Peer Review File

Article Information: http://dx.doi.org/10.21037/apm-20-1927

Reviewer A

The authors included 478 patients with first-onset CE. They found that hs-CRP was also a prognostic factor in this population. However, there are plenty of weakness should be considered.

Major:

1) The abstract: it is unnecessary to describe methods over much. There are only three sentences for results. It is bewildered why to make the conclusion (the independent predictor?). In addition, the definition of endpoints is unclear. Some references for it?

Response:

We thank the reviewer for this insightful comment about our Abstract. As advised, we have simplified the Methodssubsection, while more information was added to the Results subsection to make the conclusion clearer. References have also been added for the definition of endpoints.

Changes to the manuscript:

The following text was changed in the Abstract section:

Methods

"We recruited 478 patients with first-onset cardioembolic stroke. High-sensitivity C-reactive protein and other biochemical markers were measured within 24h after admission. High-sensitivity C-reactive protein levels were grouped into quartiles (<2.31,2.31 to <6.09,6.09 to <22.30,and ≥22.30 mg/L). Stroke severity was assessed using the modified Rankin scale, with modified Rankin scale scores of 0 to 2 classified as a good outcome, and scores of 3 to 6 as a poor outcome. Composite endpoints included poor outcomes, vascular death, myocardial infarction, and recurrent stroke (ischemic or hemorrhagic). At 3-month and 1-year follow-ups, we used multivariate logistic regression analysis to assess the relationship between baseline high-sensitivity C-reactive protein levels, modified Rankin scale scores, and composite endpoints."

Results

"Among 478 patients with cardioembolic stroke, the median high-sensitivity C-reactive protein level was 6.09 mg/L. Regarding the primary outcome, we found that high-sensitivity C-reactive protein levels ≥22.30 mg/L were positively correlated with poor outcomes at the 3-month and 1-year follow-ups(OR 3.862;95% CI (1.675–8.904), p=0.002 and OR 5.479; 95% CI[1.692–17.744],p=0.005,respectively). The secondary outcomes paralleled the results of the primary outcomes at the 3-month and 1-year follow-ups (OR 3.381, 95% CI(1.620–7.058),p=0.001 and OR 3.181;95% CI(1.475–

6.860), p = 0.003,respectively).”

The following text was changed in the Study Outcome section

Study Outcome

“Stroke severity was assessed using the modified Rankin scale (mRS), with mRS scores of 0 to 2 classified as a good outcome, and scores of 3 to 6 as a poor outcome (15-16).”

References:

[15]Winter Y, Wolfram C, Schaeg M, et al. Evaluation of costs and outcome in cardioembolic stroke or TIA. J Neurol 2009;256:954-963.
[16]Qiu R, Gao Y, Hou D, et al. Association between hs-CRP Levels and the Outcomes of Patients with Small-Artery Occlusion. Front Aging Neurosci 2016;8:191.

2) The introduction part is not written well. The reason to conduct this study is unclear. Some sentences are arbitrary with evidences, such as “Stroke-related neuroinflammatory processes are associated with adverse outcomes and post-stroke complications.”“High-sensitivity c-reactive protein(hs-CRP) has recently received greater attention in the diagnosis, treatment, and outcomes of ischemic stroke”.

Response:

We thank the reviewer for pointing this out. I have reviewed the relevant literature and revised the Introduction according to the reviewer’s comment.

Changes to the manuscript:

Background

Cardioembolic stroke is a severe subtype of cerebral infarction, accounting for 20% of cerebral infarctions(1). This condition is characterized by high in-hospital mortality rates (27.3%)(2), neurological deficits at hospital discharge, and a high risk of stroke recurrence(26%)(3). However, the prognostic indicators for patients with cardioembolic stroke remain unclear. Acute strokes may trigger an inflammatory response, leading to elevated CRP levels(4), which may be associated with poor prognosis as they reflect inflammation or tissue damage(5). Therefore, CRP is a potential predictor of future cardiovascular and cerebrovascular events and prognostic markers after the event. However, compared with CRP, hs-CRP can be measured quantitatively and accurately detect low-level inflammation(6), and it can reflect minute changes in inflammation better than CRP can. Hs-CRP also has greater clinical significance in evaluating the relationship between acute inflammation and prognosis (7). Also, hs-CRP is more widely used in clinical practice, especially in the risk stratification of cardiovascular disease (CVD) (8) and stroke (9). To further clarify the

role of hs-CRP in cardioembolic stroke, we assessed hs-CRP levels as a predictor of cardioembolic stroke prognosis. Therefore, the purpose of our study was to analyze the association between hs-CRP levels in the acute phase of cardioembolic stroke and its outcomes.

References:

[4]McColl BW, Allan SM, Rothwell NJ. Systemic inflammation and stroke: aetiology, pathology and targets for therapy. Biochem Soc Trans 2007;35:1163–5

[5] den Hertog HM, van Rossum JA, van der Worp HB, et al. C-reactive protein in the very early phase of acute ischemic stroke: association with poor outcome and death. J Neurol 2009;256:2003–8.

[6] VanGilder RL, Davidov DM, Stinehart KR, et al. C-reactive protein and long-term ischemic stroke prognosis. J. Clin. Neurosci 2014; 4:547–53. doi: 10.1016/j.jocn.2013.06.015

[7] Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499-511.

[8] Andersson J, Johansson L, Ladenvall P, et al. C-reactive protein is a determinant of first-ever stroke: prospective nested case-referent study. Cerebrovasc Dis 2009;27:544-51.

[9] Kong H, Qian YS, Tang XF, et al. C-reactive protein (CRP) gene polymorphisms, CRP levels and risk of incident essential hypertension: findings from an observational cohort of Han Chinese. Hypertens Res2012;35:1019-23.

3) The methods: the informed consent and ethics are missed. The methods part are only to describe the research approach. Some results are written in this part, such as “At the 3-month follow-up, 75 patients had recurrent stroke (ischemic or hemorrhagic), myocardial infarction (MI), or vascular death, and were included in the composite endpoint event, patients were 67 dead for other reasons and 1 patient did not attend the follow-up review. Therefore, data from 444 patients was analyzed. At the 1-year follow-up, 141 patients had recurrent stroke (ischemic or hemorrhagic), MI, or vascular death, and were included in the composite endpoint event, 14 patients were dead for other reasons, 35 patients did not attend the follow-up. Finally, data from 395 patients was analyzed in this study (Figure1).” “Through above examinations, we found 398 cases with atrial fibrillation (chronic 288 cases, paroxysmal 82 cases, persistent 28 cases) and 4 cases with atrial flutter among all patients.” “At the 3-month follow-up, the analysis results of primary and secondary outcomes were represented by model 1 and model 2, respectively. At 1-year follow-up, we used model 3 and model 4 to represent the analysis results of primary and secondary outcomes, respectively (Figure 2).”

Response:

We thank the reviewer for this insightful comment. We have added a statement on

informed consent and ethical approval to the Methods section.The sentences in the Methods sectioncontaining study findings have been moved to the Results section.

Changes to the manuscript:

The following text was added in the Methods section

Ethics

"This study was approved by the Ethics Committee of Tianjin Huanhu Hospital, and all procedures met the provisions of the Declaration of Helsinki (as revised in 2013). All participants or their legal representatives provided written informed consent at the study onset."

Results

Detailed Demographic and Baseline Data

"The detailed demographic and baseline data are presented in **Table 1**. A total of 478 cardioembolic stroke patients (270 men [56.5%]; median age: 71 years) were enrolled in our study. There were 398 patients with atrial fibrillation (chronic: 288 cases, paroxysmal: 82 cases, persistent: 28 cases) and 4 patients with atrial flutter. The patients with higher hs-CRP levels were mostly male and elderly, compared with those with lower hs-CRP levels ($p<0.05$). While obesity differed across the four groups, there was no significant trend. Hypertension, diabetes, dyslipidemia, smoking, alcohol consumption, and HbA1c were evenly distributed in the four groups. There were statistically significant differences in hs-CRP levels and NIHSS scores at admission among the four groups, whereby higher hs-CRP levels corresponded to a higher NIHSS score($p<0.001$). Furthermore, the NIHSS score at discharge was also positively correlated with baseline CRP levels ($p<0.001$). Details are provided in **Table 1**."

Results at a 3-Month Follow-up

At the 3-month follow-up, 75 patients had experienced vascular death, myocardial infarction(MI), and recurrent stroke (ischemic or hemorrhagic), and were included in the composite endpoint event. Thirty-three patients died due to other reasons, and one patient did not attend the follow-up review. Therefore, data from 444 patients were analyzed(**Figure 1**). Univariate analyses demonstrated that age, sex, hs-CRP, and NIHSS scores were associated with poor outcomes (**Table 2**). After adjusting for confounding factors such as diabetes, hypertension, dyslipidemia, alcohol consumption, smoking, obesity, and HbA1c, the primary outcomes in only the fourth quartile of hs-CRP (hs-CRP ≥22.30 mg/L) and NIHSS scores were associated with poor outcomes (OR 3.862, 95% CI (1.675–8.904), p=0.00,and OR 5.438, 95% CI (3.619–8.172), $p<0.001$,respectively)(**Figure 2, model 1**). The secondary outcomes were similar to the primary outcomes: the fourth quartile of hs-CRP (≥22.30mg/L) and NIHSS scores had

a significant tendency to increase the risk of composite endpoints (fourth quartile of hs-CRP: OR 3.381, 95% CI (1.620–7.058), p = 0.001 and NIHSS score: OR 4.246, 95% CI(3.015–5.978), p<0.001) (**Figure 2, model 2**).There was a significant difference in outcomes among patients grouped by hs-CRP quartile at 3-month follow-up, with higher levels of hs-CRP(≥22.30 mg/L) being significantly correlated with a poor outcome and composite endpoints after adjusting for confounders (**Figure 2**).

Results at 1-Year Follow-Up

"At the 1-year follow-up, 141 patients had vascular death, MI, and recurrent stroke (ischemic or hemorrhagic), and were included in the composite endpoint event. Fourteen patients died due to other reasons, and 35 patients did not attend the follow-up. Finally, data from 395 patients were analyzed(Figure1). Univariate analyses demonstrated that age, sex, hs-CRP, and NIHSS scores were associated with poor outcomes (Table 2). After adjusting for confounding factors, such as diabetes, hypertension, dyslipidemia, alcohol consumption, smoking, obesity, and HbA1c, the three higher quartiles of hs-CRP and NIHSS scores were associated with poor outcomes for the primary endpoint (p=0.043, p=0.049, p=0.005, and p<0.001, respectively) (Figure 2, model 3). Furthermore, the fourth quartile of hs-CRP(≥22.30 mg/L) was significantly associated with poor outcomes (OR 5.479, 95% CI [1.692–17.744], p=0.005)in the multivariate analysis(Figure 2,model 3). Regarding the secondary outcome, the fourth quartile of hs-CRP (≥22.30 mg/L) and NIHSS scores were positively correlated with the composite endpoints (OR 3.181, 95% CI(1.475–6.860), p=0.003 and OR 3.416, 95% CI(2.407–4.848), p<0.001,respectively) (Figure 2, model 4). At the 1-year follow-up, higher hs-CRP levels (≥22.30 mg/L) had a significant correlation with poor outcomes and composite endpoints."

4) The definition of endpoints is unclear, such as "The secondary outcome was based on dividing patients into good outcomes and composite endpoints." Are there some references to support this definition?

Response:
We thank the reviewer for this insightful comment. There are several studies that support this definition; we have included this the Study Outcomes subsection as follows.

Changes to the manuscript:

Study Outcome

"Stroke severity was assessed using the modified Rankin scale (mRS), with mRS scores of 0 to 2 classified as a good outcome, and scores of 3 to 6 as a poor outcome(15,16). Composite endpoints included poor outcomes, vascular death, myocardial infarction, and recurrent stroke (ischemic or hemorrhagic). The primary outcome was determined

by separating patients into two groups according to the mRS score. The secondary outcome was based on dividing patients into good outcomes and composite endpoints(17,18).”

References:

[15] Winter Y, Wolfram C, Schaeg M, et al. Evaluation of costs and outcome in cardioembolic stroke or TIA. J Neurol 2009;256:954-63.
[16] Qiu R, Gao Y, Hou D, et al. Association between hs-CRP Levels and the Outcomes of Patients with Small-Artery Occlusion. Front Aging Neurosci 2016;8:191.
[17]Zhu B, Pan Y, Jing J, et al. Neutrophil counts, neutrophil ratio, and new stroke in minor ischemic stroke or TIA. Neurology 2018;90:e1870-e8.
[18] Freeman WD, Aguilar MI. Prevention of cardioembolic stroke. Neurotherapeutics. 2011;8:488-502.

5) Why all continuous variables presenting as median? not SD?

Response:

Thank you for your question. Age, hs-CRP, and HbA1c are continuous variables in this study, but all of them had non-normally distributed data in the pre-analysis. In such non-normal variables, reporting the median and interquartile range ispreferable to mean and standard deviation(1).

References:

[1] Habibzadeh F. Statistical Data Editing in Scientific Articles. J Korean Med Sci 2017;32:1072-6. doi: 10.3346/jkms.2017.32.7.1072

6) The results: the baseline table includes few variables. Many risk factor: such as cardiac function, AF, renal function are not mentioned. The description is less rigorous, such as “After adjusting for confounders”. How many confound

Response 1:

We thank the reviewer for this insightful comment. Patients with abnormal liver and kidney function or cardiac insufficiency were excluded. The risk factors analyzed in our study have beenused frequently in the previous literature and are recognized risk factors for stroke.Meanwhile, atrial fibrillation or paroxysmal atrial fibrillation is considered supporting evidence for the diagnosis of cardiogenic stroke;thus, it was not included in this study as a risk factor. Information on the confounding factors has also been provided for a more thorough description, as found below.

Response 2:

We thank the reviewer for this insightful comment.I have modified themanuscriptto make our presentation more precise.

Changes to the manuscript:

"After adjusting for confounding factors such as diabetes, hypertension, dyslipidemia, alcohol consumption, smoking, obesity, and HbA1c, the primary outcomes in only the fourth quartile of hs-CRP (hs-CRP ≥22.30 mg/L) and NIHSS scores were associated with poor outcomes (OR 3.862, 95% CI (1.675–8.904), p=0.00,and OR 5.438, 95% CI (3.619–8.172), p<0.001,respectively)(**Figure 2, model 1**)."

7) The forth quartile of hs-CRP is a significant factor. However, it is not enough. What about hs-CRP as continuous variable?

Response:

We thank the reviewer for this insightful comment. We conducted a pre-analysis prior to this data analysis. In the current study, we classified hs-CRP levels into quartiles (<2.31, 2.31 to <6.09, 6.09 to <22.30, and ≥22.30 mg/L). In the pre-analysis, we also classified the levels, but in tertiles (<3.49, 3.49 to <13.47, and ≥13.47mg/L). During pre-analysis, hs-CRP in the highest group(hs-CRP≥13.47) was associated with poor outcomes(3 months: p=0.004； 1 year: p=0.019) and composite endpoints(3 months: p=0.006；1 year: p=0.032)in the logistic regression analysis. However, at 1-year follow-up, these correlations were weaker than they were at 3 months. Becausethe data was skewed and partial, we reclassified hs-CRP levels into quartiles and repeated the analysis—we found that the correlation became more obvious. In the logistic regression analysis, hs-CRP in the fourth quartile(hs-CRP ≥22.30 mg/L) was evidently associated with poor outcomes(3 months: p=0.002;1 year: p=0.005)and composite endpoints(3 months:p=0.001;1 year: p=0.003).In addition, quartile classification is a more commonly used statistical layering method, and it may make our results more comparable with other studies (1,2). When CRP was analyzed as a continuous variable, it reflected the increased risk of poor prognosis for patients with cerebral embolism for every 1mg/L increase in CRP level. However, in our study, due to the sample size limitation, a quartile grouping study was of more clinical value and guiding significance.As such, we used the quartile classification to perform the analysis in this study.

References:

[1] Matsuo R, Ago T, Hata J, et al. Plasma C-Reactive Protein and Clinical Outcomes after Acute Ischemic Stroke: A Prospective Observational Study. PLoS One 2016;11:e0156790. doi:10.1371/journal.pone.0156790

[2]Ridker PM, Rifai N, Clearfield M, et al. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. N Engl J Med 2001;344:1959-65.

8) I suggest to add KM curve.

Response:

Thank you for the constructive feedback. Three months and one-year post-stroke are two official time points used to evaluate the short-term and long-term effects in stroke patients. In our hospital, follow-ups were carried out at the two mentioned time points. All the laboratory and physical examinations were inputted in the electronic medical records database. It would have been too tedious for us to calculate each patient's survival time, and we regret that we were unable to add a KM curve to the manuscript. For this study, the design of a case-control study may be more suitable than a survival analysis.

9) The discussion: Does it really a first study? [Neurol Sci. 2008 Sep;29(4):245-9.] The story line issuperficial. Is it all the difference attributed to small sample size? In addition, the comparison of CRP and hs-CRP is insufficient.

Response:
We thank the reviewer for this feedback. After further literature review, I found that these different results may be due to the subjects' race, age, or gender differences. Meanwhile, the differences between CRP and hs-CRP have been further discussed, as found below.

Changes to the manuscript:

"In the present study, the median hs-CRP was 6.09 mg/L, which was quite higher than what was observed in our previous studies on small-artery occlusion (1.54 mg/L) and large-artery arteriosclerosis (2.49 mg/L) (16, 22). Several other studies also found higher plasma CRP levels in patients with cardioembolic stroke than in other TOAST subtypes(23,24), and den Hertog et al.(5) reported that cardioembolic stroke was more often observed in patients with CRP ≥7 mg/L. There are several potential mechanisms underlying this increased CRP in patients with cardioembolic stroke. First, CRP is generally elevated in patients with cardiac disease(25), especially in patients with atrial fibrillation (26), which is one of the major causes of cardioembolic stroke(27). This might explain the pre-stroke and elevated baseline CRP levels in cardioembolic stroke. Second, in the acute phase of cerebral infarction, the extent of brain injury and blood-brain barrier (BBB) disruption may determine the degree of acute-phase inflammatory response. It has been confirmed that the degree of BBB destruction in cardioembolic stroke patients is more serious than that in patients with other stroke subtypes(28), and this could be related to CRP levels(29).While these mechanisms may play a role in the increased CRP we observed, two studies have reported no differences in CRP levels among stroke subtypes(30,31). These conflicting results may be related to differences in the race, age, or gender of the subjects(32,33)."

References:
[32] Kelley-Hedgepeth A, Lloyd-Jones DM, Colvin A, et al. Ethnic differences in C-reactive protein concentrations. Clin Chem 2008;54:1027-37.

[33] Ahonen TM, Kautiainen HJ, Keinänen-Kiukaanniemi SM, et al. Gender difference among smoking, adiponectin, and high-sensitivity C-reactive protein. Am J Prev Med 2008;35:598-601.

The differences between CRP and hs-CRP have also been further discussed.

"However, compared with CRP, hs-CRP can be measured quantitatively and accurately detect low-level inflammation(6), and it can reflect minute changes in inflammation better than CRP can. Hs-CRP also has greater clinical significance in evaluating the relationship between acute inflammation and prognosis (7). Also, hs-CRP is more widely used in clinical practice, especially in the risk stratification of cardiovascular disease (CVD) (8) and stroke (9)."

References:

[6] VanGilder RL, Davidov DM, Stinehart KR, et al. C-reactive protein and long-term ischemic stroke prognosis. J. Clin. Neurosci 2014;4:547–53. doi: 10.1016/j.jocn.2013.06.015
[7] Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499-511
[8] Andersson J, Johansson L, Ladenvall P, et al. C-reactive protein is a determinant of first-ever stroke: prospective nested case-referent study. Cerebrovasc Dis 2009;27:544-51.
[9] Kong H, Qian YS, Tang XF, et al. C-reactive protein (CRP) gene polymorphisms, CRP levels and risk of incident essential hypertension: findings from an observational cohort of Han Chinese. Hypertens Res2012;35:1019-23. doi: 10.1038/hr.2012.89

Minor:

1) A lot of verbal error and grammatical mistakes exist. For example, "predict" should be "predicts" (title); "This study aimed to" should be "This study was aimed to" (abstract); "studies on hs-CRP level and outcomes of CE patients have not been reported" should be "value of hs-CRP level on the outcomes of CE patients have not been reported" (introduction).....

Response:

We thank the reviewer for this helpful suggestion. We have invited the editors of Editage to revise the manuscript.

Reviewer B

In this study, the authors investigated the association between hs-CRP levels in 478 cardioembolic strokes and outcome. Their results show that elevated hs-CRP levels represent an independent predictor of poor outcome; this association is particularly evident when hs-CRP 돟 22.30 mg/L.

This paper is a confirmatory study. It is well know that CRP represent a predictor of outcome in all types of ischemic stroke (including stroke from cardiombolism). Furthermore the present paper has some severe methodological limitations; the authors didn't take into account the possible role of some confounding factors such as the therapy and the concomitant infections.

Response:
We thank the reviewer for this insightful comment. Patients who received thrombolysis or mechanical thrombectomy were excluded from the study, as were patients who had concomitant infections. Thrombolysis or mechanical thrombectomy may affect the immune response after stroke(1-2), thereby influencing the correlation between CRP and CE prognosis.

References:
[1]Ye L, Cai R, Yang M, et al. Reduction of the systemic inflammatory induced by acute cerebral infarction through ultra-early thrombolytic therapy. Exp Ther Med 2015;10:1493-8. doi:10.3892/etm.2015.2672
[2]Kim S, Yi HJ, Lee DH, et al. Association of High-sensitivity C-reactive Protein with Patient Prognosis Following Mechanical Thrombectomy for Acute Ischemic Stroke [published online ahead of print, 2020 May 17]. Curr Neurovasc Res 2020;10.2174/1567202617666200517110949.

Changes to the manuscript:
The following text was changed in the Patient Selection section.

"Patients with thrombolysis or mechanical thrombectomy, concomitant infection, abnormal liver and kidney function, or cardiac insufficiency were not included in the study; thus, data from 9746 patients were analyzed."