

Original Article

Prognostic implications of retinopathy and a high plasma von Willebrand factor concentration in type 2 diabetic subjects with microalbuminuria

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Abstract

Background. Microalbuminuria in subjects with type 2 diabetes may be heterogeneous with respect to clinical features, renal histology, and prognosis. There may be at least two types of microalbuminuria in diabetes, namely with and without generalized endothelial dysfunction. We investigated whether, among microalbuminuric subjects with type 2 diabetes, the presence of generalized endothelial dysfunction, as indicated by the presence of retinopathy or a high plasma von Willebrand factor (vWf) level, has prognostic implications.

Methods. In 173 type 2 diabetic subjects of a population-based cohort, we assessed the urinary albumin-to-creatinine ratio, the plasma vWf level, and the presence of retinopathy. The main outcome was cardiovascular mortality.

Results. The absolute difference in 7 years' cardiovascular mortality between microalbuminuric (albumin-to-creatinine ratio 2.0–30.0 mg/mmol) and normoalbuminuric subjects was higher in the presence as compared to the absence of retinopathy (55.6 vs 11.1%). The age- and sex-adjusted relative risk (95% confidence interval) of cardiovascular mortality, as compared to normoalbuminuric subjects without retinopathy, was 1.1 (0.1–9.2) for normoalbuminuric subjects with retinopathy, 1.8 (0.5–6.7) for microalbuminuric subjects without retinopathy, and 9.8 (3.1–30.9) for microalbuminuric subjects with retinopathy. The absolute difference in risk of 7 years' cardiovascular mortality between microalbuminuric and normoalbuminuric subjects was higher in the

presence as compared to the absence of a high (>1.89 IU/ml) vWf level (49.8 vs 16.4%). The age- and sex-adjusted relative risk of cardiovascular mortality, as compared to normoalbuminuric subjects without a high vWf level, was 1.5 (0.4–5.5) for normoalbuminuric subjects with a high vWf level, 2.6 (0.7–9.6) for microalbuminuric subjects without a high vWf level, and 12.0 (2.9–49.5) for microalbuminuric subjects with a high vWf level. These differences in risk of cardiovascular mortality did not change materially after further adjustment for known duration of diabetes, hypertension, creatinine clearance, level of glycated haemoglobin and high-density lipoprotein cholesterol, and presence of cardiovascular disease. Analysis of all-cause instead of cardiovascular mortality showed a similar difference in risk of mortality between microalbuminuric subjects with or without retinopathy or a high vWf level.

Conclusions. Among type 2 diabetic subjects with microalbuminuria, the presence of retinopathy or a high plasma vWf level affects the risk of cardiovascular death. Although larger studies are necessary, these findings support the concept that microalbuminuria in type 2 diabetes can occur in the absence or the presence of generalized endothelial dysfunction, and that the latter is a much more 'malignant' condition than the former.

Keywords: endothelial dysfunction; heterogeneity; microalbuminuria; retinopathy; type 2 diabetes; von Willebrand factor

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Introduction

Microalbuminuria is associated with an increased risk of cardiovascular mortality among type 2 diabetic

subjects [1–5]. Recently it has been suggested that microalbuminuria is heterogeneous with respect to renal histology, clinical features, and prognosis [6]. A biopsy study among type 2 diabetic subjects with persistent microalbuminuria showed that ‘typical’ diabetic glomerulopathy was present in only 29.4%, whereas ‘atypical’ lesions or normal renal structure were present in 41.2 and 29.4% respectively [7]. In addition, diabetic subjects with ‘typical’ diabetic glomerulopathy, as compared to those with ‘atypical’ lesions or normal renal structure, more often had proliferative retinopathy; they also had higher plasma levels of von Willebrand factor (vWf), a marker of endothelial dysfunction [7,8]. Moreover, Stehouwer *et al.* [9] have shown that microalbuminuria in type 2 diabetic subjects was associated with an increased risk of cardiovascular events *only* in the presence of high levels of vWf. Thus, there may be at least two types of microalbuminuria in diabetes, namely *without* and *with* generalized endothelial dysfunction, the latter being characterized by more severe retinopathy, higher vWf concentrations, and a poorer cardiovascular prognosis [6].

We hypothesized that cardiovascular disease risk is considerably higher in microalbuminuric type 2 diabetic subjects with retinopathy or a high vWf concentration than in either normoalbuminuric subjects or in microalbuminuric subjects without retinopathy or without a high vWf concentration. We therefore investigated, in a population-based cohort of type 2 diabetic subjects, the association of microalbuminuria with cardiovascular and all-cause mortality in the absence or presence of retinopathy or high levels of vWf.

Subjects and methods

The study population consisted of 173 type 2 diabetic subjects (of whom 106 were newly diagnosed) participating in a population-based survey on disturbances of glucose tolerance among Caucasian subjects aged 50–75 years, conducted from October 1989 until February 1992, which has been described previously [10]. Type 2 diabetes was defined as a mean fasting glucose level of ≥ 7.8 mmol/l and/or a mean 2-h post-load glucose level of ≥ 11.1 mmol/l, based on two oral glucose tolerance tests, or treatment with oral blood glucose-lowering agents or insulin (WHO). The study was approved by the local ethics committee and each participant gave informed consent.

Microalbuminuria was defined as an albumin-to-creatinine ratio 2.0–30.0 mg/mmol in an early morning first voided spot urine [11]. In a random sample of 38 subjects (23%), the albumin-to-creatinine ratio was based on the mean of two measurements [11]. vWf concentrations were assessed (in duplicate) in deep-frozen (-70°C) heparin plasma by enzyme-linked immunosorbent assay [12] using polyclonal antibodies from Dako (Glostrup, Denmark), and expressed as percentage of vWf in pooled plasma of healthy volunteers. By comparison to the 4th International Standard for von Willebrand factor in plasma (NIBSC code 97/586) the pooled citrated plasma contained 1.03 IU/ml of vWf antigen. A high level of vWf was defined as a vWf level in the upper

tertile of this population (>1.89 IU/ml). Retinopathy was assessed by direct (60 dioptre) and indirect ophthalmoscopy ($n=171$) and, in a representative sample, by fundus photography ($n=123$). Both findings were graded according to the modified Arlie House classification [13]. The fundus photographs were independently graded by two ophthalmologists, and in case of disagreement the decision of a third ophthalmologist was taken to be conclusive. Any retinopathy was defined as the presence of one or more haemorrhages, microaneurysms, soft and hard exudates, neovascularization and/or laser coagulation scars in at least one eye.

We obtained data on blood pressure, weight, height, body mass index and smoking habits [10]. Hypertension was defined as diastolic pressure ≥ 95 mmHg, systolic pressure ≥ 160 mmHg and/or the use of antihypertensive drugs [11]. Current smoking was defined as currently smoking cigarettes and/or cigars. We also obtained data on plasma glycated haemoglobin and C-reactive protein, soluble vascular cell adhesion molecule-1 and serum creatinine, homocysteine, total cholesterol, high-density lipoprotein cholesterol and triglycerides [10,11,14–16]. Serum low-density lipoprotein cholesterol was calculated by the Friedewald formula and the creatinine clearance was calculated by the Cockcroft–Gault formula [11]. We obtained an ankle–brachial blood pressure index and a resting electrocardiogram [10]. Subjects were classified as having cardiovascular disease when they had a Minnesota code 1.1–1.3, 4.1–4.3, 5.1–5.3 or 7.1 on the electrocardiogram at rest and/or had undergone coronary bypass surgery or angioplasty [11]; and/or had an ankle–brachial pressure index less than 0.9 and/or had undergone a peripheral arterial bypass or amputation [10,11].

Follow-up data on the subjects’ vital status on 1 January 1999 were collected from the mortality register of the municipality of Hoorn. For each subject, we determined whether or not death had occurred, and if so, when. For all subjects who had died, the cause of death was extracted from the medical records of the general practitioner and the local hospital and classified according to the International Classification of Diseases, 9th edn. Cardiovascular mortality was defined as codes 390–459. Information on the cause of death could not be obtained for three (8%) of the deceased subjects and one subject was lost to follow-up.

All analyses were performed with SPSS 7.5 for Windows 95. Our main intent was to analyse whether the presence of retinopathy or a high vWf level influenced the difference in cardiovascular mortality risk between micro- and normoalbuminuric subjects. Subjects were therefore stratified by the presence or absence of microalbuminuria and retinopathy and, in a separate analysis, by the presence or absence of microalbuminuria and a high vWf concentration. We chose normoalbuminuric subjects without retinopathy or high vWf concentration as the reference group. Cardiovascular survival was calculated by the Kaplan–Meier method and differences were tested by the log-rank test. Determinants of cardiovascular and all-cause mortality were determined by Cox’ proportional hazards multiple regression analyses. Results are described as relative risks (hazard ratios) with 95% confidence intervals. Two-sided P values <0.05 were considered statistically significant.

Results

Of the 173 subjects, five had macroalbuminuria and nine were excluded because they used an angiotensin-converting enzyme inhibitor. In addition, 10 urine

samples were lost, retinopathy could not be assessed in two subjects, and no plasma was available in four subjects. The analyses including retinopathy were thus based on 147 subjects, and those including vWf on 145.

The prevalence of microalbuminuria was 28/154 (18%). Among the 28 subjects with microalbuminuria, nine (32%) had retinopathy, and seven (25%) had a high vWf level. Of the nine subjects with microalbuminuria and retinopathy, four (44%) had vWf >1.89 IU/ml (upper tertile, i.e. 'high') and eight (89%) had vWf >1.50 IU/ml.

The mean duration of follow-up was 7.0 (2.1) years with a range of 0.6–9.2 years. After adjustment for age and sex, the relative risk of cardiovascular mortality for subjects with microalbuminuria, as compared to those with normoalbuminuria, was 3.6 (1.4–9.5) (Figure 1). After additional adjustment for glycated haemoglobin, hypertension, and known duration of diabetes, the relative risk was 2.8 (1.0–8.1). Further adjustment for high-density lipoprotein cholesterol level, creatinine clearance, and the presence of cardiovascular disease did not materially change the results (data not shown).

Retinopathy

Microalbuminuric subjects *with* retinopathy, as compared to microalbuminuric subjects *without* retinopathy and to normoalbuminuric subjects *with* or *without* retinopathy, were older, had higher levels of glycated haemoglobin and systolic blood pressure, a longer known duration of diabetes, and a higher prevalence of hypertension (Table 1). Seven years' cardiovascular mortality was higher in microalbuminuric subjects with retinopathy than in microalbuminuric subjects without retinopathy and in normoalbuminuric subjects with and without retinopathy (55.6% *vs* 15.8%, 0% and

4.7% respectively; $P=0.0009$; Figure 2). The absolute difference in 7 years' cardiovascular mortality between microalbuminuric and normoalbuminuric subjects was much higher in the presence as compared to the absence of retinopathy (55.6 *vs* 11.1% respectively; Table 2).

Table 2 shows that, as compared to normoalbuminuric subjects without retinopathy, microalbuminuric subjects with retinopathy had a significantly increased risk of cardiovascular mortality (age- and sex-adjusted relative risk, 9.8), but microalbuminuric subjects without retinopathy and normoalbuminuric subjects with retinopathy did not. As shown in Table 2, these relative risks were not importantly affected by adjustment for hypertension, known duration of diabetes, glycated haemoglobin, high-density lipoprotein cholesterol, creatinine clearance, and presence of cardiovascular disease. Additional adjustment for body mass index, smoking habits and level of cholesterol, triglycerides and homocysteine did not materially change the results (data not shown). Analyses performed in subjects with and without retinopathy separately gave similar results (e.g. relative risk of cardiovascular mortality associated with microalbuminuria, 9.3 and 1.9 respectively, after adjustment for age and sex).

High levels of vWf

Microalbuminuric subjects with a high vWf level, as compared to microalbuminuric subjects without a high vWf level and to normoalbuminuric subjects with or without a high vWf level, were older, had higher levels of glycated haemoglobin and systolic blood pressure and a higher prevalence of hypertension and retinopathy (Table 1). Seven years' cardiovascular mortality was higher in microalbuminuric subjects with a high

Fig. 1. Cardiovascular survival (Kaplan–Meier) according to the presence or absence of microalbuminuria. *Difference between the groups after adjustment for age and sex, log-rank test.

Table 1. Baseline characteristics of type 2 diabetic subjects according to the absence or presence of microalbuminuria, retinopathy, and a high vWf plasma concentration

	Normoalbuminuria		Microalbuminuria		Normoalbuminuria		Microalbuminuria	
	without retinopathy	with retinopathy	without retinopathy	with retinopathy	with vWf ≤ 1.89 IU/ml	with vWf >1.89 IU/ml	with vWf ≤ 1.89 IU/ml	with vWf >1.89 IU/ml
Gender (male/female)	59/48	7/5	11/8	8/1	40/36	22/19	13/8	6/1
Age (years)	65 \pm 7	67 \pm 5	67 \pm 6	70 \pm 4 \ddagger	65 \pm 6	66 \pm 7	67 \pm 5	71 \pm 5 \ddagger
Glycated haemoglobin (%)	6.8 \pm 1.8	7.9 \pm 2.7	7.7 \pm 2.0	8.6 \pm 1.2 \ddagger	6.8 \pm 1.7	7.4 \pm 2.2	7.8 \pm 1.7	8.6 \pm 2.0 \ddagger
Systolic blood pressure (mmHg)	139 \pm 17	146 \pm 24	150 \pm 19	159 \pm 19 \ddagger	140 \pm 19	141 \pm 16	152 \pm 20	157 \pm 16 \ddagger
Hypertension (%)	47	58	84	89 \ddagger	47	46	81	100 \ddagger
Known diabetes duration (years)*	0.0 (0.0–3.0)	0.0 (0.0–15.5)	1.0 (0.0–5.9)	7.7 (2.4–10.3) \ddagger	0.0 (0.0–2.5)	0.0 (0.0–6.5)	2.2 (0.0–7.7)	5.0 (1.2–9.0)
Body mass index (kg/m ²)	28.3 \pm 4.2	28.9 \pm 3.0	28.5 \pm 5.2	31.4 \pm 3.2	28.7 \pm 3.4	27.6 \pm 5.1	29.5 \pm 4.7	29.2 \pm 5.3
ACR (mg/mmol)*	<2.0	<2.0	4.7 (2.7–10.2)	4.6 (3.2–5.0)	<2.0	<2.0	3.9 (2.5–7.1)	4.9 (4.6–8.9)
Creatinine clearance (ml/min)	81.7 \pm 18.5	81.9 \pm 18.7	78.8 \pm 19.8	73.3 \pm 22.4	83.5 \pm 18.0	79.2 \pm 19.7	79.5 \pm 19.6	69.6 \pm 22.4
Total cholesterol (mmol/l)	6.4 \pm 1.3	7.1 \pm 1.4	6.5 \pm 1.5	6.7 \pm 0.9	6.4 \pm 1.3	6.4 \pm 1.3	6.3 \pm 1.3	7.4 \pm 1.2
Triglycerides (mmol/l)*	1.9 (1.4–2.7)	2.1 (1.5–3.7)	1.8 (1.2–3.1)	2.3 (1.4–4.6)	2.1 (1.4–2.8)	1.8 (1.4–2.7)	1.7 (1.2–2.7)	3.1 (1.9–6.8)
HDL cholesterol (mmol/l)	1.14 \pm 0.27	1.13 \pm 0.15	1.15 \pm 0.24	1.13 \pm 0.42	1.12 \pm 0.24	1.17 \pm 0.32	1.14 \pm 0.25	1.17 \pm 0.44
LDL cholesterol (mmol/l)	4.2 \pm 1.1	4.8 \pm 1.1	4.2 \pm 1.1	4.2 \pm 1.2	4.2 \pm 1.1	4.2 \pm 1.3	4.1 \pm 1.1	4.5 \pm 1.2
Cardiovascular disease (%)	27	67	26	56	32	32	29	57
vWf (IU/ml)	1.67 \pm 0.74	1.65 \pm 0.79	1.22 \pm 0.65	1.90 \pm 0.68	1.23 \pm 0.41	2.48 \pm 0.47	1.13 \pm 0.50	2.35 \pm 0.48
vWf >1.89 IU/ml (%)	35	42	16	44	0	100	0	100
Retinopathy (%)	0	100	0	100	9	12	24	57 \ddagger

Mean (SD) unless otherwise indicated. *Median (IQR). Differences between the three groups were tested with ANOVA. In case of a significant difference, the Student–Newman–Keuls test was used to distinguish differences among the groups. Possible trends were tested by regression analyses; $\ddagger P < 0.05$, $\ddagger\ddagger P < 0.01$. vWf, von Willebrand factor; ACR, albumin-to-creatinine ratio; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Fig. 2. Cardiovascular survival (Kaplan–Meier) according to the presence or absence of microalbuminuria and retinopathy. *Difference among the groups after adjustment for age and sex, log-rank test. \ddagger Difference between the groups after adjustment for age and sex, log-rank test. ① Normoalbuminuria, retinopathy absent; ② normoalbuminuria, retinopathy present; ③ microalbuminuria, retinopathy absent; ④ microalbuminuria, retinopathy present. Percentages shown at the right indicate survival after 7.0 (2.1) years.

Table 2. Relative risk of cardiovascular mortality associated with the presence of normo- or microalbuminuria in the absence or presence of retinopathy or a high (>1.89 IU/ml) vWf plasma concentration

	Cardiovascular mortality (relative risk (95% CI))*			Cardiovascular mortality (relative risk (95% CI))*		
	Normoalbuminuria with retinopathy	Microalbuminuria without retinopathy	Microalbuminuria with retinopathy	Normoalbuminuria with a high vWf	Microalbuminuria without a high vWf	Microalbuminuria with a high vWf
Model; variables adjusted for:						
(i) Age and sex	1.1 (0.1–9.2)	1.8 (0.5–6.7)	9.8 (3.1–30.9)§,	1.5 (0.4–5.5)	2.6 (0.7–9.6)	12.0 (2.9–49.5)§,¶, #
(ii) As (i) plus hypertension, known duration of diabetes, and GHb††	1.1 (0.1–8.8)	1.4 (0.4–5.8)	8.7 (2.2–35.0)§,	1.5 (0.4–5.6)	2.2 (0.6–8.6)	11.0 (2.1–57.3)§,¶, #
(iii) As (ii) plus HDL cholesterol, creatinine clearance, CVD†	1.3 (0.2–10.7)	1.5 (0.3–6.8)	8.6 (2.1–36.1)§,	1.4 (0.4–5.7)	2.6 (0.6–11.2)	9.0 (1.6–50.5)§,

*Relative risk (95% confidence interval) of cardiovascular mortality associated with microalbuminuria obtained with Cox regression analyses. Normoalbuminuric subjects without retinopathy or a high (>1.89 IU/ml) vWf level are the reference group. Model (ii): as model (i) plus other potential risk factors selected on pathophysiological grounds. Model (iii) as model (ii) plus all risk factors associated with cardiovascular mortality in the present study ($P < 0.2$). †Adjustment for these risk factors one by one (with age and sex already in the model) gave similar results (data not shown). ‡Additional adjustment for smoking habits, body mass index (BMI) and level of cholesterol, triglycerides and homocysteine gave similar results (data not shown). § $P < 0.05$ vs reference group. || $P < 0.05$ vs microalbuminuria without retinopathy. ¶ $P < 0.05$ vs normoalbuminuria with a high vWf level. # $P < 0.05$ vs microalbuminuria without a high vWf level. Potential confounders measured on a continuous scale were used as such in the regression models, except for high-density lipoprotein (HDL) cholesterol and BMI, because the association of these variables with mortality was non-linear. Therefore a low HDL cholesterol was defined as a level below 0.9 mmol/l and obesity as a BMI above 27 kg/m² for men and above 26 kg/m² for women. Levels of triglyceride were log-transformed because of a better fit of the regression model. GHb, glycated haemoglobin; CVD, cardiovascular disease.

vWf level than in microalbuminuric subjects without a high vWf level and in normoalbuminuric subjects with and without a high vWf level (57.1% vs 19.0%, 7.3% and 2.6% respectively; $P=0.001$; Figure 3). The absolute difference in 7 years' cardiovascular mortality between microalbuminuric and normoalbuminuric subjects was higher in the presence as compared to the absence of a high vWf level (49.8 vs 16.4% respectively).

Table 2 shows that, as compared to normoalbuminuric subjects without a high vWf level, microalbuminuric subjects with a high vWf level had a significantly increased risk of cardiovascular mortality (age- and sex-adjusted relative risk, 12.0), but microalbuminuric subjects without a high vWf level and normoalbuminuric subjects with a high vWf level did not. As shown in Table 2, these relative risks were not importantly affected by adjustment for hypertension, known duration of diabetes, glycated haemoglobin, high-density lipoprotein cholesterol, creatinine clearance, and presence of cardiovascular disease. Additional adjustment for body mass index, smoking habits and level of cholesterol, triglyceride, and homocysteine did not materially change the results (data not shown). Analyses performed in subjects with and those without a high vWf level separately gave similar results (e.g. relative risk of cardiovascular mortality associated with microalbuminuria, 10.6 and 2.8 respectively, after adjustment for age and sex).

Additional analyses

Microalbuminuria defined as albumin-to-creatinine ratio >3.0 mg/mmol, or defined as an albumin-to-creatinine ratio >2.5 mg/mmol for men and an

albumin-to-creatinine ratio >3.5 mg/mmol for women, did not materially change the results (data not shown). Consideration of all-cause instead of cardiovascular mortality somewhat decreased the relative risks associated with the presence of microalbuminuria with retinopathy or a high vWf level, but did not diminish the difference in risk of mortality between microalbuminuric subjects with or without retinopathy or a high vWf level (data not shown). A high vWf level defined as vWf level >1.50 IU/ml slightly altered the relative risks and somewhat diminished the difference in risk of cardiovascular mortality between microalbuminuric subjects with or without a high vWf level. For example, the relative risk of cardiovascular mortality after adjustment for age, sex, and other risk factors as in model 3 of Table 2, as compared to subjects with normoalbuminuria without a vWf level >1.50 IU/ml, was 0.9 (0.2–3.9) for subjects with normoalbuminuria with a vWf level >1.50 IU/ml; 2.0 (0.4–10.5) for subjects with microalbuminuria without vWf level >1.50 IU/ml; and 4.3 (0.8–22.0) for subjects with microalbuminuria with a vWf level >1.50 IU/ml. ABO blood group is a determinant of vWf level. For example, among subjects with a vWf level >1.89 IU/ml, 35% had blood group O and 65% had blood group non-O. The mean vWf levels among subjects with blood group O and blood group non-O were 1.45 (0.73) IU/ml and 1.78 (0.78) IU/ml respectively. Additional adjustment for ABO blood group, however, did not materially affect the results (data not shown).

Finally, we investigated whether these differences in risk of cardiovascular mortality between microalbuminuric subjects with or without retinopathy and with or without a high vWf level were specific for

Fig. 3. Cardiovascular survival (Kaplan–Meier) according to the presence or absence of microalbuminuria and high levels of vWf (vWf >1.89 IU/ml). *Difference among the groups after adjustment for age and sex, log-rank test; †difference between the groups after adjustment for age and sex, log-rank test. ① Normoalbuminuria; vWf levels ≤ 1.89 IU/ml; ② normoalbuminuria; vWf levels >1.89 IU/ml; ③ microalbuminuria; vWf levels ≤ 1.89 IU/ml; ④ microalbuminuria; vWf levels >1.89 IU/ml. Percentages shown at the right indicate survival after 7.0 (2.1) years

retinopathy and a high vWf level or that they were related to a general increase in risk. We therefore stratified the subjects by the presence or absence of microalbuminuria and cardiovascular disease; microalbuminuria and hypertension; microalbuminuria and a high glycated haemoglobin level ($>7.5\%$, i.e. highest tertile); microalbuminuria and a high C-reactive protein level (marker of low-grade inflammation, >3.37 mg/l, i.e. highest tertile); and microalbuminuria and a high soluble vascular cell adhesion molecule-1 level (marker of generalized vascular dysfunction; >1548 ng/ml, i.e. highest tertile), and then performed similar analyses. In summary, none of these stratification procedures showed microalbuminuria to be more strongly associated with cardiovascular mortality in the presence than in the absence of cardiovascular disease, hypertension, or a high glycated haemoglobin, C-reactive protein, or soluble vascular cell adhesion molecule-1 level. For example, the relative risk of cardiovascular mortality associated with the presence of microalbuminuria was 4.9 in subjects with and 4.4 in those without cardiovascular disease after adjustment for age and sex.

Discussion

There is consensus that cardiovascular mortality is higher in type 2 diabetic subjects with microalbuminuria than in those with normoalbuminuria [1–5]. In agreement with this concept, the present study found that microalbuminuria was associated with a 3.6-fold increase in cardiovascular mortality. An important new finding was that the relative risk of cardiovascular mortality associated with the presence of microalbuminuria differed markedly according to the presence or absence of retinopathy or a high plasma vWf concentration: it was 9.3 in the presence of retinopathy, as compared to 1.9 in its absence; and it was 10.5 in the presence of a high vWf level, as compared to 2.8 in its absence. These findings lend considerable support to the concept of heterogeneity of microalbuminuria with respect to generalized endothelial dysfunction and, moreover, are of direct clinical importance.

We found that the presence of retinopathy or a high vWf level strongly affected cardiovascular mortality associated with the presence of microalbuminuria. This could not be explained by more severe microalbuminuria, because the albumin-to-creatinine ratio was similar whether or not retinopathy or a high vWf level was present (Table 1). This could also not be explained by a worse profile of cardiovascular risk factors, since adjustment for these risk factors in the analyses did not materially change the results (Table 2). We favour the interpretation that, given the presence of microalbuminuria, the additional presence of retinopathy or a high vWf level reflects generalized endothelial dysfunction, a key concept in the pathogenesis of atherothrombotic disease [17], and that this explains the very high cardiovascular mortality

among type 2 diabetic patients with both microalbuminuria and either retinopathy or a high vWf level (Figures 1 and 2). Thus 89% of subjects with microalbuminuria and retinopathy had vWf levels >1.50 IU/ml (the conventional upper limit of the normal vWf range).

It might be argued that the relative mortality risk associated with microalbuminuria may increase with the absolute background mortality risk. We investigated this possibility by comparing the relative mortality risk associated with microalbuminuria among subjects with and without other risk factors, such as a history of cardiovascular disease, or risk indicators, such as a high C-reactive protein level. Relative mortality risks associated with microalbuminuria were similar regardless of the presence of such factors. We conclude, therefore, that heterogeneity of microalbuminuria with respect to prognosis seems to be specific for the presence or absence of retinopathy or a high vWf level, i.e. endothelial dysfunction. The proximate causes of generalized endothelial dysfunction in type 2 diabetes mellitus are unknown, although there are many possible candidates, such as increased protein kinase C activity, non-enzymatic glycation and oxidative stress, alterations in redox potential, and increased expression of transforming growth factor- β and/or tumour necrosis factor- α [18].

Microalbuminuria in the absence of retinopathy or a high vWf level was associated with about a twofold increased risk of cardiovascular mortality compared to normoalbuminuria, and therefore is not an entirely benign condition. It may be that microalbuminuria without retinopathy or a high vWf level is linked to cardiovascular disease through mechanisms other than generalized endothelial dysfunction, or that retinopathy and a high vWf level are insufficiently sensitive markers of the presence of generalized endothelial dysfunction. These issues require further investigation.

Regardless of their pathophysiological explanation, our findings strongly suggest that the presence of retinopathy or a high vWf level can be used clinically to identify microalbuminuric type 2 diabetic subjects at particularly high risk of cardiovascular death and thus to focus preventive measures [19,20]. Regular ophthalmological examinations among microalbuminuric type 2 diabetic patients may therefore be important not only for ophthalmological reasons but also for cardiovascular risk stratification. Furthermore, measurement of vWf concentration in these patients deserves serious consideration, although more studies are necessary to refine the definition of a high vWf concentration.

Microalbuminuria (mostly) and a high vWf level (entirely) were defined on the basis of only one measurement and fundus photography was performed in only 72% of subjects. These circumstances increase the chance of misclassification, which, however, was likely to be non-differential with regard to outcome and which, therefore would, if anything, lead to an underestimation of the strength of the reported associations.

This study tested a pre-specified and very specific hypothesis [6]. The results were robust in that various statistical adjustments did not affect the basic finding, i.e. the difference in cardiovascular mortality between microalbuminuric subjects with *vs* those without generalized endothelial dysfunction. We therefore consider it unlikely that confounding by unmeasured variables would seriously affect our conclusions. Nevertheless, our study was too small to assess with sufficient precision above what threshold of vWf level the mortality risk increased. Analyses with vWf >1.50 IU/ml (the conventional upper limit of normal) and >1.89 IU/ml generally gave similar results, except that the latter criterion sharpened the contrast between microalbuminuric patients with and without a 'high' vWf level. This may be due to the statistical phenomenon that a higher as compared to a lower cut-off will generally reduce the effect of misclassification if the group with levels above the cut-off is substantially smaller than the group with levels below the cut-off. Finally, the overlap between microalbuminuric subjects with retinopathy and with a high vWf level was not complete. This may be the play of chance, but we cannot exclude that there are true differences between these categories.

We conclude that the risk of cardiovascular death among microalbuminuric type 2 diabetic patients is much higher when retinopathy or a high vWf concentration is present than when these factors are absent. This supports the concept that microalbuminuria in type 2 diabetes mellitus can occur in the absence or the presence of generalized endothelial dysfunction, and that the latter is a much more 'malignant' condition than the former.

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