Author's response to reviews

Title: Glycated hemoglobin independently or in combination with fasting plasma glucose versus oral glucose tolerance test to detect abnormal glycometabolism in acute ischemic stroke: A Chinese cross-sectional study

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Author's response to reviews: see over
Dear Editor-in-Chief,

Thank you very much for letting us know that our manuscript (MS: 1698631941117330) entitled “Glycated hemoglobin independently or in combination with fasting plasma glucose versus oral glucose tolerance test to detect abnormal glycometabolism in acute ischemic stroke: A Chinese cross-sectional study” has been reviewed. We greatly appreciated the careful review and helpful comments from the reviewers. We have made major changes that we believe to have improved the clarity of the writing, the presentation of our findings, and the interpretation and integration of existing literature. All the changes were highlighted for your review in the revised version. Please see our responses to each comment in italic blue words.

Reviewer 1

Several minor revisions:

In the abstract

Q1. I do not understand the last sentence of the conclusion: “significant to secondary stroke prevention” Maybe not appropriate for the results of the study.

R1--Thank you very much. We revised the content as follows (Page 3): Our results advocate the use of HbA1c as screening tool for the diagnosis of prediabetes.

In the Introduction section:

Q2. “Although the sensitivity of HbA1c for detecting DM was not as high as that of OGTT, HbA1c was recommended as a method to screen for DM by the American Diabetes Association (ADA) because its low sensitivity was offset by its advantages of familiarity to clinician, simple manipulation, and without requiring the patient to fast, etc.” Does not read well. Maybe try: “HbA1c is an easy method to screen for DM but compared to OGTT, its sensitivity for detecting DM is low. However, the ADA endorses the use of HbA1C based on its familiarity to clinicians, simple manipulation, and no need to fast.”

R2--Thank you for your valuable input. We revised the content as follows (Page 4):
**HbA1c** is an easy method to screen for diabetes mellitus (DM) but compared to oral glucose tolerance test (OGTT), its sensitivity for detecting DM is low. However, the American Diabetes Association (ADA) endorses the use of HbA1c based on its familiarity to clinicians, simple manipulation, and no need to fast.

Q3. Define FPG and OGTT early in the introduction.
R3--Thank you. We defined the FPG in the introduction as follows (Page 4):

*The test of fasting plasma glucose (FPG) requires no caloric intake for at least eight hours.*

We have removed the OGTT definition from ‘Method’ part to ‘Introduction’ as follows (Page 4):

*According to the World Health Organization (WHO) criteria in 1997, OGTT is one method to diagnose abnormal glucose levels. OGTT following an overnight fast for at least eight hours was performed via an oral intake of a standard dose of 75g anhydrous glucose dissolved in water. Fasting plasma glucose levels were measured prior to administering the anhydrous glucose and postprandial glucose was evaluated two hours later. Patients were not allowed or recommended to have a special diet during the two hours. Fasting plasma glucose and 2-h plasma glucose (PG2h) were used in combination to diagnose DM or prediabetes [4-5].*

Q4. Could you shorten the introduction? There are some redundancies.
R4--Thank you for your valuable comment! We delete the paragraph 4:

“HbA1c, FPG, and OGTT have no full concordance either in diagnosing DM or in identifying prediabetes [2]. Due to the varying diagnostic percentage among the three methods, there is a great need to compare their accuracy in diagnosing DM and prediabetes.”

We shortened the original paragraph 6 “It has been estimated that a high prevalence of
abnormal glucose regulation… Due to these limitations, a method aside from OGTT is needed to diagnose abnormal glycometabolism” as follows (Page 5):

‘Since a high prevalence of abnormal glycometabolism diagnosed using OGTT among Chinese patients with acute stroke has been estimated [11] and both DM and a high ‘normal’ glycaemia status are high risks for stroke [12], there is a great need to find an efficient screening test to identify abnormal glycometabolism in patients with acute ischemic stroke. However, OGTT is limited in the clinical practice because it is inconvenient, time-consuming, relatively expensive, and requires the patient to fast [3]. A method aside from OGTT is needed to diagnose abnormal glycometabolism quickly and easily.’

We deleted the following sentences from the original paragraph 5 “Results among these studies have been inconsistent. Internationally, Hjellestad et al. …Despite the findings from these studies, a study with a sample consisting of patients nationwide from China is still needed” and integrated the original paragraphs 5 and 7 as follows (Page 5):

‘Up to date, although several recent studies worldwide have compared the ability of diagnosing abnormal glycometabolism between OGTT and HbA1c with or without FPG based on disease spectrums and patient populations [6-10], the results were inconsistent and a similar study focus on acute ischemic stroke patients was still lacking [13].

In the present study, we aimed to compare the diagnostic accuracy among HbA1c, FPG, and OGTT for newly-diagnosed DM and pre-diabetes among patients with acute ischemic stroke. An ideal cut-off value of HbA1c was also pursued to better diagnose abnormal glycometabolism. HbA1c or combining HbA1c and FPG was hypothesized to be a better method than OGTT for screening abnormal glycometabolism.’

Discussion:

Q5-- I’d like the authors to comment on the limitations of OGTT as the gold standard. In the ACROSS-China study OGTT was performed within 2 weeks of the acute stroke. Patients were also given medications in the hospitals. These 2 factors can effect the
results of the test.

R5--Thank you for your comment. We rewrote the content as follows (Page 16):

‘However, OGTT was performed on the 14th day after stroke onset and patients might receive medications, which might affect the test results. Since OGTT was used as the golden method in the present study, the results might be affected.’

Q6-- Please delete: “In short, HbA1c combined with FPG for diagnosing DM was recommended in acute ischemic stroke patients without previous DM.” And “Based on our results, we recommend the use of HbA1C as pre-diabetes screening tool in acute ischemic stroke patients.” You might use: Our results advocate the use of HbA1C as screening tool for the diagnosis of pre-diabetes.

R6- Thank you very much! We revised the content as follows (Page 15): ‘Our results advocate the use of HbA1c as screening tool for the diagnosis of prediabetes.’

Q7. The clinical significant paragraph can be deleted or shorten.

R7--Thank you! We deleted the ‘Clinical Significant’ paragraph.

Q8. Please improve the English grammar and flow of the manuscript.

R8--We have examined the manuscript carefully and done language editing.

Reviewer 2

Q1. The status of hyperglycemia in the acute stage of stroke has been frequently reported (Katja P et al, Stroke. 2012; 43: 898-902). The authors use the combination of HbA1c and FPG as a diagnostic tool in the acute stroke and OGTT as the outcome of measurement. The time from the onset to each measurement should be presented in the manuscript.

R1--Thank you! We have added two sentences as follows (Page 6):

Blood samples were collected for the evaluation of FPG and HbA1c within 24 hours after admission. OGTT was done on the 14th day after stroke onset.
Q2. The diagnosis of DM by American Diabetes Association requires the measurement twice of the FPG $\geq 126\text{mg/dl}$.

R2--Thank you for your valuable input! All the patients were required to repeat test FPG on the next day if a FPG $\geq 126\text{mg/dl}$ was met. We have added the explanation as follows (Page 6-7):

*Patients who had FPG of $\geq 7.0\text{ mmol/L}$ were required to repeat FPG test on the next day. A diagnosis of DM was made for a patient only when both FPG values were $\geq 7.0\text{ mmol/L}$. When the FPG value was $\geq 7.0\text{ mmol/L}$ at the first time but 6.1 - 7.0 mmol/L at the second time, a diagnosis of prediabetes was made. When the FPG value was $\geq 7.0\text{ mmol/L}$ at the first time but <5.6mmol/L at the second time, normoglycaemia was diagnosed for the patient.*

Q3. A1C measurement needs NGSP certified and standardized to the DCCT assay. Please state explicitly if the laboratories in the participating centers meet the criteria.

R3--Thank you very much! We have added some explanation as follows (Page 7):

*The blood samples for HbA1c evaluation were separated and the plasmas were properly processed, refrigerated at -20°C, and transported to Beijing Tiantan Hospital in Beijing, China. The laboratory in Beijing Tiantan Hospital was certified by the National Glycohemoglobin Standardization Program (NGSP) for HbA1c measurement.*

Q4. The demographics of the patients could provide an overview of the population.

R4--Many thanks for your valuable comment! We have provided an overview of population as follows (Page 9-10):

*There were 1,316 patients included in the present analysis and their average age was 62.4 years old and 63.3% of them were male. Current smokers occupied 33% and moderate to severe drinkers occupied 15.5%. The mean systolic blood pressure was over 140mmHg. More than 50% of the patients had a history of hypertension. The mean levels of FPG and HbA1c were 5.7mmol/L and 6.0%, respectively. The mean level of low-density lipoprotein was over 3.0mmol/L.*
The mean age, FPG, HbA1c, white cell counts, insulin resistance index, and low-density lipoprotein were all higher in the DM group than those in the prediabetes and normoglycemia groups (all P<0.05). The differences of other variables among the groups were not statistically significant (see Additional file 1).

Q5: More FPG
R5--Thank you very much! Because the ACROSS study was ended, the FPG values cannot be added. In all ischemic stroke patients (n=2,639), only 45 patients (1.7%) did not have FPG values. We believe that the percentage of the missing values of FPG (1.7%) was low enough to not affect the results in the present analyses. All the ischemic stroke patients included in the current study (n=1,316) had complete FPG values.

Reviewer 3
Q1: Comparing the AUC of two procedures on the same set of subjects produces two correlated sets of statistics. It should be indicated that this correlation must be taken into account in the calculations of the standard errors of the AUC's.
R1: Thank you very much for your comment! We used OGTT as the golden method, and used HbA1c and FPG as the compared diagnostic tests in the present study. When HbA1c/FPG was compared with OGTT (golden method), Standard errors and 95% confidence intervals would be directly shown in the statistically results by SPSS. However, when comparing the AUCs between HbA1c and the combination of HbA1c and FPG, we need to take the correlation between the two diagnostic methods into account in the calculation of the standard errors during the Z test. SPSS has no such function to do that. Thus, we used the nonparametric Z test to do that comparison.

The calculating equation is \[ Z = \frac{A1 - A2}{\sqrt{SE1^2 + SE2^2 + SE_{12}^2 - 2SE1SE2r}} \]. When \[ Z \geq 1.96 \], P is < 0.05, the difference of the two AUCs is statistically different. Otherwise, it is not.

(See Reference 13: Hanley JA, McNeil BJ. A method of comparing the areas under
receiver operating characteristic curves derived from the same cases. Radiology 1983; 148(3):839-43)

For example, when compared diagnostic method A and B, A1 refers to the AUC of diagnostic method A, A2 refers to the AUC of diagnostic method B, SE1 refers to the standard error of method A, SE2 refers to the standard error of method B, ‘r’ is calculated according to the method as the following 5 steps:

1) Calculating ‘\( r = (r_n + r_a)/2 \)’, \( r_n \) indicates the correlation coefficient between method A and B in the ‘no disease’ group, ‘\( r_a \)’ indicates the correlation coefficient between method A and B in the ‘disease’ group. The Kendall tau correlation coefficient was used.

2) Calculating ‘\( (A1+A2)/2 \)’, A1 and A2 was the same as the description above.

3) Find the ‘r’ value from ‘Table I’ in Reference 1.

4) A1, A2, SE1 and SE2 were calculated from SPSS and shown in Table 2 in the revised version.

5) Calculate the Z value.

We compared the AUCs between HbA1c and the combination of HbA1c and FPG in diagnosing DM (The procedure of the comparison between FPG and the combination is omitted here):

\[ r_n = 0.779, \quad r_a = 0.768, \quad \text{average AUC}=0.702, \quad \text{average } r=0.770, \quad \text{look up ‘Table I’ in ‘Reference 1’, } r=0.74, \quad \text{SE}_{\text{HbA1c}} =0.018, \quad \text{SE}_{\text{combination}} =0.017 \] (see Table 2 below or in the revised version), \[ \text{AUC}_{\text{HbA1c}}=0.692, \quad \text{AUC}_{\text{combination}}=0.712. \]

\[ Z = \frac{0.712 - 0.692}{\sqrt{0.018^2 + 0.017^2 - 2 \cdot 0.018 \cdot 0.017 \cdot 0.74}} = 1.78 < 1.96, \quad P>0.05. \]

The difference of the AUCs between HbA1c and the combination was not statistically significant. We have added the above information in the revised version as follows (Page 11):

‘Compared with HbA1c or FPG alone, the combination of them increased the AUROC of diagnosing DM from 0.692 (HbA1c) and 0.652 (FPG) to 0.712 (the combination) \( \text{(AUC comparison between HbA1c and the combination, } Z=1.78 < 1.96, \quad P>0.05; \quad \text{AUC comparison between FPG and the combination, } Z=1.62 < 1.96, \quad P>0.05. \text{ Youden index of the combination was higher than either of them (Table 2).’} \)
We wanted to compare the AUCs between HbA1c and the combination of HbA1c and FPG in diagnosing prediabetes. The average AUC of HbA1c and the combination was (0.557+0.554)/2=0.556, which is <0.700, thus we could not get ‘r’ (the correlation coefficient) from ‘Table 1’ in ‘Reference 13’. Hence Z test could not be applied there. Even so, $r_n$ and $r_a$ were calculated by SPSS. Kendall tau correlation coefficient, $r_n=0.514$, $r_a=0.533$. We have added the according content as follows (Page 11):

However, such a slight difference could not be identified as statistically significant since the nonparametric Z test was not suitable (average Kendall tau correlation coefficient=0.52, average AUC was 0.556 (<0.700), the correlation coefficient ‘r’ could not be looked up in Table I of the Hanley et al. study[13], thus, Z value could not be calculated).

Accordingly, we have added some content in ‘Statistical analysis’ as follows (Page 9):

‘Because both HbA1c and FPG tests were taken in the same set of subjects, the AUROCs of the two tests we got were not independent. The comparisons between the AUROCs were performed by using the nonparametric Z test with the correlation between AUROCs taken into account [13]. The calculating equation was ‘$Z=\frac{A_1-A_2}{\sqrt{\frac{1}{n_1}+\frac{1}{n_2}}}$’’, ‘r’ was calculated manually according to the method described in the published [13]. The equation ‘$r = (rn + ra)/2$’ was used, $rn$ indicates the Kendall tau correlation coefficient between different diagnostic tools in the ‘no disease’ group, $ra$ indicates the Kendall tau correlation coefficient between different diagnostic tools in the ‘disease’ group.

And we have also added some explanations in ‘Discussion’ as follows (Page 14):

‘Although the difference between AUROC of HbA1c and that of the combination of HbA1c and FPG was slight and not statistically significant ($Z=1.78$, $P>0.05$), the AUROC was indeed changed (AUROC changed from 0.692 to 0.712) and a much bigger sample size might settle the puzzle.'
In ‘Merits and limitations’ (Page 16): *A much bigger sample size might improve the comparison between the AUROCs to better differentiate the diagnostic ability among these tools.*

We have added the standard errors and the 95% confidence intervals in Table 2 as follows (Page 24):

<table>
<thead>
<tr>
<th></th>
<th>AUROC</th>
<th>Standard Error</th>
<th>95% CI</th>
<th>P</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Youden Index</th>
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</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>DM</td>
<td>0.692</td>
<td>0.018</td>
<td>&lt;0.001</td>
<td>0.528</td>
<td>0.855</td>
<td>0.383</td>
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<tr>
<td></td>
<td>Prediabetes</td>
<td>0.557</td>
<td>0.017</td>
<td>0.001</td>
<td>0.419</td>
<td>0.695</td>
<td>0.114</td>
</tr>
<tr>
<td></td>
<td>AGM</td>
<td>0.648</td>
<td>0.016</td>
<td>&lt;0.001</td>
<td>0.711</td>
<td>0.585</td>
<td>0.296</td>
</tr>
<tr>
<td>FPG</td>
<td>DM</td>
<td>0.652</td>
<td>0.019</td>
<td>&lt;0.001</td>
<td>0.365</td>
<td>0.940</td>
<td>0.305</td>
</tr>
<tr>
<td></td>
<td>Prediabetes</td>
<td>0.515</td>
<td>0.017</td>
<td>0.388</td>
<td>0.273</td>
<td>0.756</td>
<td>0.029</td>
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<tr>
<td></td>
<td>AGM</td>
<td>0.654</td>
<td>0.015</td>
<td>&lt;0.001</td>
<td>0.521</td>
<td>0.787</td>
<td>0.308</td>
</tr>
<tr>
<td>HbA1c</td>
<td>DM</td>
<td>0.712</td>
<td>0.017</td>
<td>&lt;0.001</td>
<td>0.607</td>
<td>0.817</td>
<td>0.424</td>
</tr>
<tr>
<td></td>
<td>with FPG Prediabetes</td>
<td>0.554</td>
<td>0.017</td>
<td>0.001</td>
<td>0.579</td>
<td>0.530</td>
<td>0.109</td>
</tr>
<tr>
<td></td>
<td>AGM</td>
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<td>0.015</td>
<td>&lt;0.001</td>
<td>0.812</td>
<td>0.488</td>
<td>0.300</td>
</tr>
</tbody>
</table>

Serving as the corresponding author, I certify again that I take full responsibility for the data, analyses, interpretation, and the conduct of the research; I have full access to all the data; I have the right to publish any and/or all data; this report consists of original and unpublished work that is not under consideration for publication elsewhere. We have no conflicts of interest to disclose. I attest to the fact that all
authors have read the revised manuscript and agreed to submit to BMC NEUROLOGY

We appreciate your consideration again and hope the changes we’ve made will make you satisfied.

Kind regards,

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