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Serum fibrinogen and cardiovascular events in Chinese type 2 diabetic patients with stable coronary artery disease: a prospective observational study

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Complete List of Authors:	Yang, Sheng-Hua Du, Ying Zhang, Yan Li, Xiao-Lin Li, Sha Xu, Rui-Xia Zhu, Cheng-Gang Guo, Yuan-Lin Wu, Na-Qiong Qing, Ping Gao, Ying Cui, Chuan-Jue Dong, Qian Sun, Jing Li, Jian-Jun; Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College
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Authors: Sheng-Hua Yang, Ying Du, Yan Zhang, Xiao-Lin Li, Sha Li, Rui-Xia Xu, Cheng-Gang Zhu, Yuan-Lin Guo, Na-Qiong Wu, Ping Qing, Ying Gao, Chuan-Jue Cui, Qian Dong, Jing Sun, Jian-Jun Li

Address: Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, BeiLiShi Road 167, Beijing 100037, China

[#]**Correspondence:** Professor Jian-Jun Li, MD, PhD. Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100037, China.

Tel: 86+10+88396077;

Fax: 86+10+88396584;

E-mail:lijianjun938@126.com

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ABSTRACT

Objectives: The aim of this study was to investigate the association of serum fibrinogen with cardiovascular events (CVE) in Chinese patients with type 2 diabetes mellitus (T2DM) and stable coronary artery disease (CAD).

Design: An observational study.

Setting: FuWai Hospital in Beijing, China.

Participants: A cohort of 1466 T2DM patients with angiographic-proven stable CAD was evaluated.

Outcome measures: Baseline serum fibrinogen levels were measured and trisected into "low", "middle" and "high". Their association with CVE was explored using Cox proportional hazard models.

Results: With 20.2 months (average) follow-up, 44 (3%) were lost to follow-up, and 96 patients developed CVE. Compared with the patients without CVE, the ones who developed CVE had higher levels of fibrinogen. Univariable regression revealed a significant relation of fibrinogen to CVE (hazard ratio [HR] 1.25, 95% confidence interval [CI]: 1.06-1.47, *P*=0.010) per standard deviation increase of fibrinogen at baseline. After adjusting for multiple established cardiovascular disease (CVD) risk factors, the association persisted (HR 1.30, 95% CI: 1.02-1.66, *P*=0.037). Moreover, after adjusting for CVD risk factors, the HRs for middle and high serum fibrinogen concentration, using "low" group as reference, were 1.23 (95% CI: 0.69–2.20) and 2.20 (95% CI: 1.11–3.36, *P*= 0.049).

Conclusions: We firstly indicated that elevated fibrinogen level was independently associated with increased CVE in Chinese T2DM patients with stable CAD.

Keywords: Fibrinogen; type 2 diabetes mellitus; coronary artery disease; outcome

Strengths and limitations of this study

1. Fibrinogen has been well established to be a strong and independent cardiovascular risk factor in general population.

2. Studies on the role of fibrinogen in cardiovascular outcomes in diabetes are both limited and controversial.

3. We, for the first time, reported that baseline fibrinogen was associated with cardiovascular events (CVEs) in Chinese type 2 diabetic patients with stable coronary artery disease.

4. The number of CVEs was relatively small and the follow-up duration was relatively short, so further study is needed.

INTRODUCTION

Fibrinogen, as a marker of thrombosis and inflammation, is associated with cardiovascular diseases (CVD). It has been demonstrated that elevated fibrinogen level is significantly associated with intima-media thickness and subclinical atherosclerosis.[1-5] Also, previous study revealed that fibrinogen independently predicted future ischemic stroke risk and incident hypertension.[6-8] What's more, fibrinogen has been well established to be a strong and independent cardiovascular risk factor in general population.[8-12] Data suggested that baseline plasma fibrinogen level could predict cardiovascular events (CVE) in general individuals and nondiabetics. [3,9,10,13]

It is worthy of mentioning that individuals with type 2 diabetes mellitus (T2DM) have higher levels of plasma fibrinogen compared with those without T2DM.[8,14] The most common cause for mortality in patients with diabetes mellitus (DM) is coronary artery disease (CAD). Thereby, it is logically hypothesized that hyperfibrinogenemia could contribute to increase CVE in this disease. However, studies on the role of fibrinogen in cardiovascular outcomes in diabetes are both limited and controversial.[6,15-17]

Based on such situation and combined with a fact that no data is currently available so far with regard to the relationship between fibrinogen and CVE in T2DM patients with stable CAD, we, therefore, performed a prospective observational study of the association of baseline plasma fibrinogen and the risk of cardiovascular events in diabetic patients with stable coronary artery disease.

Patients and Methods

Study Population

In the present study, consecutive patients hospitalized in our division were screened between April 2011 and July 2015 for the following inclusion criteria: over the age of 18 years, type 2 diabetes mellitus and angiographic-proven stable coronary artery disease. Exclusion criteria were acute coronary syndrome (ACS), in-hospital cardiac death at first admission in our division, unavailable data of baseline fibrinogen concentration, the existence of any infectious or systematic inflammatory diseases, significant hematologic disorders, thyroid dysfunction, severe liver and/or renal insufficiency and malignant tumors. According to the inclusion and exclusion criteria, a total

of 1466 eligible patients were enrolled (**Figure 1**). The study was performed according to the Declaration of Helsinki, and the hospital ethics review board (Fuwai Hospital & National Center for Cardiovascular Diseases, Beijing, China) approved the protocol. All the subjects gave written informed consent.

Biochemical Analysis and Baseline Examination

Overnight fasting blood samples were drawn from each patient at admission and centrifuged at room temperature within 2 hours. As described in our previous studies,[18,19] the plasma levels of fibrinogen were quantitatively measured by the method of Claussand a Stagoautoanalyzer with STA Fibrinogen kit (Diagnostic Stago, Taverny, France). Glucose, haemoglobinA1c (HbA1c), uric acid and lipid profile were determined by standard methods. The concentrations of high-sensitivity C-reactive protein (hs-CRP) were determined using immunoturbidimetry (Beckmann Assay 360, Bera, CA, USA).

Height and weight were measured, and body mass index (BMI) (kg/m^2) was calculated. Diabetes mellitus was defined as a fasting plasma glucose ≥126 mg/dL (7.0mmol/L) in multiple determinations, and/or the current use of medication for diabetes. Hypertension was defined as repeated blood pressure measurements ≥140/90 mmHg (at least 2 times in different environments) or currently taking antihypertensive drugs. Stable CAD was defined as typical angina-like chest pain brought on by exertion and relieved by rest or sublingual nitrates or both, a positive treadmill exercise test (>1 mm ST-segment depression), and stable obstructive lesion >50% in at least 1 of the 3 major coronary arteries or major branches assessed by at least 2 independent senior interventional cardiologists who had no knowledge of the patients' clinical characteristics and biochemical results. Dyslipidemia was considered to be present in patients if they had fasting total cholesterol (TC) ≥200 mg/dL or triglyceride (TG) ≥150 mg/dL.

Definition of Events and Follow-up

The primary outcomes were CVE. CVE were defined as the cardiac death, stroke, nonfatal myocardial infarction (MI), post-discharge revascularization (PCI/CABG) due to clinical deterioration or unstable angina (UA). The follow-up data collection was performed by the trained nurses or cardiologists who were blinded to the aim of this study by means of standardized

telephone interviews at 6-month intervals. Follow-up time was calculated as the number of months from the enrollment till the last traceable hospital outpatient or inpatient record or telephone interview before March 2016, and was censored on the date of the first CVE. Death of a participant was reported by relatives or the general practitioner who treated the participant. Three experienced physicians who were blinded to any of the study data independently classified the events.

Statistical Analysis

Continuous variables were presented as mean±SD or median (25th, 75th percentiles) and categorical variables as percentages. Fibrinogen levels were trisected into "low" (≤2.91 g/L), "middle" (2.91-3.51 g/L) and "high" (≥3.51 g/L). Comparisons of continuous baseline data between two or more groups were performed with Student t test and ANOVA, respectively, and χ^2 test was used to compare categorical data. Kruskal-Wallis H test was used for nonnormal variables. Participants who were lost during follow-up were treated as censored observations and not used for follow-up analysis. Using the Cox proportional hazards model, hazard ratios (HR) of CVE, with 95% confidence intervals, were calculated using crude models as well as models adjusted for potential confounding factors. A $P<0.05$ for two sided testing was considered statistically significant. The statistical analysis was performed with SPSS version 19.0 software (SPSS Inc., Chicago, IL).

RESULTS

Baseline Characteristics

The baseline demographic, clinical characteristics, and laboratory findings of the study cohort classified by outcome status (with cardiovascular events or not) were listed in **Table 1**. Compared with the patients without CVE, the ones who developed CVE had lower left ventricle ejection fraction (LVEF) but higher levels of fibrinogen, HbA1c, highly sensitive C-reactive protein and uric acid. Meanwhile, we found that there is a tendency to higher fasting triglyceride level in CVE group (P=0.054). Additionally, there is no significant difference in age, BMI, gender, status of hypertension, smoking status and medical history between the patients with or without CVE.

Table 1 Characteristics of the study cohort with and without cardiovascular events of myocardial

infarction, cardiac death, myocardial revascularization and fatal or nonfatal stroke

	CVE (n=96)	No CVE (n=1326)	*p*
Age (years)	58.54±10.65	59.29±9.81	0.473
Body Mass Index (kg/m^2)	26.32±3.07	26.22±3.21	0.790
Gender, men, % (n)	70.8 (68)	70.7 (938)	0.984
Dyslipidemia, % (n)	88.5 (85)	80.6 (1068)	0.055
Hypertension, % (n)	78.1 (75)	72.9 (966)	0.265
Current smoking, % (n)	56.3 (54)	51.2 (679)	0.343
Previous PCI, %(n)	22.9 (22)	24.0 (318)	0.810
Previous CABG, %(n)	1.0 (1)	3.4 (45)	0.208
Family history of CAD, % (n)	17.7 (17)	14.8 (195)	0.224
Systolic Blood Pressure (mmHg)	124.94±14.64	127.86±16.41	0.117
Diastolic Blood Pressure (mmHg)	74.93±9.12	77.01±10.17	0.071
Left Ventricle Ejection Fraction (%)	60.4±10.2	63.7±8.0	**<0.001**
Total Cholesterol (mmol/L)	4.10±1.16	4.10±1.21	0.962
HDL-cholesterol (mmol/L)	0.98±0.28	1.03±0.27	0.126
LDL-cholesterol (mmol/L)	2.44±0.99	2.46±0.96	0.827
Triglycerides (mmol/L)	1.79 (1.28, 2.53)	1.56 (1.18, 2.21)	0.054
Apo A1 (g/L)	1.36±0.37	1.32±0.28	0.140
Apo B (g/L)	0.99±0.34	0.94±0.33	0.113
HbA1c (%)	7.89±1.54	7.50±1.33	**0.007**
Glucose (mmol/L)	7.52±2.68	7.18±2.28	0.155
Highly Sensitive C-reactive Protein, mg/L	1.83 (1.08, 4.47)	1.54 (0.77, 3.23)	**0.017**
Uric acid (umol/L)	368.39±96.44	340.26±93.36	**0.005**
Fibrinogen (g/L)	3.52±0.85	3.30±0.8	**0.015**
Medication			
Statin, % (n)	69.8 (67)	71.9 (953)	0.707
Aspirin, % (n)	88.1 (84)	86.7 (1149)	0.794
Beta-blocker, % (n)	52.4 (50)	48.2 (639)	0.601
Calcium Channel Blocker	11.5 (11)	20.7 (275)	0.166
ACEI/ARB	30.0 (29)	26.5 (351)	0.521

Data are presented as mean ± SD, median (25th, 75th percentiles) or % (n). CVE: cardiovascular events; Apo: apolipoprotein; HbA1c: Glycosylated Hemoglobin, TypeA1C; ACEI/ARB: Angiotensin Converting EnzymeInhibitor / Angiotensin IIReceptor Blocker

Table 2 compared the baseline cardiovascular risk profiles of the study population, divided into "low" (≥2.91 g/L), "middle" (2.91-3.51 g/L) and "high" (≥3.51 g/L) subgroups on the basis of the fibrinogen concentration. The patients with middle and high levels of fibrinogen had higher percentage of CVE, female, and higher levels of hs-CRP, total cholesterol, LDL-C, HbA1c and fasting glucose compared with the "low" group. No significant difference was found in age, BMI,

blood pressure and smoking status.

Table 2. Baseline characteristics of the cohort of the diabetic patients with stable CAD according to plasma fibrinogen levels at baseline

Variables	Fibrinogen Tertiles(g/L)			*p*
	Low (≤2.91) (n=476)	Middle (2.91-3.51) (n=476)	High (≥3.51) (n=470)	
Age (years)	58.65±9.66	59.16±9.69	59.91±10.22	0.142
BMI (kg/m^2)	26.21±3.08	26.18±2.92	26.30±3.58	0.838
Gender, men, % (n)	78.4 (373)	68.5 (326)	65.3 (307)	**<0.001**
Dyslipidemia, % (n)	80.5 (383)	81.1 (385)	81.9 (385)	0.848
Hypertension, % (n)	71.4 (340)	72.0 (342)	76.4 (359)	0.170
Current smoking, % (n)	53.2 (253)	53.3 (253)	48.3 (227)	0.219
SBP (mmHg)	126.82±15.45	127.57±17.08	127.58±17.04	0.723
DBP (mmHg)	76.60±10.00	77.07±10.26	76.64±10.15	0.731
TC (mmol/L)	3.86±1.05	4.17±1.17	4.27±1.24	**<0.001**
HDL-C (mmol/L)	1.02±0.25	1.03±0.26	1.03±0.30	0.739
LDL-C (mmol/L)	2.28±0.89	2.52±0.94	2.60±1.01	**<0.001**
Triglycerides (mmol/L)	1.57 (1.12, 2.13)	1.60 (1.24, 2.34)	1.54 (1.19, 2.17)	0.092
HbA1C (%)	7.23±1.23	7.52±1.28	7.84±1.46	**<0.001**
Glucose (mmol/L)	6.81±2.10	7.22±.32	7.58±2.43	**<0.001**
Hs-CRP, mg/L	0.88 (0.52, 1.45)	1.54 (0.82, 2.71)	3.64 (1.83, 8.34)	**<0.001**
Uric acid (umol/L)	340.93±95.56	339.06±86.97	346.60±98.62	0.441
CVD events, % (n)	5.0 (24)	5.7 (27)	9.6 (45)	**0.011**
Fibrinogen (g/L)	2.55±0.31	3.21±0.16	4.20±0.74	**<0.001**

Data are presented as mean ± SD, median (25th, 75th percentiles) or % (n). CAD, coronary artery disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; hs-CRP, high-sensitivity C-reactive protein; CVD events: myocardial infarction, cardiac death, myocardial revascularization and fatal or nonfatal stroke.

Outcomes

The average follow-up time was 20.2 months (range from 0.4 to 49.3). During the follow-up period, 96 patients experienced cardiovascular event: 19 (19.8%) nonfatal myocardial infarctions (MIs), 55 (57.3%) myocardial revascularization procedures (PCI or CABG) because of clinical deterioration, 15 (15.6%) strokes and 7 (7.3%) cardiac deaths. Patients suffered ACS and underwent revascularization procedures were assigned once in the analysis. Of the 1466 diabetic patients with stable coronary artery disease, 44 (3%) were lost to follow-up. Therefore, the data of 1422 participants were used for follow-up analysis.

Hazard Ratios of Fibrinogen for Study Outcomes

Univariable regression revealed significant association between serum fibrinogen level at baseline and CVE (HR=1.25, 95% confidence interval [CI]: 1.06-1.47, *P*=0.010) per standard deviation (SD) increase of serum fibrinogen at baseline. After adjusting for multiple established CVD risk factors, the association persisted (HR=1.30, 95% CI: 1.02-1.66, *P*=0.037) (Table 3). Unadjusted HR for developing a CVE was significantly increased in the "high" group compared with the "low" group at baseline, and similar results were also found after adjusting for sex and age (Figure 2). After adjusting for established CVD risk factors, the HR for middle and high serum fibrinogen concentration, using "low" group as reference, were 1.23 (95% CI: 0.69–2.20) and 2.20 (95% CI: 1.11–3.36) (*P* for trend 0.049)(**Table 3**).

Table 3. Hazard Ratios for Cardiovascular Disease Events Association with the Plasma Levels of Fibrinogen (low, middle and high)

Model	Hazard Ratio (95% CI)			*p* for trend
	Low (Reference)	Middle	High	
Crude	1.00	1.10 (0.64, 1.91)	1.99 (1.21, 3.26)	0.008
Model 1	1.00	1.10 (0.64, 1.92)	2.00 (1.22, 3.30)	0.007
Model 2	1.00	1.09 (0.63, 1.89)	1.97 (1.19, 3.26)	0.009
Model 3	1.00	1.14 (0.65, 2.00)	2.15 (1.22, 3.80)	0.012
Model 4	1.00	1.23 (0.69, 2.19)	2.10 (1.16, 3.80)	0.031
Model 5	1.00	1.23 (0.69, 2.20)	2.02 (1.11, 3.68)	0.049
	1 SD fibrinogen increase			*p*
Crude	1.25 (1.06, 1.48)			0.010
Model 1	1.26 (1.06, 1.49)			0.008
Model 2	1.25 (1.05, 1.49)			0.013
Model 3	1.36 (1.07, 1.72)			0.011
Model 4	1.34 (1.05, 1.71)			0.017
Model 5	1.30 (1.02, 1.66)			0.037

Model 1: adjusted for sex, and age; Model 2: Model 1+ total cholesterol, high-density lipoprotein cholesterol, hypertension, and smoking; Model 3: Model 2+ triglycerides, hs-CRP, and HbA1c; Model 4: Model 3+uric acid, body mass index, and family history of CVD, and Model 5: Model 4+Left Ventricle Ejection Fraction. CVD, cardiovascular disease; SD, standard deviation

DISCUSSION

In this prospective observational study in single center with a relative large sample size, we, for the first time, found that baseline fibrinogen concentration was independently associated with

CVE in Chinese type 2 diabetic patients with stable CAD. Although the study might be limited by follow-up duration, the present data provided novel and important information with regard to the key issue whether the baseline fibrinogen concentration can be a marker for predicting the clinical outcomes in T2DM patients with stable CAD.

Sufficient evidence has demonstrated that plasma fibrinogen is a risk factor for cardiovascular disease in general population,[3,6,8-10] but the role of fibrinogen in diabetes remains controversial. Some previous studies demonstrated that hyperfibrinogenemia was an important risk factor for vascular complications in diabetes.[20] For instance, a prospective cohort of 2329 patients with type 1 diabetes revealed that fibrinogen was significant and independent predictor of coronary heart disease in men.[15] Moreover, increased fibrinogen level was shown to be associated with diabetic nephropathy in type 2 diabetics[21,22] and in type 1 diabetics.[15,23,24] Gargano Heart Study (GHS) showed that fibrinogen was a predictor of incident major CVE after adjusting for sex, age, smoking habit and BMI status in diabetics.[25] In parallel, Bruno et al reported that fibrinogen had an independent predictive effect on cardiovascular mortality in T2DM.[26] Furthermore, Stehouwer et al. revealed that baseline fibrinogen concentrations were strongly and independently associated with risk of all-cause death in type 2 diabetics.[27] On the contrary, some studies reported that the association between fibrinogen and incident CAD in patients with T2DM was non-significant via multivariable analyses.[17,28] As we well known, the major cause of death in T2DM is macrovascular complications, especially CAD. Hence, in the present study, we enrolled angiographic-proven CAD patients with T2DM and tried to examine the role of fibrinogen in such a study population of type 2 diabetic patients with CAD. We found that elevated fibrinogen level at baseline was associated with increased CVE in T2DM patients with stable CAD. Additionally, we also found that fibrinogen was higher among those patients who developed a cardiovascular event compared with those who did not, which is in agreement with previous studies.[9,13] To our knowledge, this is the first time to investigate the relationship between fibrinogen and CVE in T2DM patients with stable CAD.

Recently the ADVANCE study (Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation) reported that baseline fibrinogen level was associated with an increased risk of macrovascular events and death in univariate analysis, but after further adjustment, fibrinogen was not an independent predictor of macrovascular.[17] In the

present study, our finding is not consistent with the ADVANCE study, and we speculate that some reasons could explain it. Firstly, the inclusive criteria are different: we chose the patients with T2DM and stable CAD, while the ADVANCE study chose the subjects with T2DM and a history of CVD (stroke, myocardial infarction, transient ischemic attack, unstable angina, coronary or peripheral revascularization, amputation, macroalbuminuria, proliferative retinopathy or photocoagulation, macular edema, or blindness in one eye) or one or more additional cardiovascular risk factors. Secondly, despite the patients from many countries in the ADVANCE study, Chinese and Indians were excluded. Actually, there are ethnic differences in fibrinogen levels.[29-32] Thus, whether the results also persist in Chinese is undetermined. Finally, the definitions of outcomes are different. In ADVANCE study, major macrovascular events were cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke, while our cardiovascular events were defined as cardiac death, stroke, nonfatal myocardial infarction (MI), post-discharge revascularization (PCI/CABG) due to clinical deterioration or unstable angina (UA). Additionally, our previous cross-sectional study has revealed that plasma fibrinogen is associated with severity of coronary artery disease evaluated using Gensini score in diabetic patients,[19] which partly supports our present finding.

There were several limitations of the present study. Firstly, the number of events is relatively small to assess the prognostic value of fibrinogen. The conclusion needs to be testified by large-scale study in the future. Secondly, the follow-up duration was relatively short and longer follow-up will be required. Thirdly, this sample population was collected prospectively from a single center in China, and studies from multiple centers are needed to test our finding.

In conclusion, we found that fibrinogen was associated with cardiovascular events in patients with type 2 diabetes with stable CAD in Chinese population. Further studies are required to clarify the issue completely.

Contributors S-H Y analyzed the data and drafted the manuscript. J-J L planned, designed the study, participated in fundraising and corrected the final version. YD, YZ, X-L L and SL participated in collecting data and interpreted the results. R-X X, C-G Z, Y-L G and N-Q W participated in the study design, interpretation of the results and manuscript review. PQ, YG, C-J

C and QD participated in collecting data and reviewing manuscript. All authors read and have approved the final manuscript.

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Competing interests The authors declare that they have no completing interests.

Patient consent Obtained.

Ethics approval The study was performed according to the Declaration of Helsinki, and the hospital ethics review board (Fuwai Hospital & National Center for Cardiovascular Diseases, Beijing, China) approved the protocol.

Data sharing statement The technical appendix, statistical code and data set are available from the corresponding author.

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Figure legends

Figure 1 Flow chart of the study. CAD, coronary artery disease.

Figure 2 Unadjusted (A) and adjusted by sex and age (B) cumulative hazard of cardiovascular events based on the levels of fibrinogen (low, middle and high).

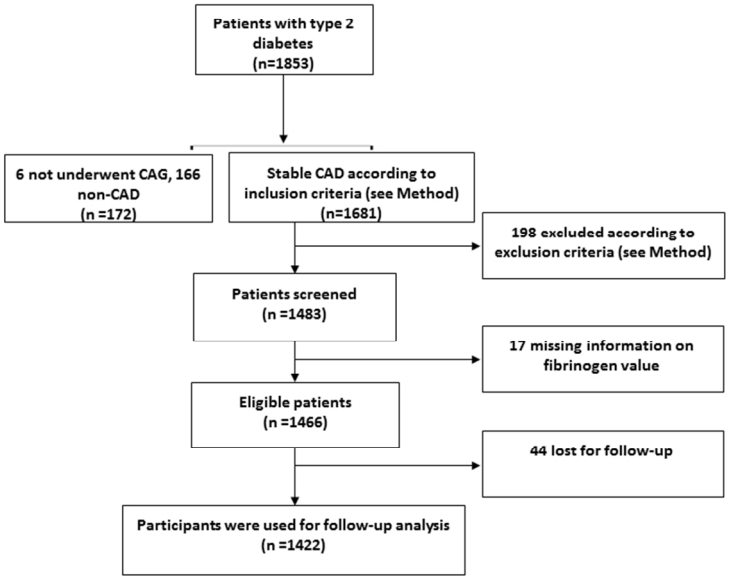

Figure 1 Flow chart of the study. CAD, coronary artery disease.

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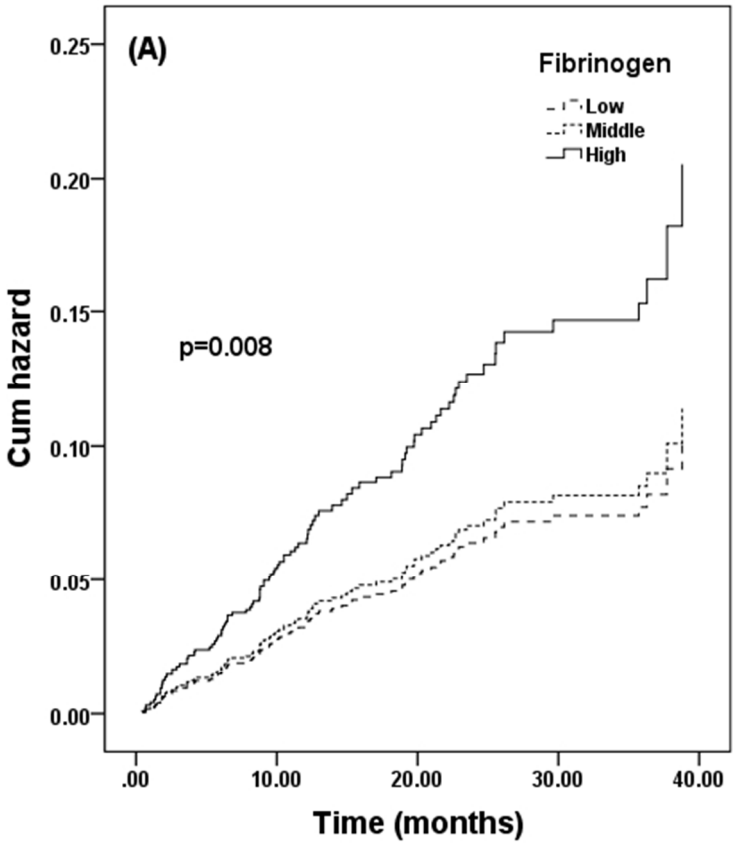

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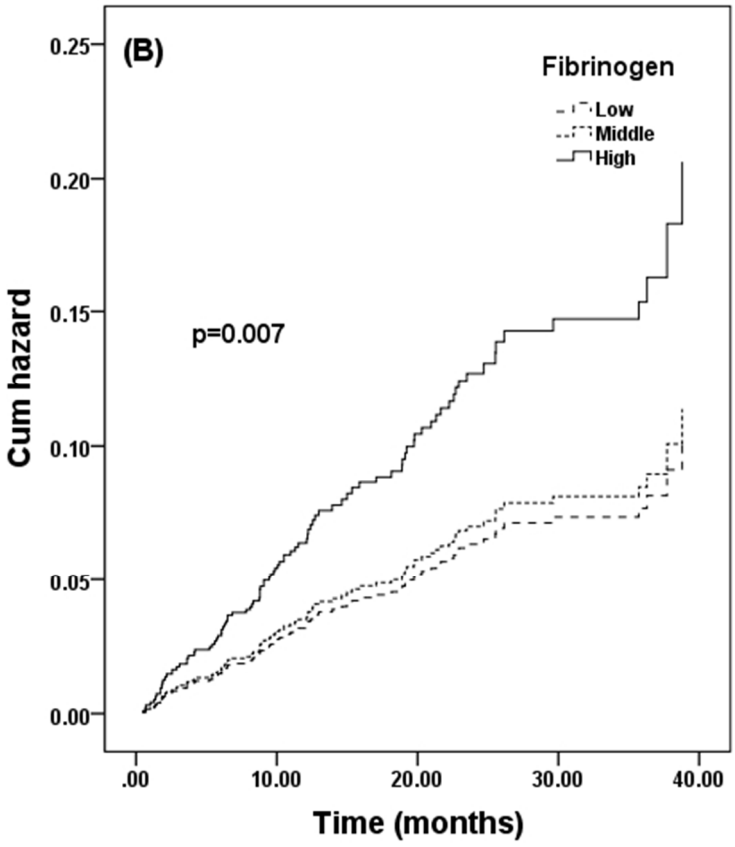

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3,4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	3
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	3
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*4*
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	3, 5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	5
		(*c*) Explain how missing data were addressed	4
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	4
		(*e*) Describe any sensitivity analyses	4

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	4
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*7*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6-8
		(*b*) Report category boundaries when continuous variables were categorized	6-8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	6-8
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7,8
Discussion			
Key results	18	Summarise key results with reference to study objectives	8,9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9,10
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10,11

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Serum fibrinogen and cardiovascular events in Chinese type 2 diabetic patients with stable coronary artery disease: a prospective observational study

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Complete List of Authors:	Yang, Sheng-Hua; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Du, Ying; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College Zhang, Yan; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Li, Xiao-Lin; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Li, Sha; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Xu, Rui-Xia; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Zhu, Cheng-Gang ; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Guo, Yuan-Lin; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Wu, Na-Qiong; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College Qing, Ping; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Gao, Ying; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College Cui, Chuan-Jue; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Dong, Qian; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College

	Sun, Jing ; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Li, Jian-Jun; Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College
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Title: Serum fibrinogen and cardiovascular events in Chinese type 2 diabetic patients with stable coronary artery disease: a prospective observational study

Authors: Sheng-Hua Yang, Ying Du, Yan Zhang, Xiao-Lin Li, Sha Li, Rui-Xia Xu, Cheng-Gang Zhu, Yuan-Lin Guo, Na-Qiong Wu, Ping Qing, Ying Gao, Chuan-Jue Cui, Qian Dong, Jing Sun, Jian-Jun Li

Address: Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, BeiLiShi Road 167, Beijing 100037, China

[#]**Correspondence:** Professor Jian-Jun Li, MD, PhD. Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100037, China.

Tel: 86+10+88396077;

Fax: 86+10+88396584;

E-mail:lijianjun938@126.com

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ABSTRACT

Objectives: The aim of this study was to investigate the association of serum fibrinogen with cardiovascular events (CVE) in Chinese patients with type 2 diabetes mellitus (T2DM) and stable coronary artery disease (CAD).

Design: An observational study.

Setting: FuWai Hospital in Beijing, China.

Participants: A cohort of 1466 T2DM patients with angiographic-proven stable CAD was evaluated.

Outcome measures: Baseline serum fibrinogen levels were measured and trisected into "low", "middle" and "high". Their association with CVE was explored using Cox proportional hazard models.

Results: With 20.2 months (average) follow-up, 44 (3%) were lost to follow-up, and 96 patients developed CVE. Compared with the patients without CVE, the ones who developed CVE had higher levels of fibrinogen. Univariable regression revealed a significant relation of fibrinogen to CVE (hazard ratio [HR] 1.25, 95% confidence interval [CI]: 1.06-1.47, *P*=0.010) per standard deviation increase of fibrinogen at baseline. After adjusting for multiple established cardiovascular disease (CVD) risk factors, the association persisted (HR 1.30, 95% CI: 1.02-1.66, *P*=0.037). Moreover, after adjusting for CVD risk factors, the HRs for middle and high serum fibrinogen concentration, using "low" group as reference, were 1.23 (95% CI: 0.69–2.20) and 2.20 (95% CI: 1.11–3.36, *P*= 0.049).

Conclusions: We firstly indicated that elevated fibrinogen level was independently associated with increased CVE in Chinese T2DM patients with stable CAD.

Keywords: Fibrinogen; type 2 diabetes mellitus; coronary artery disease; outcome

Strengths and limitations of this study

1. We, for the first time, reported that baseline fibrinogen was associated with cardiovascular events (CVEs) in Chinese type 2 diabetic patients with stable coronary artery disease.

2. The number of CVEs was relatively small and the follow-up duration was relatively short, so further study is needed.

3. We just investigated whether the one-time baseline level of fibrinogen was a predictor of short-term outcome in patients with type 2 diabetic patients with stable coronary artery disease.

INTRODUCTION

Fibrinogen, as a marker of thrombosis and inflammation, is associated with cardiovascular diseases (CVD). It has been demonstrated that elevated fibrinogen level is significantly associated with intima-media thickness and subclinical atherosclerosis.[1-5] Also, previous study revealed that fibrinogen independently predicted future ischemic stroke risk and incident hypertension.[6-8] What's more, fibrinogen has been well established to be a strong and independent cardiovascular risk factor in general population.[8-12] Data suggested that baseline plasma fibrinogen level could predict cardiovascular events (CVE) in general individuals and nondiabetics.[3,9,10,13]

It is worthy of mentioning that individuals with type 2 diabetes mellitus (T2DM) have higher levels of plasma fibrinogen compared with those without T2DM.[8,14] The most common cause for mortality in patients with diabetes mellitus (DM) is coronary artery disease (CAD). Thereby, it is logically hypothesized that hyperfibrinogenemia could contribute to increase CVE in this disease. However, studies on the role of fibrinogen in cardiovascular outcomes in diabetes are both limited and controversial.[6,15-17]

Based on such situation and combined with a fact that no data is currently available so far with regard to the relationship between fibrinogen and CVE in T2DM patients with stable CAD, we, therefore, performed a prospective observational study of the association of baseline plasma fibrinogen and the risk of cardiovascular events in diabetic patients with stable coronary artery disease.

Patients and Methods

Study Population

In the present study, consecutive patients hospitalized in our division were screened between April 2011 and July 2015 for the following inclusion criteria: over the age of 18 years, type 2 diabetes mellitus and angiographic-proven stable coronary artery disease. Exclusion criteria were acute coronary syndrome (ACS), in-hospital cardiac death at first admission in our division, unavailable data of baseline fibrinogen concentration, the existence of any infectious or systematic inflammatory diseases, significant hematologic disorders, thyroid dysfunction, severe liver and/or renal insufficiency and malignant tumors. According to the inclusion and exclusion criteria, a total

of 1466 eligible patients were enrolled (**Figure 1**). The study was performed according to the Declaration of Helsinki, and the hospital ethics review board (Fuwai Hospital & National Center for Cardiovascular Diseases, Beijing, China) approved the protocol. All the subjects gave written informed consent.

Biochemical Analysis and Baseline Examination

Overnight fasting blood samples were drawn from each patient at admission and centrifuged at room temperature within 2 hours. As described in our previous studies,[18,19] the plasma levels of fibrinogen were quantitatively measured by the method of Clauss and a Stagoauto analyzer with STA Fibrinogen kit (Diagnostic Stago, Taverny, France). Glucose, haemoglobinA1c (HbA1c), uric acid and lipid profile were determined by standard methods. The concentrations of high-sensitivity C-reactive protein (hs-CRP) were determined using immunoturbidimetry (Beckmann Assay 360, Bera, CA, USA).

Height and weight were measured, and body mass index (BMI) (kg/m^2) was calculated. Diabetes mellitus was defined as a fasting plasma glucose≥ 126 mg/dL (7.0mmol/L) in multiple determinations, and/or the current use of medication for diabetes. Hypertension was defined as repeated blood pressure measurements ≥140/90 mmHg (at least 2 times in different environments) or currently taking antihypertensive drugs. Stable CAD was defined as typical angina-like chest pain brought on by exertion and relieved by rest or sublingual nitrates or both, a positive treadmill exercise test (>1 mm ST-segment depression), and stable obstructive lesion >50% in at least 1 of the 3 major coronary arteries or major branches assessed by at least 2 independent senior interventional cardiologists who had no knowledge of the patients' clinical characteristics and biochemical results. Dyslipidemia was considered to be present in patients if they had fasting total cholesterol (TC) ≥200 mg/dL or triglyceride (TG) ≥150 mg/dL.

Definition of Events and Follow-up

The primary outcomes were CVE. CVE were defined as the cardiac death, stroke, nonfatal myocardial infarction (MI), post-discharge revascularization (PCI/CABG) due to clinical deterioration or unstable angina (UA).The follow-up data collection was performed by the trained nurses or cardiologists who were blinded to the aim of this study by means of standardized

telephone interviews at 6-month intervals. Follow-up time was calculated as the number of months from the enrollment till the last traceable hospital outpatient or inpatient record or telephone interview before March 2016, and was censored on the date of the first CVE. Death of a participant was reported by relatives or the general practitioner who treated the participant. Three experienced physicians who were blinded to any of the study data independently classified the events.

Statistical Analysis

Continuous variables were presented as mean±SD or median (25th, 75th percentiles) and categorical variables as percentages. Fibrinogen levels were trisected into "low" (≤2.91 g/L), "middle" (2.91-3.51 g/L) and "high" (≥3.51 g/L). Comparisons of continuous baseline data between two or more groups were performed with Student t test and ANOVA, respectively, and $\chi 2$ test was used to compare categorical data. Kruskal-Wallis H test was used for nonnormal variables. Test for linear trend (Jonckheere–Terpstra test) was performed by assigning median value for each tertile and treated as continuous variables.

Participants who were lost during follow-up were treated as censored observations and not used for follow-up analysis. Using the Cox proportional hazards model, hazard ratios (HR) of CVE, with 95% confidence intervals, were calculated using crude models as well as models adjusted for potential confounding factors. A $P<0.05$ for two sided testing was considered statistically significant. The statistical analysis was performed with SPSS version 19.0 software (SPSS Inc., Chicago, IL).

RESULTS

Baseline Characteristics

The baseline demographic, clinical characteristics, and laboratory findings of the study cohort classified by outcome status (with cardiovascular events or not) were listed in **Table 1**. Compared with the patients without CVE, the ones who developed CVE had lower left ventricle ejection fraction (LVEF) but higher levels of fibrinogen, HbA1c, highly sensitive C-reactive protein and uric acid. Meanwhile, we found that there is a tendency to higher fasting triglyceride level in CVE group (P=0.054). Additionally, there is no significant difference in age, BMI, gender, status of hypertension, smoking status and medical history between the patients with or without CVE.

Table 1 Characteristics of the study cohort with and without cardiovascular events of myocardial infarction, cardiac death, myocardial revascularization and fatal or nonfatal stroke

	CVE (n=96)	No CVE (n=1326)	*p*
Age (years)	58.54±10.65	59.29±9.81	0.473
Body Mass Index (kg/m^2)	26.32±3.07	26.22±3.21	0.790
Gender, men, % (n)	70.8 (68)	70.7 (938)	0.984
Dyslipidemia, % (n)	88.5 (85)	80.6 (1068)	0.055
Hypertension, % (n)	78.1 (75)	72.9 (966)	0.265
Current smoking, % (n)	56.3 (54)	51.2 (679)	0.343
Duration of diabetes (years)	10.24±6.66	8.64±6.50	0.151
Previous PCI, %(n)	22.9 (22)	24.0 (318)	0.810
Previous CABG, %(n)	1.0 (1)	3.4 (45)	0.208
Family history of CAD, % (n)	17.7 (17)	14.8 (195)	0.224
Systolic Blood Pressure (mmHg)	124.94±14.64	127.86±16.41	0.117
Diastolic Blood Pressure (mmHg)	74.93±9.12	77.01±10.17	0.071
Left Ventricle Ejection Fraction (%)	60.4±10.2	63.7±8.0	**＜0.001**
Total Cholesterol (mmol/L)	4.10±1.16	4.10±1.21	0.962
HDL-cholesterol (mmol/L)	0.98±0.28	1.03±0.27	0.126
LDL-cholesterol (mmol/L)	2.44±0.99	2.46±0.96	0.827
Triglycerides (mmol/L)	1.79 (1.28, 2.53)	1.56 (1.18, 2.21)	0.054
Apo A1 (g/L)	1.36±0.37	1.32±0.28	0.140
Apo B (g/L)	0.99±0.34	0.94±0.33	0.113
HbA1c (%)	7.89±1.54	7.50±1.33	**0.007**
Glucose (mmol/L)	7.52±2.68	7.18±2.28	0.155
Highly Sensitive C-reactive Protein, mg/L	1.83 (1.08, 4.47)	1.54 (0.77, 3.23)	**0.017**
Uric acid (umol/L)	368.39±96.44	340.26±93.36	**0.005**
Fibrinogen (g/L)	3.52±0.85	3.30±0.8	**0.015**
Medication			
Statin, % (n)	69.8 (67)	71.9 (953)	0.707
Aspirin, % (n)	88.1 (84)	86.7 (1149)	0.794
Beta-blocker, % (n)	52.4 (50)	48.2 (639)	0.601
Calcium Channel Blocker	11.5 (11)	20.7 (275)	0.166
ACEI/ARB	30.0 (29)	26.5 (351)	0.521
OADs, % (n)	48.9 (47)	54.3 (720)	0.296
Insulin, % (n)	27.1 (26)	25.8 (342)	0.813
OADs+insulin, % (n)	13.5 (13)	19.9 (264)	0.215

Data are presented as mean ± SD, median (25th, 75th percentiles) or % (n). CVE: cardiovascular events; Apo: apolipoprotein; HbA1c: Glycosylated Hemoglobin, TypeA1C; ACEI/ARB: Angiotensin Converting EnzymeInhibitor / Angiotensin IIReceptor Blocker; OADs, oral anti-diabetic drugs.

Table 2 compared the baseline cardiovascular risk profiles of the study population, divided into

"low" (≥2.91 g/L), "middle" (2.91-3.51 g/L) and "high" (≥3.51 g/L) subgroups on the basis of the fibrinogen concentration. The patients with middle and high levels of fibrinogen had higher percentage of CVE, female, and higher levels of hs-CRP, total cholesterol, LDL-C, HbA1c and fasting glucose compared with the "low" group. No significant difference was found in age, BMI, blood pressure and smoking status.

Table 2. Baseline characteristics of the cohort of the diabetic patients with stable CAD according to serum fibrinogen levels at baseline

Variables	Fibrinogen Tertiles (g/L)			*p*
	Low (≤2.91) (n=476)	Middle (2.91-3.51) (n=476)	High (≥3.51) (n=470)	
Age (years)	58.65±9.66	59.16±9.69	59.91±10.22	0.142
BMI (kg/m^2)	26.21±3.08	26.18±2.92	26.30±3.58	0.838
Gender, men, % (n)	78.4 (373)	68.5 (326)	65.3 (307)	**<0.001**
Dyslipidemia, % (n)	80.5 (383)	81.1 (385)	81.9 (385)	0.848
Hypertension, % (n)	71.4 (340)	72.0 (342)	76.4 (359)	0.170
Current smoking, % (n)	53.2 (253)	53.3 (253)	48.3 (227)	0.219
SBP (mmHg)	126.82±15.45	127.57±17.08	127.58±17.04	0.723
DBP (mmHg)	76.60±10.00	77.07±10.26	76.64±10.15	0.731
TC (mmol/L)	3.86±1.05	4.17±1.17	4.27±1.24	**<0.001**
HDL-C (mmol/L)	1.02±0.25	1.03±0.26	1.03±0.30	0.739
LDL-C (mmol/L)	2.28±0.89	2.52±0.94	2.60±1.01	**<0.001**
Triglycerides (mmol/L)	1.57 (1.12, 2.13)	1.60 (1.24, 2.34)	1.54 (1.19, 2.17)	0.092
HbA1C (%)	7.23±1.23	7.52±1.28	7.84±1.46	**<0.001**
Glucose (mmol/L)	6.81±2.10	7.22±.32	7.58±2.43	**<0.001**
Hs-CRP, mg/L	0.88 (0.52, 1.45)	1.54 (0.82, 2.71)	3.64 (1.83, 8.34)	**<0.001**
Uric acid (umol/L)	340.93±95.56	339.06±86.97	346.60±98.62	0.441
CVD events, % (n)	5.0 (24)	5.7 (27)	9.6 (45)	**0.011**
Duration of diabetes (years)	9.03±6.82	8.89±6.03	10.83±6.83	0.356
Fibrinogen (g/L)	2.55±0.31	3.21±0.16	4.20±0.74	**<0.001**

Data are presented as mean ± SD, median (25th, 75th percentiles) or % (n). CAD, coronary artery disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; hs-CRP, high-sensitivity C-reactive protein; CVD events: myocardial infarction, cardiac death, myocardial revascularization and fatal or nonfatal stroke.

Outcomes

The average follow-up time was 20.2 months (range from 0.4 to 49.3). During the follow-up period, 96 patients experienced cardiovascular event: 19 (19.8%) nonfatal myocardial infarctions

(MIs), 55 (57.3%) myocardial revascularization procedures (PCI or CABG) because of clinical deterioration, 15 (15.6%) strokes and 7 (7.3%) cardiac deaths. We calculated the number of new events per 100 person years, namely, 2.0047/100 per 100 person years. Patients suffered ACS and underwent revascularization procedures were assigned once in the analysis. Of the 1466 diabetic patients with stable coronary artery disease, 44 (3%) were lost to follow-up. Therefore, the data of 1422 participants were used for follow-up analysis.

Hazard Ratios of Fibrinogen for Study Outcomes

Univariable regression revealed significant association between serum fibrinogen level at baseline and CVE (HR=1.25, 95% confidence interval [CI]: 1.06-1.47, *P*=0.010) per standard deviation (SD) increase of serum fibrinogen at baseline. After adjusting for multiple established CVD risk factors, the association persisted (HR=1.30, 95% CI: 1.02-1.66, *P*=0.037) (Table 3). Unadjusted HR for developing a CVE was significantly increased in the "high" group compared with the "low" group at baseline, and similar results were also found after adjusting for sex and age (Figure 2). After adjusting for established CVD risk factors, the HR for middle and high serum fibrinogen concentration, using "low" group as reference, were 1.23 (95% CI: 0.69–2.20) and 2.20 (95% CI: 1.11–3.36)(*P* for trend 0.049)(**Table 3**).

Table 3 Hazard Ratios for Cardiovascular Disease Events Association with the Plasma Levels of Fibrinogen (low, middle and high)

	1 SD fibrinogen increase			*p*
Crude	1.25 (1.06, 1.48)			0.010
Model 1	1.26 (1.06, 1.49)			0.008
Model 2	1.25 (1.05, 1.49)			0.013
Model 3	1.36 (1.07, 1.72)			0.011
Model 4	1.34 (1.05, 1.71)			0.017
Model 5	1.30 (1.02, 1.66)			0.037
Model	Hazard Ratio (95% CI)			*p* for trend
	Low (Reference)	Middle	High	
Crude	1.00	1.10 (0.64, 1.91)	1.99 (1.21, 3.26)	0.008
Model 1	1.00	1.10 (0.64, 1.92)	2.00 (1.22, 3.30)	0.007
Model 2	1.00	1.09 (0.63, 1.89)	1.97 (1.19, 3.26)	0.009
Model 3	1.00	1.14 (0.65, 2.00)	2.15 (1.22, 3.80)	0.012
Model 4	1.00	1.23 (0.69, 2.19)	2.10 (1.16, 3.80)	0.031

Model 5	1.00	1.23 (0.69, 2.20)	2.02 (1.11, 3.68)	0.049

Model 1: adjusted for sex, and age; Model 2: Model 1+ total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, hypertension, and smoking; Model 3: Model 2+ triglycerides, hs-CRP, and HbA1c; Model 4: Model 3+uric acid, body mass index, and family history of CVD, and Model 5: Model 4+Left Ventricle Ejection Fraction. CVD, cardiovascular disease; SD, standard deviation

DISCUSSION

In this prospective observational study in single center with a relative large sample size,we, for the first time, found that baseline fibrinogen concentration was independently associated with CVE in Chinese type 2 diabetic patients with stable CAD. Although the study might be limited by follow-up duration, the present data provided novel and important information with regard to the key issue whether the baseline fibrinogen concentration can be a marker for predicting the clinical outcomes in T2DM patients with stable CAD.

Sufficient evidence has demonstrated that plasma fibrinogen is a risk factor for cardiovascular disease in general population,[3,6,8-10] but the role of fibrinogen in diabetes remains controversial. Some previous studies demonstrated that hyperfibrinogenemia was an important risk factor for vascular complications in diabetes.[20] For instance, a prospective cohort of 2329 patients with type 1 diabetes revealed that fibrinogen was significant and independent predictor of coronary heart disease in men.[15] Moreover, increased fibrinogen level was shown to be associated with diabetic nephropathy in type 2 diabetics[21,22] and in type 1 diabetics.[15,23,24] Gargano Heart Study (GHS) showed that fibrinogen was a predictor of incident major CVE after adjusting for sex, age, smoking habit and BMI status in diabetics.[25] In parallel, Bruno et al reported that fibrinogen had an independent predictive effect on cardiovascular mortality in T2DM.[26] Furthermore, Stehouwer et al. revealed that baseline fibrinogen concentrations were strongly and independently associated with risk of all-cause death in type 2 diabetics.[27] On the contrary, some studies reported that the association between fibrinogen and incident CAD in patients with T2DM was non-significant via multivariable analyses.[17,28] As we well known, the major cause of death in T2DM is macrovascular complications, especially CAD. Hence, in the present study, we enrolled angiographic-proven CAD patients with T2DM and tried to examine the role of fibrinogen in such a study population of type 2 diabetic patients with CAD. We found that elevated fibrinogen level

at baseline was associated with increased CVE in T2DM patients with stable CAD. Additionally, we also found that fibrinogen was higher among those patients who developed a cardiovascular event compared with those who did not, which is in agreement with previous studies.[9,13] To our knowledge, this is the first time to investigate the relationship between fibrinogen and CVE in T2DM patients with stable CAD.

Recently the ADVANCE study (Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation) reported that baseline fibrinogen level was associated with an increased risk of macrovascular events and death in univariate analysis, but after further adjustment, fibrinogen was not an independent predictor of macrovascular.[17] In the present study, our finding is not consistent with the ADVANCE study, and we speculate that some reasons could explain it. Firstly, the inclusive criteria are different: we chose the patients with T2DM and stable CAD, while the ADVANCE study chose the subjects with T2DM and a history of CVD (stroke, myocardial infarction, transient ischemic attack, unstable angina, coronary or peripheral revascularization, amputation, macroalbuminuria, proliferative retinopathy or photocoagulation, macular edema, or blindness in one eye) or one or more additional cardiovascular risk factors. Secondly, despite the patients from many countries in the ADVANCE study, Chinese and Indians were excluded. Actually, there are ethnic differences in fibrinogen levels.[29-32] Thus, whether the results also persist in Chinese is undetermined. Finally, the definitions of outcomes are different. In ADVANCE study, major macrovascular events were cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke, while our cardiovascular events were defined as cardiac death, stroke, nonfatal myocardial infarction (MI), post-discharge revascularization (PCI/CABG) due to clinical deterioration or unstable angina (UA). Additionally, our previous cross-sectional study has revealed that plasma fibrinogen is associated with severity of coronary artery disease evaluated using Gensini score in diabetic patients,[19] which partly supports our present finding. Furthermore, in the present study, we found that 3.515(g/L) is a cut-off value of fibrinogen to predict CVEs in our cohort, which is consistent with previous study.[33]

There were several limitations of the present study. Firstly, the number of events is relatively small to assess the prognostic value of fibrinogen. The conclusion needs to be testified by large-scale study in the future. Secondly, the follow-up duration was relatively short and longer

follow-up will be required. Thirdly, this sample population was collected prospectively from a single center in China. Fibrinogen varies in race,[29] so we do not know if our results would apply to other ethnic groups and studies from multiple centers are needed to test our finding. Finally, we just collected one-time baseline level of fibrinogen.

In conclusion, we found that fibrinogen was associated with cardiovascular events in patients with type 2 diabetes with stable CAD in Chinese population. Further studies are required to clarify the issue completely.

Contributors S-H Y analyzed the data and drafted the manuscript. J-J L planned, designed the study, participated in fundraising and corrected the final version. YD, YZ, X-L L and SL participated in collecting data and interpreted the results. R-X X, C-G Z, Y-L G and N-Q W participated in the study design, interpretation of the results and manuscript review. PQ, YG, C-J C and QD participated in collecting data and reviewing manuscript. All authors read and have approved the final manuscript.

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Competing interests The authors declare that they have no completing interests.

Patient consent Obtained.

Ethics approval The study was performed according to the Declaration of Helsinki, and the hospital ethics review board (Fuwai Hospital & National Center for Cardiovascular Diseases, Beijing, China) approved the protocol.

Data sharing statement The technical appendix, statistical code and data set are available from the corresponding author.

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Figure legends

Figure 1 Flow chart of the study. CAD, coronary artery disease.

Figure 2 Unadjusted (A) and adjusted by sex and age (B) cumulative hazard of cardiovascular events based on the levels of fibrinogen (low, middle and high).

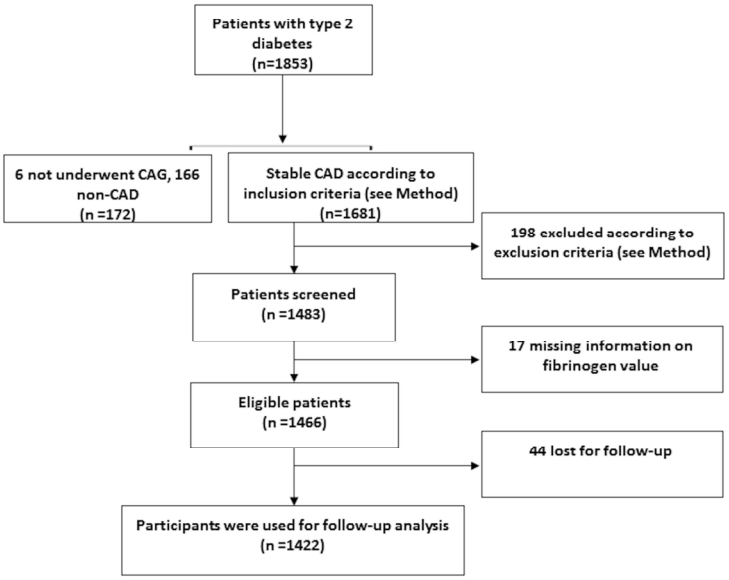

Figure 1 Flow chart of the study. CAD, coronary artery disease.

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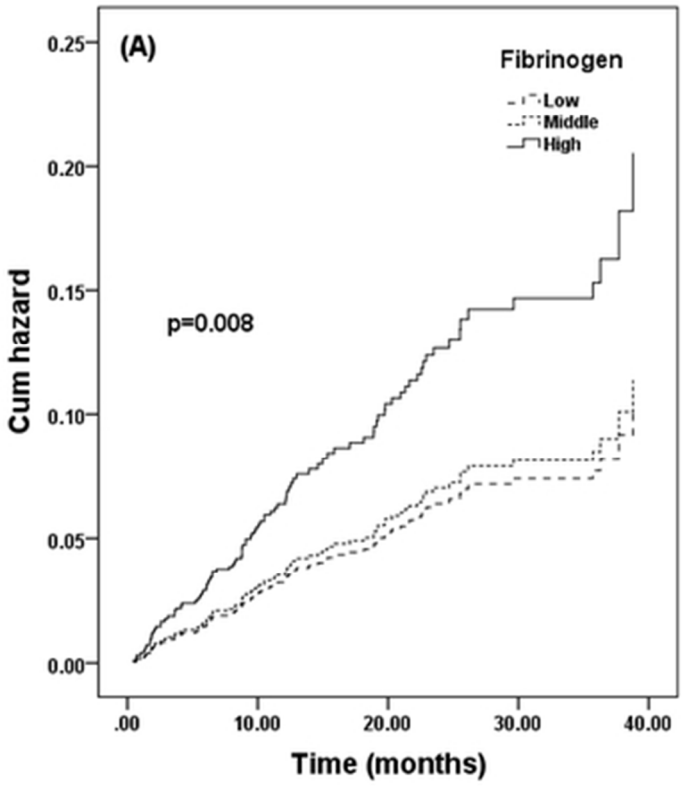

Figure 2 Unadjusted (A) and adjusted by sex and age (B) cumulative hazard of cardiovascular events based on the levels of fibrinogen (low, middle and high).

39x33mm (300 x 300 DPI)

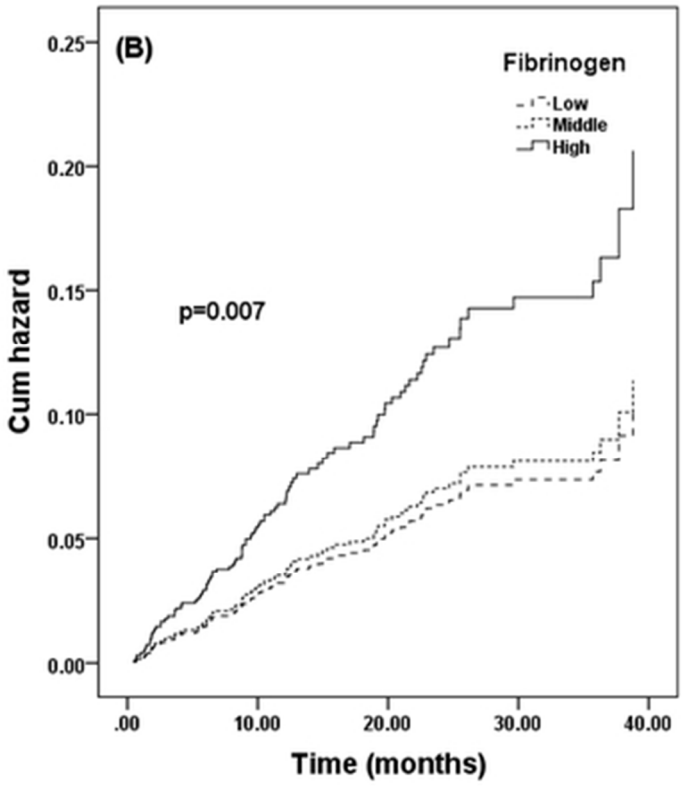

Figure 2 Unadjusted (A) and adjusted by sex and age (B) cumulative hazard of cardiovascular events based on the levels of fibrinogen (low, middle and high).

39x33mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3,4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	3
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	3
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*4*
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	3, 5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	5
		(*c*) Explain how missing data were addressed	4
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	4
		(*e*) Describe any sensitivity analyses	4

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	4
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*7*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6-8
		(*b*) Report category boundaries when continuous variables were categorized	6-8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	6-8
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7,8
Discussion			
Key results	18	Summarise key results with reference to study objectives	8,9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9,10
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10,11

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Serum fibrinogen and cardiovascular events in Chinese type 2 diabetic patients with stable coronary artery disease: a prospective observational study

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Complete List of Authors:	Yang, Sheng-Hua; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Du, Ying; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College Zhang, Yan; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Li, Xiao-Lin; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Li, Sha; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Xu, Rui-Xia; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Zhu, Cheng-Gang ; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Guo, Yuan-Lin; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Wu, Na-Qiong; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College Qing, Ping; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Gao, Ying; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College Cui, Chuan-Jue; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Dong, Qian; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College

	Sun, Jing ; Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease Li, Jian-Jun; Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Cardiovascular medicine, Diabetes and endocrinology
Keywords:	Fibrinogen, type 2 diabetes mellitus, coronary artery disease, outcome

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Title: Serum fibrinogen and cardiovascular events in Chinese type 2 diabetic patients with stable coronary artery disease: a prospective observational study

Authors: Sheng-Hua Yang, Ying Du, Yan Zhang, Xiao-Lin Li, Sha Li, Rui-Xia Xu, Cheng-Gang Zhu, Yuan-Lin Guo, Na-Qiong Wu, Ping Qing, Ying Gao, Chuan-Jue Cui, Qian Dong, Jing Sun, Jian-Jun Li[#]

Address: Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, BeiLiShi Road 167, Beijing 100037, China

[#]**Correspondence:** Professor Jian-Jun Li, MD, PhD. Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100037, China.

Tel: 86+10+88396077;

Fax: 86+10+88396584;

E-mail:lijianjun938@126.com

Word count: 2525

ABSTRACT

Objectives: The aim of this study was to investigate the association of serum fibrinogen with cardiovascular events (CVE) in Chinese patients with type 2 diabetes mellitus (T2DM) and stable coronary artery disease (CAD).

Design: An observational study.

Setting: FuWai Hospital in Beijing, China.

Participants: A cohort of 1466 T2DM patients with angiographic-proven stable CAD was evaluated.

Outcome measures: Baseline serum fibrinogen levels were measured and trisected into "low", "middle" and "high". Their association with CVE was explored using Cox proportional hazard models.

Results: With 20.2 months (average) follow-up, 44 (3%) were lost to follow-up, and 96 patients developed CVE. Compared with the patients without CVE, the ones who developed CVE had higher levels of fibrinogen. Univariable regression revealed a significant relation of fibrinogen to CVE (hazard ratio [HR] 1.25, 95% confidence interval [CI]: 1.06-1.47, *P*=0.010) per standard deviation increase of fibrinogen at baseline. After adjusting for multiple established cardiovascular disease (CVD) risk factors, the association persisted (HR 1.30, 95% CI: 1.02-1.66, *P*=0.037). Moreover, after adjusting for CVD risk factors, the HRs for middle and high serum fibrinogen concentration, using "low" group as reference, were 1.23 (95% CI: 0.69–2.20) and 2.20 (95% CI: 1.11–3.36, *P*= 0.049).

Conclusions: We firstly indicated that elevated fibrinogen level was independently associated with increased CVE in Chinese T2DM patients with stable CAD.

Keywords: Fibrinogen; type 2 diabetes mellitus; coronary artery disease; outcome

Strengths and limitations of this study

1. We, for the first time, reported that baseline fibrinogen was associated with cardiovascular events (CVEs) in Chinese type 2 diabetic patients with stable coronary artery disease.

2. The number of CVEs was relatively small and the follow-up duration was relatively short, so further study is needed.

3. We just investigated whether the one-time baseline level of fibrinogen was a predictor of short-term outcome in patients with type 2 diabetic patients with stable coronary artery disease.

INTRODUCTION

Fibrinogen, as a marker of thrombosis and inflammation, is associated with cardiovascular diseases (CVD). It has been demonstrated that elevated fibrinogen level is significantly associated with intima-media thickness and subclinical atherosclerosis.[1-5] Also, previous study revealed that fibrinogen independently predicted future ischemic stroke risk and incident hypertension.[6-8] What's more, fibrinogen has been well established to be a strong and independent cardiovascular risk factor in general population.[8-12] Data suggested that baseline plasma fibrinogen level could predict cardiovascular events (CVE) in general individuals and nondiabetics.[3,9,10,13]

It is worthy of mentioning that individuals with type 2 diabetes mellitus (T2DM) have higher levels of plasma fibrinogen compared with those without T2DM.[8,14] The most common cause for mortality in patients with diabetes mellitus (DM) is coronary artery disease (CAD). Thereby, it is logically hypothesized that hyperfibrinogenemia could contribute to increase CVE in this disease. However, studies on the role of fibrinogen in cardiovascular outcomes in diabetes are both limited and controversial.[6,15-17]

Based on such situation and combined with a fact that no data is currently available so far with regard to the relationship between fibrinogen and CVE in T2DM patients with stable CAD, we, therefore, performed a prospective observational study of the association of baseline plasma fibrinogen and the risk of cardiovascular events in diabetic patients with stable coronary artery disease.

Patients and Methods

Study Population

In the present study, consecutive patients hospitalized in our division were screened between April 2011 and July 2015 for the following inclusion criteria: over the age of 18 years, type 2 diabetes mellitus and angiographic-proven stable coronary artery disease. Exclusion criteria were acute coronary syndrome (ACS), in-hospital cardiac death at first admission in our division, unavailable data of baseline fibrinogen concentration, the existence of any infectious or systematic inflammatory diseases, significant hematologic disorders, thyroid dysfunction, severe liver insufficiency (transaminases > ten times upper reference levels) and/or severe renal insufficiency (end-stage renal disease or chronic dialysis treatment) and malignant tumors. According to the inclusion and exclusion criteria, a total of 1466 eligible patients were enrolled (**Figure 1**). The study was performed according to the Declaration of Helsinki, and the hospital ethics review board (Fuwai Hospital & National Center for Cardiovascular Diseases, Beijing, China) approved

the protocol. All the subjects gave written informed consent.

Biochemical Analysis and Baseline Examination

Overnight fasting blood samples were drawn from each patient at admission and centrifuged at room temperature within 2 hours. As described in our previous studies,[18,19] the plasma levels of fibrinogen were quantitatively measured by the method of Clauss and a Stagoauto analyzer with STA Fibrinogen kit (Diagnostic Stago, Taverny, France). Glucose, haemoglobinA1c (HbA1c), uric acid and lipid profile were determined by standard methods. The concentrations of high-sensitivity C-reactive protein (hs-CRP) were determined using immunoturbidimetry (Beckmann Assay 360, Bera, CA, USA).

Height and weight were measured, and body mass index (BMI) (kg/m^2) was calculated. Diabetes mellitus was defined as a fasting plasma glucose≥ 126 mg/dL (7.0mmol/L) in multiple determinations, and/or the current use of medication for diabetes. Hypertension was defined as repeated blood pressure measurements ≥140/90 mmHg (at least 2 times in different environments) or currently taking antihypertensive drugs. Stable CAD was defined as typical angina-like chest pain brought on by exertion and relieved by rest or sublingual nitrates or both, a positive treadmill exercise test (>1 mm ST-segment depression), and stable obstructive lesion >50% in at least 1 of the 3 major coronary arteries or major branches assessed by at least 2 independent senior interventional cardiologists who had no knowledge of the patients' clinical characteristics and biochemical results. Dyslipidemia was considered to be present in patients if they had fasting total cholesterol (TC) ≥200 mg/dL or triglyceride (TG) ≥150 mg/dL.

Definition of Events and Follow-up

The primary outcomes were CVE. CVE were defined as the cardiac death, stroke, nonfatal myocardial infarction (MI), post-discharge revascularization (PCI/CABG) due to clinical deterioration or unstable angina (UA).The follow-up data collection was performed by the trained nurses or cardiologists who were blinded to the aim of this study by means of standardized telephone interviews at 6-month intervals. Follow-up time was calculated as the number of months from the enrollment till the last traceable hospital outpatient or inpatient record or telephone interview before March 2016, and was censored on the date of the first CVE. Death of a

participant was reported by relatives or the general practitioner who treated the participant. Three experienced physicians who were blinded to any of the study data independently classified the events.

Statistical Analysis

Continuous variables were presented as mean±SD or median (25th, 75th percentiles) and categorical variables as percentages. Fibrinogen levels were trisected into "low" (≤2.91 g/L), "middle" (2.91-3.51 g/L) and "high" (≥3.51 g/L). Comparisons of continuous baseline data between two or more groups were performed with Student t test and ANOVA, respectively, and $\chi 2$ test was used to compare categorical data. Kruskal-Wallis H test was used for nonnormal variables. Test for linear trend (Jonckheere–Terpstra test) was performed by assigning median value for each tertile and treated as continuous variables.

Participants who were lost during follow-up were treated as censored observations and not used for follow-up analysis. Using the Cox proportional hazards model, hazard ratios (HR) of CVE, with 95% confidence intervals, were calculated using crude models as well as models adjusted for potential confounding factors. A $P<0.05$ for two sided testing was considered statistically significant. The statistical analysis was performed with SPSS version 19.0 software (SPSS Inc., Chicago, IL).

RESULTS

Baseline Characteristics

The baseline demographic, clinical characteristics, and laboratory findings of the study cohort classified by outcome status (with cardiovascular events or not) were listed in **Table 1**. Compared with the patients without CVE, the ones who developed CVE had lower left ventricle ejection fraction (LVEF) but higher levels of fibrinogen, HbA1c, highly sensitive C-reactive protein and uric acid. Meanwhile, we found that there is a tendency to higher fasting triglyceride level in CVE group (P=0.054). Additionally, there is no significant difference in age, BMI, gender, status of hypertension, smoking status and medical history between the patients with or without CVE.

Table 1 Characteristics of the study cohort with and without cardiovascular events of myocardial infarction, cardiac death, myocardial revascularization and fatal or nonfatal stroke

	CVE (n=96)	No CVE (n=1326)	*p*

Age (years)	58.54±10.65	59.29±9.81	0.473
Body Mass Index (kg/m^2)	26.32±3.07	26.22±3.21	0.790
Gender, men, % (n)	70.8 (68)	70.7 (938)	0.984
Dyslipidemia, % (n)	88.5 (85)	80.6 (1068)	0.055
Hypertension, % (n)	78.1 (75)	72.9 (966)	0.265
Current smoking, % (n)	56.3 (54)	51.2 (679)	0.343
Duration of diabetes (years)	10.24±6.66	8.64±6.50	0.151
Previous PCI, %(n)	22.9 (22)	24.0 (318)	0.810
Previous CABG, %(n)	1.0 (1)	3.4 (45)	0.208
Family history of CAD, % (n)	17.7 (17)	14.8 (195)	0.224
Systolic Blood Pressure (mmHg)	124.94±14.64	127.86±16.41	0.117
Diastolic Blood Pressure (mmHg)	74.93±9.12	77.01±10.17	0.071
Left Ventricle Ejection Fraction (%)	60.4±10.2	63.7±8.0	**<0.001**
Total Cholesterol (mmol/L)	4.10±1.16	4.10±1.21	0.962
HDL-cholesterol (mmol/L)	0.98±0.28	1.03±0.27	0.126
LDL-cholesterol (mmol/L)	2.44±0.99	2.46±0.96	0.827
Triglycerides (mmol/L)	1.79 (1.28, 2.53)	1.56 (1.18, 2.21)	0.054
Apo A1 (g/L)	1.36±0.37	1.32±0.28	0.140
Apo B (g/L)	0.99±0.34	0.94±0.33	0.113
HbA1c (%)	7.89±1.54	7.50±1.33	**0.007**
Glucose (mmol/L)	7.52±2.68	7.18±2.28	0.155
Highly Sensitive C-reactive Protein, mg/L	1.83 (1.08, 4.47)	1.54 (0.77, 3.23)	**0.017**
Uric acid (umol/L)	368.39±96.44	340.26±93.36	**0.005**
eGFR (ml/min)	82.03±24.55	80.12±25.58	0.471
Fibrinogen (g/L)	3.52±0.85	3.30±0.8	**0.015**
Medication			
Statin, % (n)	69.8 (67)	71.9 (953)	0.707
Aspirin, % (n)	88.1 (84)	86.7 (1149)	0.794
Beta-blocker, % (n)	52.4 (50)	48.2 (639)	0.601
Calcium Channel Blocker	11.5 (11)	20.7 (275)	0.166
ACEI/ARB	30.0 (29)	26.5 (351)	0.521
OADs, % (n)	48.9 (47)	54.3 (720)	0.296
Insulin, % (n)	27.1 (26)	25.8 (342)	0.813
OADs+insulin, % (n)	13.5 (13)	19.9 (264)	0.215

Data are presented as mean ± SD, median (25th, 75th percentiles) or % (n). CVE: cardiovascular events; Apo: apolipoprotein; HbA1c: Glycosylated Hemoglobin, TypeA1C; eGFR, estimated glomerular filtration rate; ACEI/ARB: Angiotensin Converting EnzymeInhibitor / Angiotensin IIReceptor Blocker; OADs, oral anti-diabetic drugs.

Table 2 compared the baseline cardiovascular risk profiles of the study population, divided into "low" (≥2.91 g/L), "middle" (2.91-3.51 g/L) and "high" (≥3.51 g/L) subgroups on the basis of the fibrinogen concentration. The patients with middle and high levels of fibrinogen had higher

percentage of CVE, female, and higher levels of hs-CRP, total cholesterol, LDL-C, HbA1c and fasting glucose compared with the "low" group. No significant difference was found in age, BMI, blood pressure and smoking status.

Table 2. Baseline characteristics of the cohort of the diabetic patients with stable CAD according to serum fibrinogen levels at baseline

Variables	Fibrinogen Tertiles (g/L)			*p*
	Low (≤2.91) (n=476)	Middle (2.91-3.51) (n=476)	High (≥3.51) (n=470)	
Age (years)	58.65±9.66	59.16±9.69	59.91±10.22	0.142
BMI (kg/m^2)	26.21±3.08	26.18±2.92	26.30±3.58	0.838
Gender, men, % (n)	78.4 (373)	68.5 (326)	65.3 (307)	**<0.001**
Dyslipidemia, % (n)	80.5 (383)	81.1 (385)	81.9 (385)	0.848
Hypertension, % (n)	71.4 (340)	72.0 (342)	76.4 (359)	0.170
Current smoking, % (n)	53.2 (253)	53.3 (253)	48.3 (227)	0.219
SBP (mmHg)	126.82±15.45	127.57±17.08	127.58±17.04	0.723
DBP (mmHg)	76.60±10.00	77.07±10.26	76.64±10.15	0.731
TC (mmol/L)	3.86±1.05	4.17±1.17	4.27±1.24	**<0.001**
HDL-C (mmol/L)	1.02±0.25	1.03±0.26	1.03±0.30	0.739
LDL-C (mmol/L)	2.28±0.89	2.52±0.94	2.60±1.01	**<0.001**
Triglycerides (mmol/L)	1.57 (1.12, 2.13)	1.60 (1.24, 2.34)	1.54 (1.19, 2.17)	0.092
HbA1C (%)	7.23±1.23	7.52±1.28	7.84±1.46	**<0.001**
Glucose (mmol/L)	6.81±2.10	7.22±.32	7.58±2.43	**<0.001**
Hs-CRP, mg/L	0.88 (0.52, 1.45)	1.54 (0.82, 2.71)	3.64 (1.83, 8.34)	**<0.001**
Uric acid (umol/L)	340.93±95.56	339.06±86.97	346.60±98.62	0.441
CVD events, % (n)	5.0 (24)	5.7 (27)	9.6 (45)	**0.011**
Duration of diabetes (years)	9.03±6.82	8.89±6.03	10.83±6.83	0.356
Gensini score	28 (19, 49)	30 (16, 56)	33 (17, 61)	0.315
Fibrinogen (g/L)	2.55±0.31	3.21±0.16	4.20±0.74	**<0.001**

Data are presented as mean ± SD, median (25th, 75th percentiles) or % (n). CAD, coronary artery disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; hs-CRP, high-sensitivity C-reactive protein; CVD events: myocardial infarction, cardiac death, myocardial revascularization and fatal or nonfatal stroke.

Outcomes

The average follow-up time was 20.2 months (range from 0.4 to 49.3). During the follow-up period, 96 patients experienced cardiovascular event: 19 (19.8%) nonfatal myocardial infarctions (MIs), 55 (57.3%) myocardial revascularization procedures (PCI or CABG) because of clinical deterioration, 15 (15.6%) strokes and 7 (7.3%) cardiac deaths. We calculated the number of new

events per 100 person years, namely, 2.0047/100 per 100 person years. Patients suffered ACS and underwent revascularization procedures were assigned once in the analysis. Of the 1466 diabetic patients with stable coronary artery disease, 44 (3%) were lost to follow-up. Therefore, the data of 1422 participants were used for follow-up analysis.

Hazard Ratios of Fibrinogen for Study Outcomes

Univariable regression revealed significant association between serum fibrinogen level at baseline and CVE (HR=1.25, 95% confidence interval [CI]: 1.06-1.47, *P*=0.010) per standard deviation (SD) increase of serum fibrinogen at baseline. After adjusting for multiple established CVD risk factors, the association persisted (HR=1.30, 95% CI: 1.02-1.66, *P*=0.037) (Table 3). Unadjusted HR for developing a CVE was significantly increased in the "high" group compared with the "low" group at baseline, and similar results were also found after adjusting for sex and age (Figure 2). After adjusting for established CVD risk factors, the HR for middle and high serum fibrinogen concentration, using "low" group as reference, were 1.23 (95% CI: 0.69–2.20) and 2.20 (95% CI: 1.11–3.36)(*P* for trend 0.049)(**Table 3**).

Table 3 Hazard Ratios for Cardiovascular Disease Events Association with the Plasma Levels of Fibrinogen (low, middle and high)

	1 SD fibrinogen increase			*p*
Crude	1.25 (1.06, 1.48)			0.010
Model 1	1.26 (1.06, 1.49)			0.008
Model 2	1.25 (1.05, 1.49)			0.013
Model 3	1.36 (1.07, 1.72)			0.011
Model 4	1.34 (1.05, 1.71)			0.017
Model 5	1.30 (1.02, 1.66)			0.037
Model	Hazard Ratio (95% CI)			*p* for trend
	Low (Reference)	Middle	High	
Crude	1.00	1.10 (0.64, 1.91)	1.99 (1.21, 3.26)	0.008
Model 1	1.00	1.10 (0.64, 1.92)	2.00 (1.22, 3.30)	0.007
Model 2	1.00	1.09 (0.63, 1.89)	1.97 (1.19, 3.26)	0.009
Model 3	1.00	1.14 (0.65, 2.00)	2.15 (1.22, 3.80)	0.012
Model 4	1.00	1.23 (0.69, 2.19)	2.10 (1.16, 3.80)	0.031
Model 5	1.00	1.23 (0.69, 2.20)	2.02 (1.11, 3.68)	0.049

Model 1: adjusted for sex, and age; Model 2: Model 1+ total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, hypertension, and smoking; Model 3: Model 2+

triglycerides, hs-CRP, and HbA1c; Model 4: Model 3+uric acid, body mass index, and family history of CVD, and Model 5: Model 4+Left Ventricle Ejection Fraction. CVD, cardiovascular disease; SD, standard deviation

DISCUSSION

In this prospective observational study in single center with a relative large sample size,we, for the first time, found that baseline fibrinogen concentration was independently associated with CVE in Chinese type 2 diabetic patients with stable CAD. Although the study might be limited by follow-up duration, the present data provided novel and important information with regard to the key issue whether the baseline fibrinogen concentration can be a marker for predicting the clinical outcomes in T2DM patients with stable CAD.

Sufficient evidence has demonstrated that plasma fibrinogen is a risk factor for cardiovascular disease in general population,[3,6,8-10] but the role of fibrinogen in diabetes remains controversial. Some previous studies demonstrated that hyperfibrinogenemia was an important risk factor for vascular complications in diabetes.[20] For instance, a prospective cohort of 2329 patients with type 1 diabetes revealed that fibrinogen was significant and independent predictor of coronary heart disease in men.[15] Moreover, increased fibrinogen level was shown to be associated with diabetic nephropathy in type 2 diabetics[21,22] and in type 1 diabetics.[15,23,24] Gargano Heart Study (GHS) showed that fibrinogen was a predictor of incident major CVE after adjusting for sex, age, smoking habit and BMI status in diabetics.[25] In parallel, Bruno et al reported that fibrinogen had an independent predictive effect on cardiovascular mortality in T2DM.[26] Furthermore, Stehouwer et al. revealed that baseline fibrinogen concentrations were strongly and independently associated with risk of all-cause death in type 2 diabetics.[27] On the contrary, some studies reported that the association between fibrinogen and incident CAD in patients with T2DM was non-significant via multivariable analyses.[17,28] As we well known, the major cause of death in T2DM is macrovascular complications, especially CAD. Hence, in the present study, we enrolled angiographic-proven CAD patients with T2DM and tried to examine the role of fibrinogen in such a study population of type 2 diabetic patients with CAD. We found that elevated fibrinogen level at baseline was associated with increased CVE in T2DM patients with stable CAD. Additionally, we also found that fibrinogen was higher among those patients who developed a cardiovascular event compared with those who did not, which is in agreement with previous studies.[9,13] To our

knowledge, this is the first time to investigate the relationship between fibrinogen and CVE in T2DM patients with stable CAD.

Recently the ADVANCE study (Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation) reported that baseline fibrinogen level was associated with an increased risk of macrovascular events and death in univariate analysis, but after further adjustment, fibrinogen was not an independent predictor of macrovascular.[17] In the present study, our finding is not consistent with the ADVANCE study, and we speculate that some reasons could explain it. Firstly, the inclusive criteria are different: we chose the patients with T2DM and stable CAD, while the ADVANCE study chose the subjects with T2DM and a history of CVD (stroke, myocardial infarction, transient ischemic attack, unstable angina, coronary or peripheral revascularization, amputation, macroalbuminuria, proliferative retinopathy or photocoagulation, macular edema, or blindness in one eye) or one or more additional cardiovascular risk factors. Secondly, despite the patients from many countries in the ADVANCE study, Chinese and Indians were excluded. Actually, there are ethnic differences in fibrinogen levels.[29-32] Thus, whether the results also persist in Chinese is undetermined. Finally, the definitions of outcomes are different. In ADVANCE study, major macrovascular events were cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke, while our cardiovascular events were defined as cardiac death, stroke, nonfatal myocardial infarction (MI), post-discharge revascularization (PCI/CABG) due to clinical deterioration or unstable angina (UA). Additionally, our previous cross-sectional study has revealed that plasma fibrinogen is associated with severity of coronary artery disease evaluated using Gensini score in diabetic patients,[19] which partly supports our present finding. Furthermore, in the present study, we found that 3.515(g/L) is a cut-off value of fibrinogen to predict CVEs in our cohort, which is consistent with previous study.[33]

There were several limitations of the present study. Firstly, the number of events is relatively small to assess the prognostic value of fibrinogen. The conclusion needs to be testified by large-scale study in the future. Secondly, the follow-up duration was relatively short and longer follow-up will be required. Thirdly, this sample population was collected prospectively from a single center in China. Fibrinogen varies in race,[29] so we do not know if our results would apply to other ethnic groups and studies from multiple centers are needed to test our finding. Finally, we

just collected one-time baseline level of fibrinogen.

In conclusion, we found that fibrinogen was associated with cardiovascular events in patients with type 2 diabetes with stable CAD in Chinese population. Further studies are required to clarify the issue completely.

Contributors S-H Y analyzed the data and drafted the manuscript. J-J L planned, designed the study, participated in fundraising and corrected the final version. YD, YZ, X-L L and SL participated in collecting data and interpreted the results. R-X X, C-G Z, Y-L G and N-Q W participated in the study design, interpretation of the results and manuscript review. PQ, YG, C-J C and QD participated in collecting data and reviewing manuscript. All authors read and have approved the final manuscript.

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Competing interests The authors declare that they have no completing interests.

Patient consent Obtained.

Ethics approval The study was performed according to the Declaration of Helsinki, and the hospital ethics review board (Fuwai Hospital & National Center for Cardiovascular Diseases, Beijing, China) approved the protocol.

Data sharing statement The technical appendix, statistical code and data set are available from the corresponding author.

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Figure legends

Figure 1 Flow chart of the study. CAD, coronary artery disease.

Figure 2 Unadjusted (A) and adjusted by sex and age (B) cumulative hazard of cardiovascular events based on the levels of fibrinogen (low, middle and high).

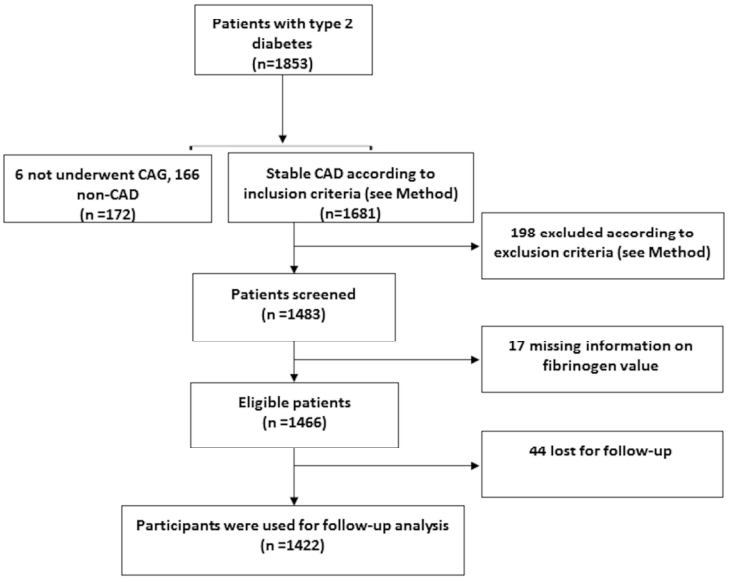

Figure 1 Flow chart of the study. CAD, coronary artery disease.

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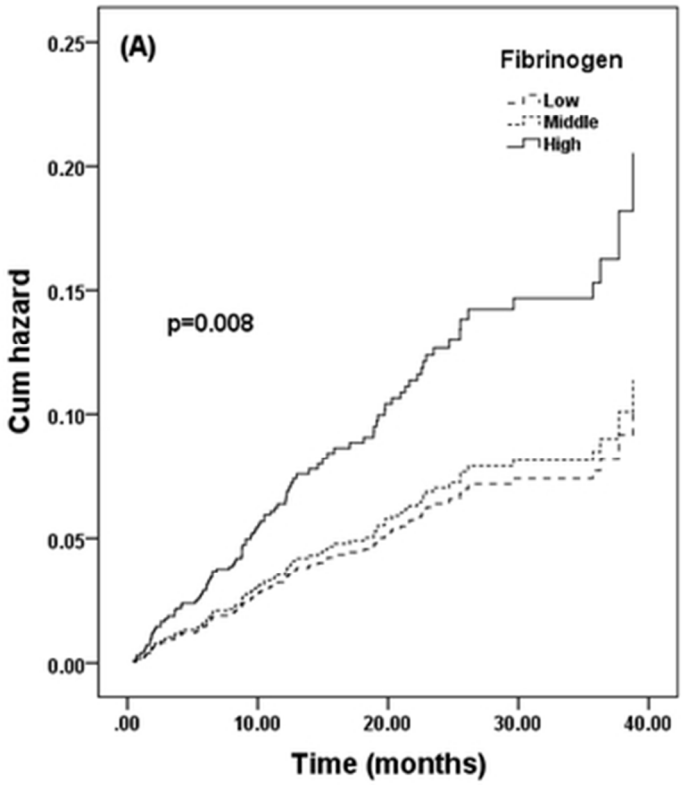

Figure 2 Unadjusted (A) and adjusted by sex and age (B) cumulative hazard of cardiovascular events based on the levels of fibrinogen (low, middle and high).

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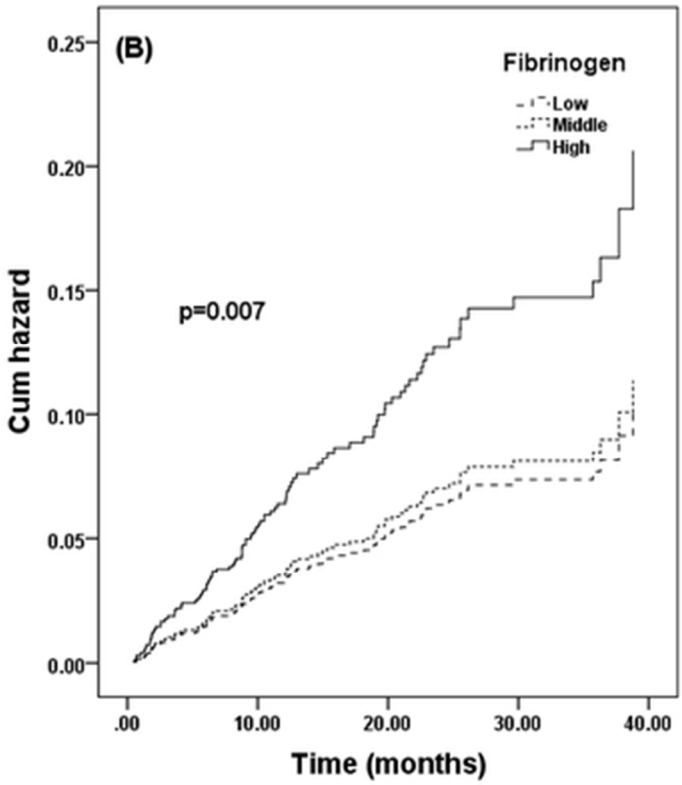

Figure 2 Unadjusted (A) and adjusted by sex and age (B) cumulative hazard of cardiovascular events based on the levels of fibrinogen (low, middle and high).

39x33mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3,4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	3
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	3
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*4*
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	3, 5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	5
		(*c*) Explain how missing data were addressed	4
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	4
		(*e*) Describe any sensitivity analyses	4

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	4
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*7*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6-8
		(*b*) Report category boundaries when continuous variables were categorized	6-8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	6-8
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7,8
Discussion			
Key results	18	Summarise key results with reference to study objectives	8,9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9,10
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10,11

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.