiovascular mortality was to a large extent explained by other risk factors (Table 5). Second, we found that the RR of mortality for CRP was stronger in the first few years of follow-up, whereas Ridker et al[18] found that this risk was stable in time, at least up to 8 years of follow-up. Third, we did not find a linear association of CRP level with mortality, whereas others did.[18,19] Fourth, although we did find evidence for a stronger association of CRP with mortality risk among women and among subjects aged >65 years, we could not demonstrate interactions, with regard to mortality risk, between CRP and current smoking,[16,49] total cholesterol levels,[50] or IHD.[16] Fifth, the determinants of CRP that we found (Table 1) were in line with some[51] but not all previous studies.[17] Taken together, these data suggest that the association between CRP and cardiovascular mortality differs between populations. This hypothesis requires further study.

We observed similar associations of vWf and CRP with RR of cardiovascular mortality among nondiabetic and diabetic subjects. NIDDM is associated with a 2- to 4-fold increase of cardiovascular mortality.[23] Various hypotheses have been put forward to explain the mechanism through which diabetes accelerates atherothrombosis. Potential glucose-mediated mechanisms include increased oxidative stress, increased concentrations of advanced glycation end products, and activation of the diacylglycerol–protein kinase C pathway,[5,52] which can directly or indirectly induce endothelial dysfunction, an acute phase response, and a procoagulant state.[5,52] In other words, the associations of vWf and CRP with cardiovascular mortality could be different between diabetic and nondiabetic subjects, because the underlying pathophysiological conditions that cause increased levels of vWf and CRP might be dissimilar. However, from our data, it seems that once levels of vWf and CRP are increased, their associations with cardiovascular mortality are similar among diabetic and nondiabetic subjects.

As has been shown previously, we found higher RRs of mortality associated with vWf[12,53] among women than among men. Furthermore, vWf was a stronger predictor in subjects aged >65 years and in the presence of hypertension or a low level of HDL cholesterol. We could neither prove nor disprove the presence of interaction between vWf and these last 3 variables; therefore, more comprehensive studies are necessary to address these issues.

The levels of vWf and CRP were both measured once, which may have led to nondifferential misclassification and, therefore, an underestimation of the RRs associated with mortality. The present study was too limited to assess, with much precision, above what threshold vWf and CRP are associated with mortality. Furthermore, a longer follow-up duration as necessary to establish or exclude definitively the

existence of time dependence in the associations of vWf and CRP with mortality risk. Although our data support the concept that levels of vWf and CRP predict cardiovascular mortality through different pathophysiological mechanisms, we did not investigate these possible mechanisms.

We have shown that the level of vWf is a strong independent predictor of cardiovascular and all-cause mortality in the general population, whereas the association of CRP with mortality is confounded by other risk factors. The risk estimates of cardiovascular mortality associated with vWf and CRP were mutually independent, suggesting that vWf and CRP predict mortality through different pathways. This, along with our finding that the risk predicted by vWf seems time dependent (stronger in the first few years of follow-up), gives support to the hypothesis that vWf is more likely to be a marker of risk for atherothrombotic than for atherosclerotic disease. From a therapeutic point of view, this is of clinical relevance, because antithrombotic agents have been shown to reduce the risk of a first myocardial infarction[54] and to provide a favorable outcome after a myocardial infarction.[44] Therefore, we suggest that the use of antithrombotic agents among subjects with high vWf levels might be particularly effective in reducing the risk of myocardial infarction. Randomized clinical trials are necessary to investigate this hypothesis.

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