Peer Review File

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Comment 1: Considerable controversy on the role and function of platelet miRNA`s and specifically miRNA-223 exists with reports suggesting either inhibition or increase of miRNA-223 levels with P2Y12. While this study supports that miRNA-223 levels rather correlates with reduced platelet function, I feel that the data provided is overall preliminary and merely of an association. The low number of patients, the lack of in-depth platelet testing, and the single-center, single-surgeon approach, makes this therefore somewhat of an anecdotal report. Consequently, I fear the manuscript rather contributes to increase rather than resolve current confusion.

Reply 1: Although miR-223 has been reportedly to be involved in regulating $P2Y_{12}$ activity, the clinical value of miR-223 remains unclear. The influence of antiplatelet therapy on miR-223 was investigated in previous research, but the results were controversial. Our research tried to investigate the influence of antiplatelet therapy on miRNAs. Besides, we investigated the role of miR-223 in prediction for hemorrhage after off-pump coronary artery bypass grafting, which has not been studies before. The surgery was performed by one surgeon in order to reduce the influence of surgical procedures by different surgeons, which also limited the sample size in our research. We presented the limitations of small sample size and single-center study in our research in the discussion section.

Changes in the text: We presented the novelty and limitations of our study in the revised manuscript (see Page 10, Line 16-21; Page 14, Line 4-5)

Comment 2: The primary outcomes chest tube output and transfusion requirement are, even though they are probably the best we have, crude (It has been proposed hourly quantification of blood loss can improve the accuracy of

postoperative bleeding measurement [Colson et al. PlosOne, 2016]). Especially in the light of the low number of enrolled patients, and the lack of any metric of perioperative platelet response/reactivity, this makes for very weak quality data. Do the authors see any differences in perioperative dynamics of platelet counts or MPV as surrogate markers?

Reply 2: Many thanks for the reviewer's suggestion. Postoperative blood loss, defined as chest tube drainage (CTD) volume, can be measured easily and has been previously used as an outcome measurement in clinical research (Christensen M C et al. J Thorac Cardiovasc Surg, 2009, 138(3): 687-693), however, just as the reviewers mentioned that the CTD volume may not reflect the severity of bleeding in an accurate or timely manner. According to the reviewer's suggestion, we re-assessed the bleeding by using the definition of active bleeding described in Colson's research (Colson et al. PLoS One. 2016;11(9): e0162396.) and the universal definition of perioperative bleeding (UDPB) in adult cardiac surgery (Dyke C et al. J Thorac Cardiov Sur. 2014, 147(5): 1458-1463). We found that three patients (5.08%) experienced active bleeding and 8 patients experienced severe perioperative bleeding (13.56%) (Table 1). Univariable and multivariable analyses showed that miR-223 and BMI were independent predictors for severe perioperative bleeding defined by universal definition of perioperative bleeding. Besides, we also investigated the relationship among platelet count, mean platelet volume, plateletcrit and platelet distribution width and bleeding, but we found that these indicators were not associated with active bleeding or UDPB-defined bleeding (Table 2 & Table 3), which suggested that these platelet biochemical indicators may not predict bleeding in off-pump coronary artery bypass grafting surgery.

Changes in the text: We added some data and re-assessed bleeding by active bleeding and the universal definition of perioperative bleeding in the revised manuscript (see Page 5, Line 17 - Page 6, Line 5; Page 8, Line 15 - Page 9, Line 11; Page 11, Line 12 - Page 12, Line 4). Besides, we also analyzed perioperative dynamics of platelet counts, PCT, PDW and MPV in the

univariable and multivariable analyses (see Table 2 & Table 3, Page 20-23).

Comment 3: Successful platelet inhibition is not adequately measured. To my understanding, the gold standard for evaluating the P2Y12 receptor blockade is the VASP index measured by flow cytometry (Aleil et al. Thromb Haemost. 2005; 3,(1): 85–92).

Reply 3: The flow cytometric analysis of VASP phosphorylation seems to be suitable for assess the inhibitory effect of clopidogrel on platelet functions (Aleil et al. Thromb Haemost. 2005; 3, (1): 85–92). However, there is currently no consensus regarding the most appropriate method to quantify the on-treatment platelet reactivity and a previous study suggested that the existing platelet function tests cannot provide accurate prognostic information to identify patients at higher risk of bleeding (Breet N J, JAMA. 2010;303(8):754-762.). We chose TEG for platelet function test because in our center, TEG was the most frequently used method for platelet function test. Previous studies have investigated the reliability of thromboelastograph (TEG) as a monitoring tool for analyzing the response to antiplatelet therapy. The TEG combined with PlateletMapping assay was able to predict a subtherapeutic response that highly correlated with the method of light transmittance aggregometry (Bliden KP et al. J Am Coll Cardiol. 2007;49(6):657-666.). Because different methods for platelet function test may lead to different results, other platelet function tests should also be investigated in the future.

Changes in the text: We presented the limitation of the platelet function test in our study in the revised manuscript (see Page 14, Line 8-11).

Comment 4: miRNA-223 to my understanding is largely PMP associated. Platelets are extremely sensitive and therefore handling and processing can have significant influence. What have the authors done to ensure that platelets are not activated ex vivo? Specifically, the use of EDTA seems problematic as this anticoagulant is known to activate platelets and could lead to unpredictable

results (Ahnadi et al. Thromb Haemost. 2003 Nov;90(5):940-8.)

Reply 4: In order to avoid platelet activation, the first 2-3 mL of blood were discarded and then blood was collected into plastic tubes containing anticoagulant (Chyrchel B et al. Platelets. 2015;26(6):593-597). Previous studies suggested that platelet may be influenced in a time-dependent manner when EDTA is used as anticoagulant (Ahnadi et al. Thromb Haemost. 2003 Nov;90(5):940-8; Bath PM, et al. Blood Coagul Fibrinolysis 1996;7:157-161.). However, it is also reported that when the measurement is performed within 2 h after venipuncture, EDTA has less influence on platelet (Endler G, et al. Br J Haematol 2002; 117: 399-404.). In the present study, in order to minimize the effect of EDTA, we used the standardized sample tubes with the same amounts of EDTA (10.8mg/6ml) in each tube and the whole blood was processed immediately to avoid swelling of platelets in EDTA blood ((Jäger B, et al. Eur J Clin Invest. 2019;49(8): e13149; Chyrchel B, et al. Platelets. 2015;26(6):593–597). The use of EDTA as anticoagulant for detection of mean platelet volume and platelet-related miRNAs was also described in many previous studies (Jäger B, et al. Eur J Clin Invest. 2019;49(8): e13149; Chyrchel B, et al. Platelets. 2015;26(6):593-597; Poon KS, et al. Sci Rep. 2017;7(1):10807; Huczek Z, et al. J Am Coll Cardiol. 2005;46(2):284-290; Yilmaz M B, et al. J Thromb Thrombolysis. 2008;26(1):49-54; Chu SG, et al. J Thromb Haemost. 2010;8(1):148-156.).

Changes in the text: We have modified out text in the revised manuscript and described the process of blood collection (see Page 6, Line 13-20).