## PEER REVIEW HISTORY



## ARTICLE DETAILS

| | |
|---|---|
| **TITLE (PROVISIONAL)** | Serum fibrinogen and cardiovascular events in Chinese type 2 diabetic patients with stable coronary artery disease: a prospective observational study |
| **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 |

## VERSION 1 - REVIEW

| | |
|---|---|
| **REVIEWER** | RONG TAO<br>Department of Cardiology, Ruijin Hospital, Jiao Tong University School of Medicine, Shanghai 200025, P. R. China |
| **REVIEW RETURNED** | 22-Nov-2016 |

| | |
|---|---|
| **GENERAL COMMENTS** | Several studies have already demonstrated that baseline plasma fibrinogen levels could predict CVE in general individuals and nondiabetics. However, the present study first evaluated the predictive value of fibrinogen for cardiovascular events especially in Chinese patients with T2DM along with stable CAD, containing a large sample size and using professional study design as well as appropriate methods.<br>I appreciate your study and honesty for study limitations. Nonetheless, I've got several questions about it.<br><br>Major points:<br>Although your study contains a large sample size of 1466 patients, only 96 patients developed CVE, which is relatively small to investigate the predictive value of fibrinogen. Since you've defined it as one of the study limitations, could you further explain how the study size was arrived at in your study design?<br><br>As shown by KM curves and COX regression analysis, patients in the high level group are at highest risk of CVE compared with both low and middle group. However, the difference between low and middle group seemed not significant. Could you establish a cut-off value of fibrinogen best predict CVE in these patients?<br><br>All of your analyses and results focused on T2DM patients with CAD and the reason of such choice is clearly clarified in your introduction and discussion. Nevertheless, I'm curious if the predictive value of fibrinogen for CVE still exists in angiographic-proven CAD patients but without T2DM.<br><br>Minor points:<br>First of all, according to your inclusion and exclusion criteria (page 4), were patients with old myocardial infarction, stroke or atrial |

| | |
|---|---|
| | fibrillation included in your study? Could these medical histories influence fibrinogens levels? Also did any comorbidity such as hypertension affect the predictive value of fibrinogen for CVE in patients with T2DM?<br><br>As shown in table 1, patients in both CVE and no CVE group use similar medication. Moreover, although I can tell the association between fibrinogen and hyperlipemia as well as blood glucose, I couldn't find the relationship between fibrinogen and medication. Could medication affect fibrinogen levels analyzed in your study as well as other studies? Moreover, why not adjust for medication such as statin use in your COX regression models?<br><br>Fibrinogen in men is shown to be much lower than that in women in table 2, also you've indicated that fibrinogen was a significant and independent predictor of coronary heart disease in men in another cohort (page 10, paragraph 2, line 4). Is the predictive value of fibrinogen significantly different between men and women in your cohort?<br><br>In table 2, LDL-C levels are significantly different among these three groups divided by fibrinogen tertiles, which means patients with higher fibrinogen level have much higher LDL-C level (low: 2.28±0.89, middle: 2.52±0.94, high: 2.60±1.01, $p<0.001$). But in your COX regression analysis, none of these 5 models contains LDL-C. Why don't you adjust for LDL-C level but choose HDL-C and triglycerides instead?<br><br>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 in your study (page 5, paragraph 3, line 2-3), also the severity of DM and disease progression could affect the occurrence rate of CVE. In table 2, fibrinogen levels seem to be positively correlated to serum glucose. Could it be influenced by medication for diabetes? |

| | |
|---|---|
| **REVIEWER** | Claudia Menzaghi<br>IRCCS "Casa Sollievo della Sofferenza", San Giovanni Rotondo, Italy |
| **REVIEW RETURNED** | 28-Nov-2016 |

| | |
|---|---|
| **GENERAL COMMENTS** | In this paper Yang and coworkers explore the association between serum fibrinogen and cardiovascular events in Chinese patients with type 2 diabetes and coronary artery disease. The authors found that high fibrinogen levels are independently associated with cardiovascular events.<br>The aims are sound, but I have several major concerns as follow:<br>Please report the incidence rate for the endpoint of interest expressed as the number of new events per 100 person years (py).<br><br>Please report number of subjects for each fibrinogen levels low, medium and high. Why the authors have not used fibrinogen tertiles?<br>Which test has been used for the comparison between serum fibrinogen levels group? And to test for linear trend in incident rate over fibrinogen groups?<br><br>The data showing an increased number of female among patients |

| | |
|---|---|
| | with middle and high fibrinogen levels are interesting. Have the authors tested for a possible interaction between fibrinogen and sex?<br><br>Please in Table 3 first describe HRs for 1 SD fibrinogen increase and after the association divided by groups. The information given by fibrinogen as continuous variable is more important from a clinical point of view.<br><br>With this number of events what is the power of the study?<br><br>Please add a sentence on the generalizability of data obtained to other population of different ethnicities and to other clinical sets.<br><br>References in the introduction need to be better organized. In fact several other papers already reported by the authors (i.e. 25, 26 ecc) have to be mentioned also in the introduction along with references 15-17. |

**VERSION 1 – AUTHOR RESPONSE**

Reviewer: 1

Question 1: Although your study contains a large sample size of 1466 patients, only 96 patients developed CVE, which is relatively small to investigate the predictive value of fibrinogen. Since you've defined it as one of the study limitations, could you further explain how the study size was arrived at in your study design?
Response: Thank you for your scientific comment. In fact, our study is a prospective registry study for CAD patients (Would you kindly find our published papers, e.g. Medicine (Baltimore). 2015;94:e2426; Ann Med. 2016;48:83-8; J Clin Lipidol. 2015;9:794-800)? The aim of this study is to investigate the role of fibrinogen in CVE in Chinese patients with CAD and T2DM. In the present study, we defined the follow-up outcomes (Page 5, Paragraph 4). Hence, this study is just one part of our prospective registry study for CAD patients, and the follow-up is still continued.

Question 2: As shown by KM curves and COX regression analysis, patients in the high level group are at highest risk of CVE compared with both low and middle group. However, the difference between low and middle group seemed not significant. Could you establish a cut-off value of fibrinogen best predict CVE in these patients?
Response: Thank you for your advice. In the present study, we found that 3.515(g/L) is a cut-off value of fibrinogen to predict CVEs in Chinese diabetics with coronary artery disease, which is consistent with previous study [1].We have added this information into the section of discussion in the revised manuscript (Please see page 11, Paragraph 2, Line 20-21).
[1] Zhang Y, Zhu CG, GuoYL, et al. Higher fibrinogen level is independently linked with the presence and severity of new-onset coronary atherosclerosis among Han Chinese population. PLoS One.2014;9:e113460.

Question 3: All of your analyses and results focused on T2DM patients with CAD and the reason of such choice is clearly clarified in your introduction and discussion. Nevertheless, I'm curious if the predictive value of fibrinogen for CVE still exists in angiographic-proven CAD patients but without T2DM.
Response: Thank you very much for your excellent question. Actually, although lots of previous studies have demonstrated that fibrinogen can predict cardiovascular events in general population [1-6], patients with diabetes were included in these studies. Importantly, our previous study has also reported that the predictive value of fibrinogen for coronary artery severity existed in Chinese patients

with angiographic-proven CAD [7]. Therefore, in the present study, we did not perform the analysis in CAD patients without DM.

[1]Ang L, Thani KB, Ilapakurti M, et al. Elevated Plasma Fibrinogen Rather Than Residual Platelet Reactivity AfterClopidogrel Pre-Treatment IsAssociatedWith an Increased Ischemic Risk During Elective Percutaneous Coronary Intervention. J Am CollCardiol. 2013;61:23-34.
[2] De Luca G, Verdoia M, Cassetti E, et al. High fibrinogen level is an independent predictor of presence and extent of coronary artery disease among Italian population. J Thromb Thrombolysis 2011;31:458-63
[3]Danesh J, Lewington S, Thompson SG, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. JAMA 2005;294:1799-809
[4] Mahmud E, Behnamfar O, Lin F, et al. Elevated Serum Fibrinogen Is Associated With 12-Month Major Adverse Cardiovascular Events Following Percutaneous Coronary Intervention. J Am CollCardiol. 2016;67:2556-7
[5] Kunutsor SK, Kurl S, Zaccardi F, et al. Baseline and long-term fibrinogen levels and risk of sudden cardiac death: A new prospective study and meta-analysis. Atherosclerosis.2016;245:171-8
[6] Collaboration TERF. C-Reactive Protein, Fibrinogen, and Cardiovascular Disease Prediction. N Engl J Med. 2012;367:1310-20.
[7]Zhang Y, Zhu CG, GuoYL, et al. Higher fibrinogen level is independently linked with the presence and severity of new-onset coronary atherosclerosis among Han Chinese population. PLoS One.2014;9:e113460.

Question 4: First of all, according to your inclusion and exclusion criteria (page 4), were patientswith old myocardial infarction, stroke or atrial fibrillationincluded in your study? Could these medical histories influence fibrinogens levels? Also did any comorbidity such as hypertension affect the predictive value of fibrinogen for CVE in patients with T2DM?
Response: Thank you very much for your scientific question. In the present study, patients with previous PCI, CABG, stroke or atrial fibrillation were also included due to stable status of the disease. Besides, there is no difference between the CVE group and non-CVE group (See Table 1). Moreover, there is no difference in the percentage of the common medicine use or hypertension status between the CVE group and non-CVE group (Table 1). Similarly, a significant difference was not found in usage rate of medicines or or hypertension status across fibrinogen level tertiles (Table 2).

Question 5: As shown in table 1, patients in both CVE and no CVE group use similar medication. Moreover, although I can tell the association between fibrinogen and hyperlipemia as well as blood glucose, I couldn't find the relationship between fibrinogen and medication. Could medication affect fibrinogen levels analyzed in your study as well as other studies? Moreover, why not adjust for medication such as statin use in your COX regression models?
Response: Thank you very much for your good question. Indeed, it is difficult to find out whether medicines can affect the predictive value of fibrinogen for CVE in this study, because we just evaluated the predictive value just according to one-time levels of fibrinogen. However, no difference in medicine use was found in various levels of fibrinogen when the baseline characters were collected (Table 2). Moreover, there was no significant difference in medicine use between the patients with or without CVE (Table 1). Since the subjects we enrolled were patients with CAD and T2DM, statin use was very common and there was no difference in statin use across fibrinogen tertiles (Table 2). Meanwhile, we found that after adjustment for statin use, fibrinogen remains associated with CVE in our cohort. In a word, our results are similar to previous studies [1-4].

[1]Luc G. C-Reactive Protein, Interleukin-6, and Fibrinogen as Predictors of Coronary Heart Disease: The PRIME Study. Arteriosclerosis, Thrombosis, and Vascular Biology. 2003;23:1255-61.
[2] Lowe G, Woodward M, Hillis G, et al. Circulating inflammatory markers and the risk of vascular complications and mortality in people with type 2 diabetes and cardiovascular disease or risk factors: the ADVANCE study. Diabetes. 2014;63:1115-23.

[3] Soedamah-Muthu SS, Chaturvedi N, Pickup JC, et al. Relationship between plasma sialic acid and fibrinogen concentration and incident micro- and macrovascular complications in type 1 diabetes. The EURODIAB Prospective Complications Study (PCS). Diabetologia. 2008;51:493-501.
[4]Kunutsor SK, Kurl S, Zaccardi F, et al. Baseline and long-term fibrinogen levels and risk of sudden cardiac death: A new prospective study and meta-analysis. Atherosclerosis. 2016;245:171-80

Question 6: Fibrinogen in men is shown to be much lower than that in women in table 2, also you've indicated that fibrinogen was a significant and independent predictor of coronary heart disease in men in another cohort (page 10, paragraph 2, line 4). Is the predictive value of fibrinogen significantly different between men and women in your cohort?
Response: Thank you very much for your excellent question. Indeed, our results showed that fibrinogen level was higher in women than that in men, which is consistent with previous studies [1-3]. Although fibrinogen level has been demonstrated to be associated with CVE in general population after adjustment for sex, few studies reported whether or not the predictive value of fibrinogen is significantly different between men and women. Soedamah-Muthu et al. reported that fibrinogen was a significant and independent predictor of coronary heart disease in menbut not in women [4], whereas, Stecet al. reported that fibrinogen level was a predictor of cardiovascular diseases in both men and women [5]. Actually, we wonder whether there is a similar result in our cohort. Unluckily, we did not evaluate if there was a significant difference about the relationship between fibrinogen and CVE in men and women because of fewer events. Thus, further study is required.
[1]Fowkes et al. Sex Differences in Susceptibility to EtiologicFactors for Peripheral AtherosclerosisImportance of Plasma Fibrinogen and Blood Viscosity. ArteriosclerThrombVasc Biol. 1994;14:862-868
[2]De Luca G, Verdoia M, Cassetti E, et al. High fibrinogen level is an independent predictor of presence and extent of coronary artery disease among Italian population. J Thromb Thrombolysis. 2011;31:458-63.
[3]Wang TJ, Gona P, Larson MG, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. N Engl J Med. 2006;355:2631-9.
[4] Soedamah-Muthu SS, Chaturvedi N, Pickup JC, et al. Relationship between plasma sialic acid and fibrinogen concentration and incident micro- and macrovascular complications in type 1 diabetes. The EURODIAB Prospective Complications Study (PCS). Diabetologia 2008;51:493-501.
[5] StecJJ, Silbershatz H, Tofler GH, et al. Association of fibrinogen with cardiovascular risk factors and cardiovascular disease in the Framingham Offspring Population. Circulation.2000;102:1634-8.

Question 7:In table 2, LDL-C levels are significantly different among these three groups divided by fibrinogen tertiles, which means patients with higher fibrinogen level have much higher LDL-C level (low: 2.28±0.89, middle: 2.52±0.94, high: 2.60±1.01, $p<0.001$). But in your COX regression analysis, none of these 5 models contains LDL-C. Why don't you adjust for LDL-C level but choose HDL-C and triglycerides instead?
Response: Thank you very much for pointing out the mistake. In fact, we had adjusted LDL-C but overlooked it because of our carelessness. We have corrected it.

Question 8: 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 in your study (page 5, paragraph 3, Line 2-3), also the severity of DM and disease progression could affect the occurrence rate of CVE. In table 2, fibrinogen levels seem to be positively correlated to serum glucose. Could it be influenced by medication for diabetes?
Response: Thank you very much for your sound question. We have added this information to Table 1 and Table 2. In Table 1 there was no difference in anti-diabetic drugs use between the patients who developed CVE or not. Similarly, a significant difference in anti-diabetic medicine use was not found across the tertiles of fibrinogen levels (Table 2). Moreover, we found that anti-diabetic drugs did not affect the relationship between fibrinogen and CVE in Cox model (data not shown).

Reviewer: 2

Question 1: Please report the incidence rate for the endpoint of interest expressed as the number of new events per 100 person years (py).
Response: Thank you for your suggestion. According to what you suggested, we calculated the number of new events per 100 person years, namely, 2.0047/100per 100 person years. We have added this information to the manuscript (page 8, paragraph 1, line 4).

Question 2: Please report number of subjects for each fibrinogen levels low, medium and high. Why the authors have not used fibrinogen tertiles?
Response: Thank you for your suggestion. We have reported the number of subjects for each fibrinogen levels low, medium and high in Table 2. Actually, we trisected fibrinogen levels into (≤2.91 g/L), (2.91-3.51 g/L) and (≥3.51 g/L) (See page 6, paragraph 2, line 2). “Low”, “Middle” and “High” levels of fibrinogen are just named artificially according to fibrinogen tertiles.

Question 3: Which test has been used for the comparison between serum fibrinogen levels group? And to test for linear trend in incident rate over fibrinogen groups?
Response: Thank you very much for your valuable question. Kruskal-Wallis H test was used for nonnormal variables such as CVE rate overfibrinogen groups, and ANOVA applied for comparisons of continuous variables. Test for linear trend (Jonckheere–Terpstra test) was performed by assigning median value for each tertile and treated as continuous variables. Moreover, we found a linear trend in CVE rate over fibrinogen groups. We have added this information to the manuscript (Page 6, Paragraph 3, Line 5-6).

Question 4: The data showing an increased number of female among patients with middle and high fibrinogen levels are interesting. Have the authors tested for a possible interaction between fibrinogen and sex?
Response: Thank you for your important question. Indeed, our results showed that the fibrinogen level was higher in women than that in men, which is in agreement with previous studies [1-4]. After testing, we have not found a possible interaction between fibrinogen and sex in our cohort. Despite all this, we cannot determine whether or not a possible interaction between fibrinogen and sex exists due to our relatively small sample, so further study is needed.

[1]Fowkes et al. Sex Differences in Susceptibility to EtiologicFactors for Peripheral AtherosclerosisImportance of Plasma Fibrinogen and Blood Viscosity. ArteriosclerThrombVasc Biol. 1994;14:862-868
[2] De Luca G, Verdoia M, Cassetti E, et al. High fibrinogen level is an independent predictor of presence and extent of coronary artery disease among Italian population. J Thromb Thrombolysis. 2011;31:458-63.
[3] Wang TJ, Gona P, Larson MG, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. N Engl J Med. 2006;355:2631-9.
[4] StecJJ, Silbershatz H, Tofler GH, et al. Association of fibrinogen with cardiovascular risk factors and cardiovascular disease in the Framingham Offspring Population. Circulation.2000;102:1634-8.

Question 5: Please in Table 3 first describe HRs for 1 SD fibrinogen increase and after the association divided by groups. The information given by fibrinogen as continuous variable is more important from a clinical point of view.
Response: Thank you very much for your valuable suggestion. We agree with you and have corrected it as what you advised.

Question 6: With this number of events what is the power of the study?

Response: Thank you for your question. We have calculated roughly the the power of the study with the help of a medical statistician, namely, 0.89, according to previous published papers [1-3].

[1] Bruno G, Merletti F, Biggeri A, et al. Fibrinogen and AER are major independent predictors of 11-year cardiovascular mortality in type 2 diabetes: the Casale Monferrato Study. Diabetologia.2005;48:427-34.
[2] Hoffmeister A, Rothenbacher D, Bazner U, et al. Role of novel markers of inflammation in patients with stable coronary heart disease. Am J Cardiol.2001;87:262-6.
[3] Luc G. C-Reactive Protein, Interleukin-6, and Fibrinogen as Predictors of Coronary Heart Disease: The PRIME Study.ArteriosclerThrombVascBiol.2003;23:1255-61.

Question 7: Please add a sentence on the generalizability of data obtained to other population of different ethnicities and to other clinical sets.
Response: Thank you for your suggestion. We agree with you and have added this information in the revised manuscript (Page 11, Paragraph 2, Line 5).

Question 8: References in the introduction need to be better organized. In fact several otherpapers already reported by the authors (i.e. 25, 26 etc) have to be mentioned also in theintroduction along with references 15-17.
Response: Thank you very much for your suggestion. We have checked the references carefully as what you advised and corrected them in the revised manuscript.

**VERSION 2 – REVIEW**

| | |
|---|---|
| **REVIEWER** | Tao, Rong<br>Department of Cardiology, Ruijin Hospital, Jiao Tong University School of Medicine, Shanghai 200025, China |
| **REVIEW RETURNED** | 18-Jan-2017 |

| | |
|---|---|
| **GENERAL COMMENTS** | Reviewer comments:<br><br>This longitudinal study executed by Yang et al., investigated the association of serum fibrinogen with cardiovascular events (CVE) in a population of Chinese T2DM with stable CAD patients. They found high tertile of serum fibrinogen was significantly associated with the occurrence of CVE in T2DM patients with or without adjustment by sex and age in a period of 20.2 months. Although the design for this study is interesting, there are a number of issues which need the authors to clarify. In addition, some important information is missing.<br><br>Major comments:<br><br>1. Since post-discharge revascularization (PCI/CABG) was related to the severity of coronary vasculopathy and the baseline severity of coronary lesions in T2DM patients was also associated with CVE, it might potentially cofound the final results. Therefore, at this point, the baseline severity of CAD in T2DM patients from tertile of fibrinogen groups is missing.<br><br>2. Fibrinogen is a heterogeneous mixture of many different molecular forms and some agents (e.g. rtPA) or eGFR levels may affect the plasma concentration of fibrinogen. What are the eGFR levels in CVE and No CVE group patients? Using uric acid levels to describe renal function is limited.<br><br>3. Figure 2B: Why did the authors choose age and sex only? What |

| | |
|---|---|
| | about other established CVD risk factors?<br><br>4. Table 3: The increase of HR from low to high tertile of fibrinogen group is rather modest after adjustment for Model 4 plus LVEF. Considering the importance of all these established risk factors, the clinical implication for the cut-off value of fibrinogen mentioned in the text seems to be limited.<br><br>Minor comments:<br><br>1. The inclusion criteria needs to be clarified. Why did all the patients conduct CAG examination?<br><br>2. What does “severe liver and/or renal insufficiency” mean? |

| | |
|---|---|
| **REVIEWER** | Claudia Menzaghi<br>IRCCS "Casa Sollievo della Sofferenza", San Giovanni Rotondo, Italy |
| **REVIEW RETURNED** | 16-Jan-2017 |

| | |
|---|---|
| **GENERAL COMMENTS** | The authors addressed all the comments and improved the paper. |

**VERSION 2 – AUTHOR RESPONSE**

Reviewer: 1
Question 1:Since post-discharge revascularization (PCI/CABG) was related to the severity of coronary vasculopathy and the baseline severity of coronary lesions in T2DM patients was also associated with CVE, it might potentially cofound the final results. Therefore, at this point, the baseline severity of CAD in T2DM patients from tertile of fibrinogen groups is missing.
Response: Thank you very much for your scientific comment. We agree with you and have added this information in Table 2 in the revised manuscript.

Question 2: Fibrinogen is a heterogeneous mixture of many different molecular forms and some agents (e.g. rtPA) or eGFR levels may affect the plasma concentration of fibrinogen. What are the eGFR levels in CVE and No CVE group patients? Using uric acid levels to describe renal function is limited.
Response: Thank you for very much your comment. We agree with you and have added this information in Table 1 of the revised manuscript.

Question 3: Figure 2B: Why did the authors choose age and sex only? What about other established CVD risk factors?
Response: Thank you for your question. Actually, we have showed the hazard ratio for CVE associated with fibrinogen after adjustment for the CVD risk factors in Table 3. Indeed, in Figure 2B, we just showed the hazard ratio for CVEassociated with fibrinogen after an adjustment for age and sex, but we think it may be reasonable, because previous papers also exhibited the figures like this (J Intern Med 2013;273(6):595-601).

Question 4:Table 3: The increase of HR from low to high tertile of fibrinogen group is rather modest after adjustment for Model 4 plus LVEF. Considering the importance of all these established risk factors, the clinical implication for the cut-off value of fibrinogen mentioned in the text seems to be limited.
Response: Thank you very much for your excellent comment. We do agree with what you said. We speculate that this phenomenon is likely due to the relatively small number of CVEs resulting from

short duration of follow-up. Thus, further study is need.

Question 5: The inclusion criteria needs to be clarified. Why did all the patients conduct CAG examination?
Response: Thank you for your scientific comment. In the present study, all patients were under CAG according to their case histories and/or chest pain and/or coronary artery CT and/or positive results of treadmill exercise test for the diagnosis or treatment of CAD.

Question 6: What does “severe liver and/or renal insufficiency” mean?
Response: Thank you very much for your suggestion. We have added the definitions of severe liver and/or renal insufficiency in Methods section in the revised manuscript.