White Blood Cell Count Is Associated With Macro- and Microvascular Complications in Chinese Patients With Type 2 Diabetes

PETER C. TONG, PHD[1]
KA-FAI LEE, MBCHB[1]
WING-YEE SO, MBCHB[1]
MARGARET H. NG, MD[2]
WING-BUN CHAN, MBCHB[1]
MATTHEW K. LO, MBCHB[1]
NORMAN N. CHAN, MD[1]
JULIANA C. CHAN, MD[1]

OBJECTIVES — There are close associations among raised white blood cell (WBC) count, coronary heart disease, and metabolic syndrome in the general population. The association between WBC count and vascular complications of diabetes has not been explored. We carried out a cross-sectional cohort study to determine the association between WBC count and the presence of macro- and microvascular complications in type 2 diabetes.

RESEARCH DESIGN AND METHODS — In this study, 3,776 patients with type 2 diabetes and normal WBC count (3.5–12.5 $\times$ 10^9/l) underwent a comprehensive assessment of complications and cardiovascular risk factors based on the European DiabCare protocol. Demographic and anthropometric parameters were recorded. Metabolic profiles, including complete blood picture and urinary albumin excretion, were measured.

RESULTS — Patients with higher WBC counts (categorized into quintiles) had adverse metabolic profiles as evidenced by higher blood pressure, BMI, HbA_{1c}, fasting plasma glucose, LDL cholesterol, triglycerides, and urinary albumin excretion, but lower HDL cholesterol (all P $<$0.001 for trend). The prevalence of macro- and microvascular complications increased in a dosage-related manner with WBC count. After adjustments for smoking and other known cardiovascular risk factors, a 1-unit (1 $\times$ 10^9/l) increment of WBC count was associated with a 15.8% (95% CI 9.3–22.6; $P < 0.001$) and 12.3% increase (5.8–19.1; $P < 0.001$) in the prevalence of macro- and microvascular complications, respectively.

CONCLUSIONS — Elevated WBC count, even within the normal range, is associated with both macro- and microvascular complications in type 2 diabetes. Chronic inflammation, as indicated by a higher WBC count, may play a linkage role in the development of macro- and microvascular complications in diabetes.

Diabetes Care 27:216–222, 2004

Diabetes is a progressive chronic disease that is emerging as a global epidemic (1,2). Diabetes imposes a tremendous burden on health economies mainly because of its devastating complications. A long duration of metabolic disturbances can cause vascular damage, leading to both macro- and microvascular complications. Patients with type 2 diabetes have an increased risk for coronary heart disease, stroke, and peripheral vascular disease. Many conventional risk factors have been shown to be important contributors to the development of diabetic complications. Nevertheless, these risk factors cannot fully account for the excess risk produced by diabetes (3). There is increasing evidence that atherosclerosis is accompanied by inflammation (4). White blood cell (WBC) count, fibrinogen, and C-reactive proteins are all positively associated with increased cardiovascular mortality, mainly from coronary heart disease and ischemic stroke (5–9). In contrast, the impact of inflammation on microangiopathy is less well established. More importantly, there has been little research on the relation between WBC count and vascular complications of diabetes, although a recent report did suggest an association between WBC count and albuminuria in type 2 diabetes (10). Therefore, we carried out a cross-sectional analysis to investigate the association of WBC count, a biomarker of inflammation, with macro- and microvascular complications in a consecutive cohort of Chinese patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

— The Prince of Wales Hospital is the teaching hospital of the Chinese University of Hong Kong and serves a population of >1.2 million. Each week, the Diabetes Clinic registers 15–20 new patients who were referred from the community and hospital clinics or discharged from the hospital and reviews 120–150 patients. Since 1995, as part of a continuous quality improvement program, all newly referred patients to the clinic underwent a comprehensive assessment of complications and risk factors based on the European DiabCare protocol (11). This included documentation of de-

From the [1]Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, N.T., Hong Kong; and the [2]Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong, The Prince of Wales Hospital, Shatin, N.T., Hong Kong.

Address correspondence and reprint requests to Dr. Peter C.Y. Tong, Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong. E-mail: ptong@cuhk.edu.hk.

Received for publication 6 July 2003 and accepted in revised form 5 October 2003.

Abbreviations: TG, triglyceride; TGF-β1, transforming growth factor-β1; UAE, urinary albumin excretion; WBC, white blood cell; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

mographic data, use of health care resources, and previous medication use.

Clinical assessments included measurement of BMI, waist-to-hip ratio (WHR), blood pressure, visual acuity, fundoscopy through dilated pupils, and foot examination using monofilament and a graduated tuning fork. Mean arterial pressure was defined as the sum of the diastolic blood pressure and 33% of the pulse pressure. Peripheral vascular disease was defined by the absence of foot pulses on palpation, confirmed by Doppler ultrasound examination of the ankle-to-brachial ratio of <0.9. Fundi were examined by a physician or ophthalmologist. Retinopathy was defined by the presence of dot and blot hemorrhages, hard exudates, cotton wool spots, neovascularization, laser scars, and a history of vitrectomy. Sensory neuropathy was diagnosed if two of the following findings were present: reduced sensation to monofilament examination in any part of the sole with normal skin, a score of ≤6/8 (age ≤65 years) or ≤4/8 (age >65 years) using the graduated tuning fork, or typical symptoms of numbness or abnormal sensation over both lower limbs. Fasting plasma blood samples were taken for measurement of glucose, lipid levels (including total cholesterol, HDL cholesterol, triglycerides [TGs], and calculated LDL cholesterol), and renal and liver function. Samples for complete blood count and HbA_{1c} were collected in EDTA tubes. Timed urine collections were obtained on two occasions. Normal albuminuria was defined as 24-h urinary albumin excretion (UAE) <30 mg/day, microalbuminuria as UAE of 30–300 mg/day, and macroalbuminuria as UAE >300 mg/day. The albuminuric status was defined by concordant results from both urine samples (12).

To minimize the confounding effect of infection, only subjects with a WBC count within the normal range (3.5–12.5 × 10^9/l) were included in the analysis. Patients presenting with symptoms suggestive of type 1 diabetes, defined as diabetic ketoacidosis, acute presentation with heavy ketonuria (3+), or continuous requirement of insulin within 1 year of diagnosis were excluded (13). At baseline, the presence of macrovascular complications was defined by a history of angina, myocardial infarction, stroke, or peripheral vascular disease. To enhance the specificity of microvascular complications of diabetes, both retinopathy and albuminuria were required to be present. Information on smoking habits was assessed by a standardized questionnaire. Patients' smoking status was classified as never having smoked, former smoker (ceased smoking for at least 1 year), or current smoker. In this study, former and current smokers were analyzed as a group and compared with those who had never smoked.

Laboratory assays

Plasma glucose was measured by a hexokinase method (Hitachi 911 automated analyzer; Boehringer Mannheim, Mannheim, Germany). HbA_{1c} was measured using an automated ion-exchange chromatographic method (Bio-Rad Laboratory, Hercules, CA; reference range 5.1–6.4%). The inter- and intra-assay coefficient of variation (CV) for HbA_{1c} was ≤3.1% at values <6.5%. Total cholesterol, TGs, and HDL cholesterol were measured by enzymatic methods on the Hitachi 911 automated analyzer using reagent kits supplied by the manufacturer. LDL cholesterol was calculated by Friedewald's equation for TG <4.5 mmol/l (14). The precision performance of these assays was within the manufacturer's specifications. Urinary creatinine (Jaffe's kinetic method) and albumin (immunoturbidimetry method) were also measured on the Hitachi 911 analyzer using reagent kits supplied by the manufacturer. The inter-assay precision CV was 12.0 and 2.3% for urinary albumin concentrations of 8.0 and 68.8 mg/l, respectively. The lowest detection limit was 3.0 mg/l. Plasma creatinine (Jaffe's kinetic method) was measured on a Dimension AR system (Dade

Table 1—*Clinical and metabolic characteristics of 3,776 subjects with type 2 diabetes, categorized according to WBC count quintiles*

	1st quintile (3.50–5.60)	2nd quintile (5.70–6.60)	3rd quintile (6.70–7.40)	4th quintile (7.50–8.60)	5th quintile (8.70–12.50)	*P*
n	723	825	719	742	767	—
Age (years)	59.4 ± 12.9	58.6 ± 13.0	58.6 ± 13.8	59.0 ± 13.8	59.4 ± 13.8	0.657
Male (%)	41.1	41.7	45.3	42.9	45.2	0.351
Smokers (%)	21.3	22.8	28.1	32.0	37.0	<0.001
Disease duration (years)	7.4 ± 6.1	7.0 ± 6.3	7.2 ± 6.4	7.2 ± 6.6	8.1 ± 7.3	0.046
Waist (cm)	82.2 ± 9.6	84.9 ± 9.6	85.8 ± 9.7	86.3 ± 10.4	87.4 ± 10.4	<0.001
BMI (kg/m^2)	23.9 ± 3.6	25.0 ± 3.8	25.1 ± 3.9	25.5 ± 4.2	25.6 ± 4.3	<0.001
Waist-to-hip ratio	0.87 ± 0.07	0.88 ± 0.06	0.89 ± 0.06	0.89 ± 0.07	0.90 ± 0.08	<0.001
Systolic blood pressure (mmHg)	132 ± 21	135 ± 20	138 ± 22	138 ± 22	138 ± 22	<0.001
Diastolic blood pressure (mmHg)	75 ± 11	77 ± 11	78 ± 12	78 ± 11	77 ± 11	<0.001
HbA_{1c} (%)	7.44 ± 1.84	7.70 ± 1.81	7.78 ± 1.81	7.92 ± 1.87	8.08 ± 1.83	<0.001
Fasting plasma glucose (mmol/l)	8.66 ± 3.51	8.83 ± 3.08	8.93 ± 3.44	9.08 ± 3.51	9.12 ± 3.68	0.004
Total cholesterol (mmol/l)	5.27 ± 1.31	5.32 ± 1.11	5.42 ± 1.18	5.48 ± 1.24	5.51 ± 1.17	<0.001
HDL cholesterol (mmol/l)	1.36 ± 0.40	1.27 ± 0.34	1.25 ± 0.37	1.23 ± 0.35	1.19 ± 0.31	<0.001
LDL cholesterol (mmol/l)	3.26 ± 0.96	3.32 ± 0.88	3.37 ± 1.00	3.42 ± 1.04	3.44 ± 0.98	<0.001
Triglycerides (mmol/l)	1.08 (0.75–1.56)	1.24 (0.90–1.95)	1.37 (0.96–2.10)	1.48 (1.00–2.24)	1.62 (1.12–2.31)	<0.001
Plasma creatinine (umol/l)	71 (60–86)	72 (60–87)	76 (62–92)	77 (63–94)	78 (64–100)	<0.001
UAE (mg/day)	13.3 (7.59–39.9)	16.1 (8.1–65.1)	22.7 (8.9–96.4)	23.8 (9.8–173.5)	42.8 (12.3–250.9)	<0.001

Data are means ± SD or median (interquartile range).

Behring, Deerfield, IL). A complete blood profile, including WBC count, was measured using an automated cell counter (GEN-S; Beckman Coulter, Miami, FL).

Statistical analysis

The analysis was performed using the SPSS (version 10.1) statistical package. Plasma TGs, plasma creatinine, and albuminuria were logarithmically transformed because of skewed distributions. All data are expressed as means ± SD or median (interquartile range), as appropriate. Student's *t* test or ANOVA was used for between-group comparisons for continuous variables, and the χ^2 test was used for categorical variables. WBC count was grouped into quintiles, and the adjusted odds ratio (OR) for these quintiles was computed with the lowest category as the referent group. The logistic regression model was used to estimate the OR (95% CI) for diabetic complications. In the regression analysis, known conventional risk factors for cardiovascular diseases were included as covariates in the final model. $P < 0.05$ (two tailed) was considered to be significant.

RESULTS — In this cross-sectional analysis of 3,776 type 2 diabetic patients, 42.1% were men and 28.7% were smokers. The mean age was 59.0 ± 13.5 years, and the duration of diabetes was 7.4 ± 6.6 years. The mean WBC count was 7.2 ± 1.7 $\times 10^9$/l. Table 1 summarizes the demographic, anthropometric, and metabolic characteristics of the study sample according to the quintiles of WBC count. Subjects with higher WBC counts had longer disease duration, higher systolic blood pressure, diastolic blood pressure, BMI, WHR, HbA_{1c}, fasting plasma glucose, LDL cholesterol, TGs, plasma creatinine, and UAE, and lower HDL cholesterol ($P < 0.001$ for trend for all). Univariate analysis revealed positive associations of WBC count with BMI, waist circumference, blood pressure, glycemic control, LDL cholesterol, TGs, and UAE, and a negative association with HDL cholesterol (all $P < 0.001$) (Table 2).

Of the 3,776 subjects, 14.4% had macrovascular complications at baseline. The prevalence rates of albuminuria and retinopathy were 42.8 and 26.5%, respectively. The frequencies of macro- and microvascular complications increased in a dosage-dependent manner as the WBC count increased ($P < 0.001$ for trend for all) (Fig. 1).

Smokers had a higher risk of having macrovascular complications (OR 1.83 [95% CI 1.51–2.21]; $P < 0.001$) and microvascular complications (1.36 [1.13–1.63]; $P = 0.001$) compared with nonsmokers. Using nonsmokers in the lowest quintile of WBC count as the referent, the age-adjusted ORs for macrovascular diseases in the highest WBC quintile were 2.88 (1.92–4.31; $P < 0.001$) for nonsmokers and 3.61 (2.33–5.59; $P < 0.001$) for former or current smokers (Fig. 2*A*). For the coexistence of retinopathy and albuminuria, the age-adjusted ORs in the highest WBC quintile were 2.54 (1.78–3.61; $P < 0.001$) for nonsmokers and 2.56 (1.73–3.81; $P = 0.003$) for former or current smokers (Fig. 3*A*).

After adjustment for sex, age, smoking status, disease duration, BMI, WHR, mean arterial pressure, HbA_{1c}, HDL cholesterol, and LDL cholesterol, patients with higher WBC counts remained at increased risk for having macrovascular (Fig. 2*B*) or microvascular complications (Fig. 3*B*) ($P < 0.001$ for trend for both). When entered as a continuous variable in the multivariate-adjusted models for both macro- and microvascular diseases, the WBC count remained a significant factor of complications after controlling for the conventional risk factors. For macrovascular complications, a 1-unit (1×10^9/l) increment of WBC count was associated with a 15.8% (95% CI 9.3–22.6; $P < 0.001$) increased risk. The correlation between WBC count and macrovascular complications remained significant after the inclusion of UAE as a covariate in the final model (1.14 [1.07–1.21]; $P < 0.001$). For microvascular complications, the risk was increased by 12.3% (5.8–19.1; $P < 0.001$) with each unit increment of WBC count.

CONCLUSIONS — In this large-scale cross-sectional study involving 3,776 Chinese patients with type 2 diabetes, WBC count, even within the normal range, was independently associated with

Table 2—*Univariate relation of WBC count with selected anthropometric and metabolic characteristics in 3,776 subjects with type 2 diabetes*

Characteristic	WBC count
Age	0.006
Duration of diabetes	0.039*
BMI	0.137†
Waist circumference	0.165†
Systolic blood pressure	0.088†
Diastolic blood pressure	0.068†
HbA_{1c}	0.120†
Fasting plasma glucose	0.048*
HDL cholesterol	−0.145†
LDL cholesterol	0.080†
Triglycerides‡	0.196†
Plasma creatinine‡	0.123†
Urinary albumin excretion‡	0.206†

Determined using Pearson's correlation coefficient. *$P < 0.05$; †$P < 0.001$; ‡log transformed.

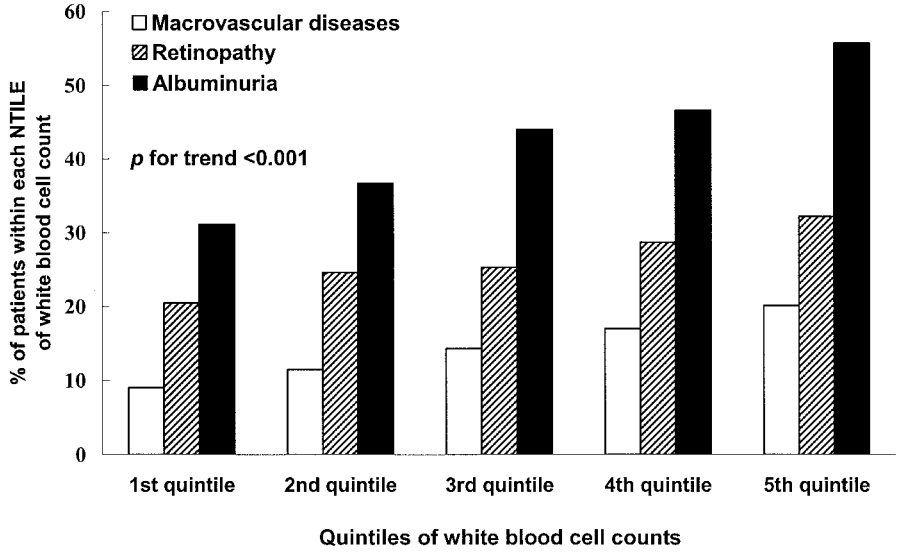

Figure 1—*Prevalence of macrovascular diseases, retinopathy, and albuminuria within each quintile of WBC count in 3,776 patients with type 2 diabetes.*

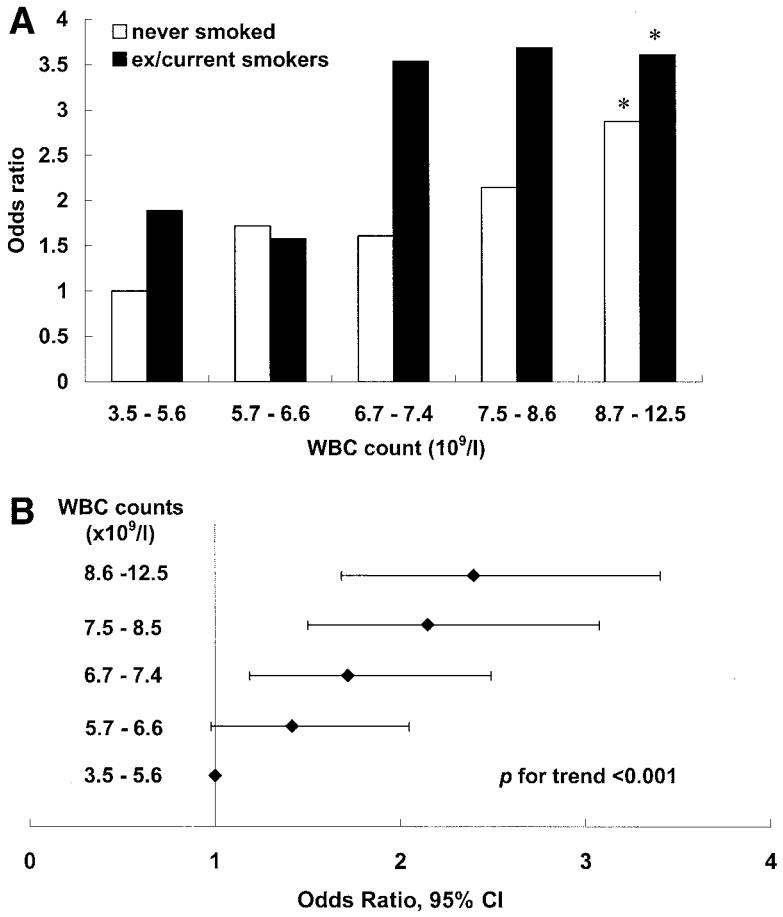

Figure 2—*Age-adjusted OR (*A*) and multivariate-adjusted OR (*B*) and 95% CI for macrovascular diseases by quintiles of WBC count in 3,776 patients with type 2 diabetes according to smoking status. OR estimates were obtained using the lowest quintile of those who never smoked as the referent.* *P *for trend <0.001.*

both macro- and microvascular complications of diabetes in a dosage-related manner and after controlling for conventional risk factors, including smoking, blood pressure, lipids, albuminuria, and glucose control as well as obesity. This finding is in agreement with current evidence regarding the association of inflammatory markers, including WBC count, with the development of metabolic syndrome, coronary heart disease, and all-cause mortality (4–8,15–20).

In this study, the prevalence of macrovascular disease was 2.4-fold higher in subjects with WBC count >8.6 $\times 10^9$/l than in those with counts <5.6 $\times 10^9$/l. Despite the potential effects of smoking on WBC count (21,22) and the controversy regarding the effect of WBC count on cardiovascular diseases independent of smoking (5,23,24), we were able to demonstrate a dosage-dependent association between WBC count and macrovascular diseases in both smokers and nonsmokers.

Chinese patients with type 2 diabetes have an excessive burden of microvascular complications (25). In our study cohort, the prevalence rates of retinopathy and albuminuria were two and three times the rate of macrovascular complications, respectively. We even defined microvascular complications as the presence of both retinopathy and albuminuria to minimize false positive classification. The OR of microvascular complications increased in a stepwise fashion with progressive quintiles of WBC count. This finding is in agreement with that of the Insulin Resistance Atherosclerosis Study, which showed an association of C-reactive protein and fibrinogen with albuminuria in subjects with type 2 diabetes (26). A recent study has also demonstrated a relation between albuminuria and WBC count (10), as is seen in our present study. By contrast, the association between fibrinogen and albuminuria was less reproducible, although the former parameter was not measured in our present study (27–31). Unlike macrovascular complications, smoking status did not appear to have an additional effect on the prevalence of microvascular complications in our study.

The close association between the WBC count and both micro- and macrovascular complications raises the hypothesis that inflammation may be a common linking factor. In support of this notion, the inflammatory process is now recognized to be a major component of atherosclerosis (4). Mononuclear leukocytes are recruited to the site of endothelial injury and form foam cells in the plaque (32). Activation of neutrophil leads to changes in rheological properties and adherence to the endothelium, all of which lead to capillary plugging and tissue ischemia (33). Furthermore, various cytokines and growth factors, such as interleukins, tumor necrosis factor-α, and transforming growth factor-β1 (TGF-β1) are released from activated leukocytes (34,35) to cause endothelial dysfunction (36,37). In this connection, the leukocyte count has been shown to be an independent predictor of both endothelium-dependent and -independent vasodilation in type 2 diabetic patients (38). Furthermore, increased secretion of TGF-β1 by mononuclear cells has been demonstrated in patients with diabetic nephropathy (39). Elevated TGF-β1 levels in the glomeruli stimulate proliferation of mesangial and epithelial cells, leading to a matrix expansion typical of glomerulosclerosis (40,41). In addition, activated leukocytes can release superoxide radicals and proteases, all of which promote oxidative stress. The latter can then activate the transcription factor nuclear factor-κB in peripheral mononuclear blood cells. All these pathways can lead to diabetic nephropathy (42). Taken together, it is plausible that low-grade chronic inflammatory responses can interact with other risk factors, leading to widespread vascular damage, endothelial dysfunction, increased oxidative stress, and increased production of growth factors and cytokines to cause micro- and macrovascular complications in type 2 diabetic patients.

Despite the circumstantial evidence, it remains plausible that the elevated WBC count may be an effect of vascular

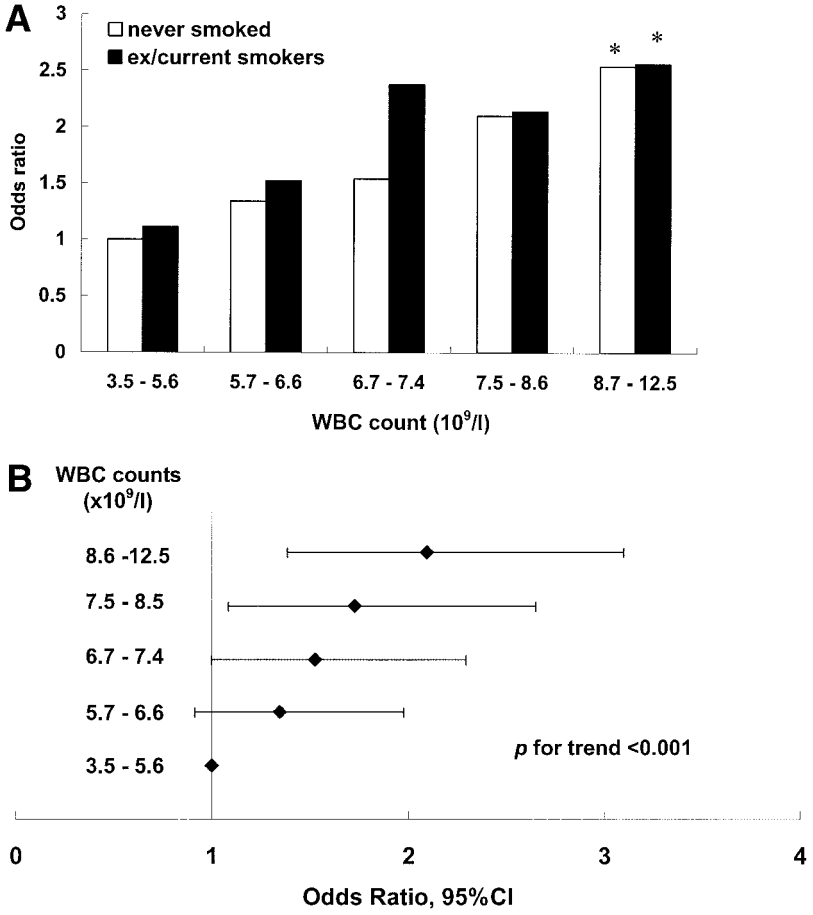

Figure 3—*Age-adjusted OR (A) and multivariate-adjusted OR (B) and 95% CI for the presence of retinopathy and albuminuria by quintiles of WBC count in 3,776 patients with type 2 diabetes according to smoking status. OR estimates were obtained using the lowest quintile of those who never smoked as the referent. *P for trend <0.001.*

complications (e.g., inflammation after micro-infarction). Nevertheless, the inflammatory response has been shown to be involved in the development of vascular occlusion and ischemic organ damage in other diseases. A notable example is sickle cell anemia, a common hereditary hemoglobinopathy caused by a single-point mutation of the β-globin chain of the hemoglobin. Elevated WBC counts have been shown to correlate with the incidence of stroke in children with sickle cell anemia (43) and may precipitate sickle crisis (44). Increased expression of adhesion molecules by leukocytes may be important in the pathogenesis of sickle cell complications. Patients with complications of sickle cell disease have a high expression of $\alpha M\beta_2$ integrin and L-selectin (45). More importantly, an improvement in symptoms after hydroxyurea therapy is accompanied by a marked drop in WBC counts without the rise in hemoglobin F (46) and reduced expression of adhesion molecules in leukocytes (45). In multivariate analysis, the reduction of total WBC count is a predictor of clinical response to hydroxyurea (47). Elevated WBC counts may therefore contribute to the development of vascular complications, although prospective studies are required to address this issue. Another limitation of the present study was the lack of measurement of C-reactive proteins, a specific marker of inflammation. Given the close association between C-reactive proteins and WBC count, we opted to measure the latter, which was more feasible in a cohort study involving large number of subjects.

In conclusion, elevated WBC count, although still within the normal range, is associated with both macro- and microvascular complications in Chinese patients with type 2 diabetes. Chronic inflammation may play a crucial role in the pathogenesis of retinopathy and cardiovascular and renal complications of diabetes. Further research is required to establish the causal relation of WBC and diabetic complications and the underlying mechanisms.

Acknowledgments— The study was supported by funding from the Hong Kong Foundation for Research and Development in Diabetes.

We thank our research nurses for their professionalism as well as all medical and nursing staff at the Diabetes and Endocrine Centre, the Prince of Wales Hospital, for their dedication.

References

1. King H, Aubert RE, Herman WH: Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections. *Diabetes Care* 21:1414–1431, 1998
2. International Diabetes Federation: *Diabetes Atlas 2000*. Brussels, International Diabetes Federation, 2000
3. Stamler J, Vaccaro O, Neaton JD, Wentworth D: Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care* 16:434–444, 1993
4. Ross R: Atherosclerosis: an inflammatory disease. *N Engl J Med* 340:115–126, 1999
5. Weijenberg MP, Feskens EJ, Kromhout D: White blood cell count and the risk of coronary heart disease and all-cause mortality in elderly men. *Arterioscler Thromb Vasc Biol* 16:499–503, 1996
6. Saito I, Folsom AR, Brancati FL, Duncan BB, Chambless LE, McGovern PG: Nontraditional risk factors for coronary heart disease incidence among persons with diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. *Ann Intern Med* 133:81–91, 2000
7. Brown DW, Giles WH, Croft JB: White blood cell count: an independent predictor of coronary heart disease mortality among a national cohort. *J Clin Epidemiol* 54:316–322, 2001
8. Noto D, Barbagallo CM, Cavera G, Cefalu AB, Caimi G, Marino G, Lo Coco L, Caldarella R, Notarbartolo A, Averna MR: Leukocyte count, diabetes mellitus and age are strong predictors of stroke in a rural population in southern Italy: an 8-year follow-up. *Atherosclerosis* 157:225–231, 2001
9. Lee CD, Folsom AR, Nieto FJ, Chambless LE, Shahar E, Wolfe DA: White blood cell count and incidence of coronary heart disease and ischemic stroke and mortality

from cardiovascular disease in African-American and white men and women: Atherosclerosis Risk in Communities Study. *Am J Epidemiol* 154:758–764, 2001
10. Cavalot F, Massucco P, Perna P, Traversa M, Anfossi G, Trovati M: White blood cell count is positively correlated with albumin excretion rate in subjects with type 2 diabetes (Letter). *Diabetes Care* 25:2354–2355, 2002
11. Piwernetz K, Home PD, Snorgaard O, Antsiferov M, Staehr-Johansen K, Krans M, the DIABCARE Monitoring Group of the St. Vincent Declaration Steering Committee: Monitoring the targets of the St. Vincent Declaration and the implementation of quality management in diabetes care: the DIABCARE initiative. *Diabet Med* 10: 371–377, 1993
12. Mogensen CE, Vestbo E, Poulsen PL, Christiansen C, Damsgaard EM, EiskjÆr H, Frøland A, Hansen KW, Nielsen S, Pedersen MM: Microalbuminuria and potential confounders. *Diabetes Care* 18:572–581, 1995
13. Laakso M, Pyorala K: Age of onset and type of diabetes. *Diabetes Care* 8:114–117, 1985
14. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18:499–502, 1972
15. Targher G, Seidell JC, Tonoli M, Muggeo M, De Sandre G, Cigolini M: The white blood cell count: its relationship to plasma insulin and other cardiovascular risk factors in healthy male individuals. *J Intern Med* 239:435–441, 1996
16. Vozarova B, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA: High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 51:455–461, 2002
17. Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, Azambuja MI, Tracy RP, Heiss G: Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities Study): a cohort study. *Lancet* 353:1649–1652, 1999
18. Freeman DJ, Norrie J, Sattar N, Neely RD, Cobbe SM, Ford I, Isles C, Lorimer AR, Macfarlane PW, McKillop JH, Packard CJ, Shepherd J, Gaw A: Pravastatin and the development of diabetes mellitus: evidence for a protective treatment effect in the West of Scotland Coronary Prevention Study. *Circulation* 103:357–362, 2001
19. Ford ES: Leukocyte count, erythrocyte sedimentation rate, and diabetes incidence in a national sample of US adults. *Am J Epidemiol* 155:57–64, 2002
20. Nakanishi N, Yoshida H, Matsuo Y, Suzuki K, Tatara K: White blood-cell count and the risk of impaired fasting glucose or type II diabetes in middle-aged Japanese men. *Diabetologia* 45:42–48, 2002
21. Sunyer J, Munoz A, Peng Y, Margolick J, Chmiel JS, Oishi J, Kingsley L, Samet JM: Longitudinal relation between smoking and white blood cells. *Am J Epidemiol* 144: 734–741, 1996
22. Petitti DB, Kipp H: The leukocyte count: associations with intensity of smoking and persistence of effect after quitting. *Am J Epidemiol* 123:89–95, 1986
23. Kannel WB, Anderson K, Wilson PW: White blood cell count and cardiovascular disease: insights from the Framingham Study. *JAMA* 267:1253–1256, 1992
24. Gillum RF, Ingram DD, Makuc DM: White blood cell count, coronary heart disease, and death: the NHANES I Epidemiologic Follow-up Study. *Am Heart J* 125:855–863, 1993
25. Chan JCN, Tomlinson B, Nicholls MG, Swaminathan R, Cheung CK, Cockram CS: Albuminuria, insulin resistance and dyslipidaemia in Chinese patients with non-insulin-dependent diabetes (NIDDM). *Diabet Med* 13:150–155, 1996
26. Festa A, D'Agostino R, Howard G, Mykkanen L, Tracy RP, Haffner SM: Inflammation and microalbuminuria in nondiabetic and type 2 diabetic subjects: the Insulin Resistance Atherosclerosis Study. *Kidney Int* 58:1703–1710, 2000
27. Winocour PH, Harland JO, Millar JP, Laker MF, Alberti KG: Microalbuminuria and associated cardiovascular risk factors in the community. *Atherosclerosis* 93:71–81, 1992
28. Gruden G, Cavallo-Perin P, Bazzan M, Stella S, Vuolo A, Pagano G: PAI-1 and factor VII activity are higher in IDDM patients with microalbuminuria. *Diabetes* 43:426–429, 1994
29. Gabazza EC, Takeya H, Deguchi H, Sumida Y, Taguchi O, Murata K, Nakatani K, Yano Y, Mohri M, Sata M, Shima T, Nishioka J, Suzuki K: Protein C activation in NIDDM patients. *Diabetologia* 39:1455–1461, 1996
30. Jensen JS, Myrup B, Borch-Johnsen K, Jensen G, Jensen T, Feldt-Rasmussen B: Aspects of haemostatic function in healthy subjects with microalbuminuria: a potential atherosclerotic risk factor. *Thromb Res* 77:423–430, 1995
31. Collier A, Rumley A, Rumley AG, Paterson JR, Leach JP, Lowe GD, Small M: Free radical activity and hemostatic factors in NIDDM patients with and without microalbuminuria. *Diabetes* 41:909–913, 1992
32. Fuster V: Lewis A. Conner Memorial Lecture: Mechanisms leading to myocardial infarction: insights from studies of vascular biology. *Circulation* 90:2126–2146, 1994
33. Ernst E, Hammerschmidt DE, Bagge U, Matrai A, Dormandy JA: Leukocytes and the risk of ischemic diseases. *JAMA* 257: 2318–2324, 1987
34. Baud L, Ardaillou R: Tumor necrosis factor alpha in glomerular injury. *Kidney Int* (Suppl.) 45:S32−S36, 1994
35. Klein NJ, Shennan GI, Heyderman RS, Levin M: Alteration in glycosaminoglycan metabolism and surface charge on human umbilical vein endothelial cells induced by cytokines, endotoxin and neutrophils. *J Cell Sci* 102:821–832, 1992
36. Vallance P, Collier J, Bhagat K: Infection, inflammation, and infarction: does acute endothelial dysfunction provide a link? *Lancet* 349:1391–1392, 1997
37. Hingorani AD, Cross J, Kharbanda RK, Mullen MJ, Bhagat K, Taylor M, Donald AE, Palacios M, Griffin GE, Deanfield JE, MacAllister RJ, Vallance P: Acute systemic inflammation impairs endothelium-dependent dilatation in humans. *Circulation* 102:994–999, 2000
38. Woodman RJ, Watts GF, Puddey IB, Burke V, Mori TA, Hodgson JM, Beilin LJ: Leukocyte count and vascular function in type 2 diabetic subjects with treated hypertension. *Atherosclerosis* 163:175–181, 2002
39. Korpinen E, Groop PH, Fagerudd JA, Teppo AM, Akerblom HK, Vaarala O: Increased secretion of TGF-β1 by peripheral blood mononuclear cells from patients with type 1 diabetes mellitus with diabetic nephropathy. *Diabet Med* 18: 121–125, 2001
40. Nakamura T, Miller D, Ruoslahti E, Border WA: Production of extracellular matrix by glomerular epithelial cells is regulated by transforming growth factor-beta 1. *Kidney Int* 41:1213–1221, 1992
41. Ziyadeh FN, Sharma K, Ericksen M, Wolf G: Stimulation of collagen gene expression and protein synthesis in murine mesangial cells by high glucose is mediated by autocrine activation of transforming growth factor-beta. *J Clin Invest* 93:536–542, 1994
42. Hofmann MA, Schiekofer S, Isermann B, Kanitz M, Henkels M, Joswig M, Treusch A, Morcos M, Weiss T, Borcea V, Abdel Khalek AK, Amiral J, Tritschler H, Ritz E, Wahl P, Ziegler R, Bierhaus A, Nawroth PP: Peripheral blood mononuclear cells isolated from patients with diabetic nephropathy show increased activation of the oxidative-stress sensitive transcription factor NF-κB. *Diabetologia* 42:222–232, 1999
43. Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, Klug PP: Mortality in sickle cell disease: life expectancy and risk factors for early death. *N Engl J Med* 330:1639–1644, 1994

44. Abboud M, Laver J, Blau CA: Granulocytosis causing sickle-cell crisis (Letter). *Lancet* 351:959, 1998
45. Okpala I, Daniel Y, Haynes R, Odoemene D, Goldman J: Relationship between the clinical manifestations of sickle cell disease and the expression of adhesion molecules on white blood cells. *Eur J Haematol* 69:135–144, 2002
46. Charache S, Terrin ML, Moore RD, Dover GJ, Barton FB, Eckert SV, McMahon RP, Bonds DR, Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia: Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. *N Engl J Med* 332:1317–1322, 1995
47. Charache S: Mechanism of action of hydroxyurea in the management of sickle cell anemia in adults. *Semin Hematol* 34: 15–21, 1997