Elevated Serum Levels of Alkaline Phosphatase and the Risk of Low Responsiveness to Clopidogrel

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Summary
The elevated serum levels of alkaline phosphatase (ALP) serve as independent predictors of stent thrombosis after percutaneous coronary intervention (PCI). Our study aims at investigating the relationship between the serum ALP and the responsiveness to clopidogrel. Patients undergoing elective PCI were enrolled for the study, and all participants received a 300-mg clopidogrel loading dose. The responsiveness to clopidogrel was determined by thromboelastography (TEG), and low responsiveness to clopidogrel was defined based on two aspects: (1) adenosine diphosphate (ADP)-induced platelet-fibrin clot strength (MA_{ADP}) of > 47 mm and (2) ADP-induced platelet inhibition rate of < 50%. A logistic regression model analysis was used to calculate the risks of responsiveness to clopidogrel as odd ratios (OR) and 95% confidence intervals (CIs). Overall, 809 patients were considered for the study. They were divided into four quartile groups based on the serum ALP levels. A positive linear trend was observed in MA_{ADP} across the ALP quartiles (P for linear trend < 0.001), whereas ADP-induced platelet inhibition rate decreased across the ALP quartiles (P for linear trend = 0.007). When multiple confounders were adjusted, the highest ALP quartile correlated with an increased risk of low responsiveness to clopidogrel compared to the lowest ALP quartile (OR, 1.423; 95% CI, 1.017-1.991; P = 0.039). In the sensitivity analysis, the association remained significant for different definitions of low responsiveness to clopidogrel. The elevated serum levels of ALP are independently associated with an increased risk of low responsiveness to clopidogrel.

Key words: Liver function, Drug response, Thromboelastography, Platelet function

Dual antiplatelet therapy with aspirin and P2Y12 adenosine diphosphate (ADP) receptor inhibitor is the cornerstone for patients undergoing percutaneous coronary intervention (PCI) or with acute coronary syndrome (ACS). The new oral P2Y12 inhibitors, prasugrel and ticagrelor, offer additional risk reduction in patients with ACS compared to clopidogrel, which may lead to an increased risk of bleeding events.1-3 Currently, clopidogrel remains the most widely used P2Y12 inhibitor globally.4-6 One of the major limitations of clopidogrel is the individual variation in the antiplatelet effects owing to it being a prodrug that converts to active metabolite through the cytochrome P450 pathway.7 An undated meta-analysis of 14 studies involving 4,465 patients with coronary artery disease (CAD) showed a significant association between low responsiveness to clopidogrel and an increased risk of death and/or recurrent thrombotic events.8 ADAPT-DES was the largest prospective multicenter registry study that investigated the relationship between the responsiveness to clopidogrel and risk of stent thrombosis, and it was determined that low responsiveness to clopidogrel was strongly related to an increase in the risk of stent thrombosis and myocardial infarction.9

Alkaline phosphatase (ALP), initially considered as a marker of liver function, is treated as a surrogate marker of cardiovascular disease.10 A meta-analysis of prospective cohort studies has indicated that circulating ALP levels are positively associated with the risk of cardiovascular disease in general population.11 For patients with CAD undergoing PCI with drug-eluting stents, the risk of cardiovascular mortality and stent thrombosis in patients with the highest serum ALP tertile was significantly higher than those with the lowest ALP tertile. This indicated serum ALP to be an independent predictor of stent thrombosis after PCI.12 However, the mechanisms underlying the relationship between ALP and stent thrombosis remain unclear. Thus, the current study aims at exploring the relationship between the serum ALP levels and the risk of low responsiveness to clopidogrel.
Methods

Patients who underwent elective PCI between January 2018 and December 2018 in the Department of Cardiology were included in this study. However, the following patients were excluded: those who used maintenance-dose clopidogrel, other ADP P2Y12 inhibitors (such as prasugrel and ticagrelor), or glycoprotein IIb/IIIa antagonists before the angiography; those with contraindications to clopidogrel; those who required emergency or urgent PCI; those with malignancy; those with documented liver disease or cirrhosis; those with hyperparathyroidism; those with bone diseases or fracture; those with end-stage renal disease requiring dialysis; and those who were pregnant. The study protocol was approved by institutional review boards. This study was conducted in accordance with the Declaration of Helsinki, and all participants signed informed consent forms.

Data related to demography, lifestyle, medical history, and the medication of each patient were collected. Body mass index (BMI) is defined as weight (kg) divided by height squared (m²). Hypertension (HTN) is defined as a systolic blood pressure of 140 mm Hg or more or a diastolic blood pressure of 90 mm Hg or more, antihypertensive medication prescription, or any documented diagnosis of HTN. Diabetes mellitus (DM) is defined as a fasting plasma glucose of 7.0 mmol/L or more, or HbA1c of 6.5% or more, taking diabetes medication, or any documented diagnosis of diabetes. The data related to the use of medication were collected directly from medical records.

Prior to PCI, a fasting venous blood sample (fasting for more than 8 hours) was collected from each patient for laboratory tests using standard methods. The laboratory tests for each patient included complete blood count; determining liver function parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), ALP, and gamma-glutamyl transferase (GGT); creatinine; high-sensitivity C-reactive protein (hs-CRP); and lipid profile, including total cholesterol (TC), triglycerides, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). The EPI-CKD creatinine equation was used to calculate the estimated glomerular filtration rate. The serum ALP was measured using the 2-amino-2-methyl-1-propanol buffer (Kehua Bio-Engineering, China).

All patients received 300-mg clopidogrel loading dose at least 12 hours (within 12-24 hours) prior to platelet function assessment. Blood samples were collected, handled, and measured within 2 hours of the assessment. The postclopidogrel platelet function was measured by using the thromboelastography (TEG) system (Lepu Medical Technology, Beijing, China). In short, modified TEG can detect the effects of antiplatelet therapy action via the ADP pathways. A TEG trace would automatically be provided by the system when blood clots develop in the channels. One of the most important parameters is ADP-induced maximum amplitude (MA_{ADP}), a representative of which represents maximal clot strength. Previous studies have confirmed that MA_{ADP} correlates with optical aggregometry, which is a gold standard method for assessing the responsiveness to clopidogrel. Further, a modified TEG mapping system can generate the platelet inhibition rate, a novel parameter that combines the speed and strength of clot formation. Low responsiveness to clopidogrel is considered when MA_{ADP} is more than 47 mm and ADP-induced platelet inhibition rate is less than 50%.

Categorical variables are presented as percentages, and continuous variables are indicated as means ± standardized deviation. Demographic data, lifestyle, medical histories, and medical treatment were compared across ALP quartile groups using either Chi-square for categorical variables or analysis of variance for continuous variables. Logistic regression analysis was used to estimate the risk of low responsiveness to clopidogrel across serum ALP quintiles with lowest quartile as the reference. Three different models of adjustments were used for regression analyses. In model 1, liver parameters were adjusted, which included ALT, AST, TBIL, and GGT. In model 2, baseline variables with significant difference among the ALP quartiles were adjusted. In model 3, all the baseline variables, including demographic data, lifestyle, medical histories, and medical treatment, were adjusted. In the sensitivity analysis, MA_{ADP} of more than 47 mm was defined as low responsiveness to clopidogrel.

All statistical tests were two-sided, and P < 0.05 was considered as the statistical significance. Statistical analyses were performed using SPSS software, version 20.0 (IBM corporation, USA).

Results

Overall, 809 patients undergoing elective PCI satisfied the eligibility criteria and were enrolled in the study (Supplemental Figure). The mean age was 60.2 ± 10.6 years, and the mean BMI was 25.9 ± 3.6 kg/m². Most of the patients were male (71.3%), and the prevalence of DM and HTN were 34.4% and 65.9%, respectively. The mean ALP level was 82.1 ± 44.6 U/L. Patients were divided into quartile groups based on the serum ALP level (quartile 1, < 65 U/L; quartile 2, 65-76 U/L; quartile 3, 77-91 U/L; and quartile 4, > 91 U/L). The median values of the serum ALP level for each quartile are as follows: quartile 1, 57.5 U/L; quartile 2, 70 U/L; quartile 3, 84 U/L; and quartile 4, 104 U/L. Data related to demography, lifestyle, medical histories, and medical treatment were compared among the four ALP quartile groups, and the results are listed in the Table. The proportion of males with diabetes decreased from the lowest to highest quartiles, whereas the WBC, PLT, ALT, AST, GGT, TC, LDL-C, and hs-CRP levels increased from the lowest to highest quartiles (where P < 0.05).

A linear trend was observed in both MA_{ADP} (quartile 1, 37.88 ± 13.18 mm; quartile 2, 39.44 ± 15.01 mm; quartile 3, 40.80 ± 12.99 mm; and quartile 4, 42.78 ± 13.73 mm; with P for linear trend < 0.001, as shown in Figure 1A) and ADP-induced platelet inhibition rate (quartile 1, 40.50% ± 22.78%; quartile 2, 38.86% ± 26.05%; quartile 3, 36.83% ± 22.95%; and quartile 4, 34.42% ± 23.25%, with P for linear trend = 0.007, as shown in Figure 1B) across all ALP quartiles.
The prevalence of low responsiveness to clopidogrel was determined to be 29.1%, 37.7%, 32.4%, and 43.8% from the lowest to highest quartiles, respectively. The unadjusted and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of risk of low responsiveness to clopidogrel are presented in Figure 2. With the lowest quartile as the reference, the risk of low responsiveness to clopidogrel independently defined as the low responsiveness to clopidogrel, the logistic regression analysis is repeated with adjustments, which further determines that the association between the highest serum ALP quartile and risk of low responsiveness to clopidogrel continues to be significant (model 1: OR, 1.406; 95% CI, 1.048-1.885; P = 0.023; model 2: OR, 1.401; 95% CI, 1.024-1.916; P = 0.035; and model 3: OR, 1.453; 95% CI, 1.039-2.031; P = 0.029), as shown in Figure 3.

### Discussion
The major findings of the current study are as follows: (1) a positive linear correlation is identified between the serum ALP level and MAADP, (2) a negative linear correlation is identified between the serum ALP level and ADP-induced platelet inhibition rate, and (3) the ALP level serves to be an independent predictor of the risk of low responsiveness to clopidogrel. This indicated that the unadjusted results of quartile 2 confounded with the other variables.

In the sensitivity analysis, when MA_ADP > 47 mm is independently defined as the low responsiveness to clopidogrel, the logistic regression analysis is repeated with adjustments, which further determines that the association between the highest serum ALP quartile and risk of low responsiveness to clopidogrel continues to be significant (model 1: OR, 1.406; 95% CI, 1.048-1.885; P = 0.023; model 2: OR, 1.401; 95% CI, 1.024-1.916; P = 0.035; and model 3: OR, 1.453; 95% CI, 1.039-2.031; P = 0.029), as shown in Figure 3.
Figure 1. Adenosine diphosphate (ADP)-induced platelet-fibrin clot strength (MAADP) and ADP-induced platelet inhibition rate across alkaline phosphatase quartiles. A: MAADP, B: ADP-induced platelet inhibition rate.

|                | ORs       | 95% CIs       | P value |
|----------------|-----------|---------------|---------|
| Unadjusted     |           |               |         |
| Quartile 1     | Reference |               |         |
| Quartile 2     | 1.462     | 1.091-1.959   | 0.011   |
| Quartile 3     | 1.050     | 0.785-1.404   | 0.741   |
| Quartile 4     | 1.349     | 1.001-1.800   | 0.042   |
| Model 1        |           |               |         |
| Quartile 1     | Reference |               |         |
| Quartile 2     | 1.467     | 1.083-1.987   | 0.013   |
| Quartile 3     | 1.075     | 0.798-1.449   | 0.633   |
| Quartile 4     | 1.392     | 1.038-1.867   | 0.027   |
| Model 2        |           |               |         |
| Quartile 1     | Reference |               |         |
| Quartile 2     | 1.191     | 0.860-1.651   | 0.292   |
| Quartile 3     | 1.117     | 0.812-1.534   | 0.497   |
| Quartile 4     | 1.386     | 1.012-1.897   | 0.042   |
| Model 3        |           |               |         |
| Quartile 1     | Reference |               |         |
| Quartile 2     | 1.108     | 0.776-1.582   | 0.571   |
| Quartile 3     | 1.117     | 0.794-1.571   | 0.526   |
| Quartile 4     | 1.423     | 1.017-1.991   | 0.039   |

Figure 2. Unadjusted and adjusted odd ratios and 95% confidence intervals of low responsiveness to clopidogrel across alkaline phosphatase quartiles. Model 1: Adjusted for alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, and gamma-glutamyl transferase (GGT). Model 2: Adjusted for gender, diabetes, white blood cell count, platelet count, ALT, AST, GGT, total cholesterol, low-density lipoprotein cholesterol, and high-sensitivity C-reaction protein. Model 3: Adjusted for all the variables listed in the Table.
low responsiveness to clopidogrel in patients undergoing elective PCI. To the best of our knowledge, the current study first determined the relationship between the serum ALP level and the risk of low responsiveness to clopidogrel.

It is well established that elevated ALP levels are associated with adverse cardiac outcomes in patients with cardiovascular diseases.17,18) In prospective Korean studies, the adjusted hazard ratios in the highest serum ALP tertile (with the lowest tertile as reference) for cardiovascular mortality, myocardial infarction, and stent thrombosis were identified to be 3.92 (95% CI, 1.37-11.20), 1.98 (95% CI, 0.91-4.29), and 2.73 (95% CI, 1.33-5.61), respectively. This indicates that stent thrombosis accounts for the adverse effect of ALP.12) Stent thrombosis is one of the most serious complications of PCI and involves several risk factors including clinical condition, lesion characteristics, procedure, and responsiveness to antiplatelet medications as indicated by previous studies.19) ALP has been considered as a regulator of vascular calcification. Previous studies have confirmed that serum ALP levels correlate with coronary calcification assessed either by coronary computed tomography or angiography.12,20) Furthermore, the inhibition of ALP by novel drugs can suppress the vascular smooth muscle cell calcification in an in vitro study.21) However, the impact of coronary calcification on the increase in the risk of stent thrombosis remains unclear. Another possible mechanism underlying the association between ALP and stent thrombosis is inflammation. In terms of drug-eluting stent, elevated CRP levels were identified to be significantly associated with an increased risk of stent thrombosis.22,23) However, in terms of increasing the risk of stent thrombosis, the effect of ALP is independent of the hs-CRP level.12) Similarly, we consider hs-CRP as a confounder in our study. The association between the elevated serum ALP level and responsiveness to clopidogrel remains significant after adjusting hs-CRP and other confounders. The results of our study suggest that responsiveness to clopidogrel might be an independent mechanism underlying the relationship be-

| ORs           | 95% CIs          | P value |
|---------------|------------------|---------|
| Unadjusted    |                  |         |
| Quartile 1    | Reference        |         |
| Quartile 2    | 1.431            | 1.069-1.916 | 0.016 |
| Quartile 3    | 1.050            | 0.785-1.404 | 0.734 |
| Quartile 4    | 1.362            | 1.021-1.817 | 0.035 |
| Model 1       |                  |         |
| Quartile 1    | Reference        |         |
| Quartile 2    | 1.430            | 1.057-1.936 | 0.021 |
| Quartile 3    | 1.075            | 0.798-1.448 | 0.634 |
| Quartile 4    | 1.406            | 1.048-1.885 | 0.023 |
| Model 2       |                  |         |
| Quartile 1    | Reference        |         |
| Quartile 2    | 1.512            | 0.840-1.609 | 0.365 |
| Quartile 3    | 1.116            | 0.813-1.532 | 0.498 |
| Quartile 4    | 1.401            | 1.024-1.916 | 0.035 |
| Model 3       |                  |         |
| Quartile 1    | Reference        |         |
| Quartile 2    | 1.085            | 0.761-1.547 | 0.653 |
| Quartile 3    | 1.118            | 0.796-1.571 | 0.521 |
| Quartile 4    | 1.453            | 1.039-2.031 | 0.029 |

Figure 3. Results of sensitivity analyses. Unadjusted and adjusted odd ratios and 95% confidence intervals of low responsiveness to clopidogrel across alkaline phosphatase quartiles using MA_{ALP} more than 47 mm independently as the definition of low responsiveness to clopidogrel. Model 1: Adjusted for alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, and gamma-glutamyl transferase (GGT). Model 2: Adjusted for gender, diabetes, white blood cell count, platelet count, ALT, AST, GGT, total cholesterol, low-density lipoprotein cholesterol, and high-sensitivity C-reaction protein. Model 3: Adjusted for all the variables listed in the Table.
tween ALP and stent thrombosis.

Several studies have investigated the relationship between ALP and platelet function. An *in vitro* study found that ALP could prevent platelet activation induced by thromboxane. However, whether the platelet aggregation induced by ADP is affected by ALP remains unclear. Further, mechanisms underlying the relationship between the elevated serum ALP and the increased risk of responsiveness to clopidogrel are also unclear. A possible mechanism is subclinical liver disease. Although patients with liver diseases were excluded from the study and the conclusions were adjusted for other liver enzymes, elevated ALP might still be a surrogate of subclinical liver disease. A prospective cohort study indicated that higher serum ALP levels can be considered to be a significant predictor for nonalcoholic fatty liver disease in certain population. Meanwhile, platelet number, activation, and aggregation have been found to increase in nonalcoholic fatty liver disease. Further, certain parameters of liver synthesis, such as cholinesterase, serum albumin, and TC levels, correlate inversely with on-treatment platelet reactivity that is assessed by the VerifyNow P2Y12 assay in patients on clopidogrel. This indicates that an impaired liver synthesis function can increase the risk of low responsiveness to clopidogrel.

Although current guidelines do not recommend the routine assessment of platelet function for patients on clopidogrel, these tests are important in certain cases, such as for patients with high ischemic risk, recurrent events, and stent thrombosis. Based on our study results, ALP can be taken into account while decision-making. Nowadays, ALP is being considered as a novel treatment target for cardiovascular issues in patients with chronic kidney disease. In a recent post hoc analysis of a phase 2 trial, a bromodomain and extra-terminal protein inhibitor, apabetalone, was found to lower serum ALP, which is associated with a lower risk of cardiovascular events compared to placebo. ALP can be considered as a target for treating low responsiveness to clopidogrel.

However, our study has certain limitations. Firstly, low responsiveness to clopidogrel is defined based only on the TEG and is not confirmed using other methods, such as light transmission aggregometry. Secondly, although adjustments have been made for multiple potential confounders, the possibility of residual confounding factors cannot be ruled out. Thirdly, data on parathyroid hormone and vitamin D levels were unavailable for consideration in the study. Finally, only the circulating ALP levels have been measured instead of ALP isoforms.

In conclusion, our study determined that elevated ALP levels are independently associated with an increase in the risk of low responsiveness to clopidogrel. This conclusion partially explains why ALP can increase the risk of stent thrombosis. However, further study is needed to investigate the underlying link between ALP and responsiveness to clopidogrel.

Disclosure

Conflicts of interest: The authors declare no competing financial interests.

Data availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

References

1. Wallentin L, Becker RC, Budaj A, et al. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. N Engl J Med 2009; 361: 1045-57.
2. Wiviott SD, Braunwald E, McCabe CH, et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. N Engl J Med 2007; 357: 2001-15.
3. Roe MT, Armstrong PW, Fox KA, et al. Prasugrel versus clopidogrel for acute coronary syndromes without revascularization. N Engl J Med 2012; 367: 1297-309.
4. De Luca L, Zezmen U, Claeyns MJ, et al. Comparison of P2Y12 receptor inhibitors in patients with ST-elevation myocardial infarction in clinical practice: a propensity score analysis of 5 contemporary European registries. Eur Heart J Cardiovasc Pharmacother 2020.
5. Sahlén A, Varenhorst C, Lagerqvist B, et al. Outcomes in patients treated with ticagrelor or clopidogrel after acute myocardial infarction: experiences from SWEDHEART registry. Eur Heart J 2016; 37: 3335-42.
6. Ahn JH, Ahn Y, Jeong MH, et al. Ticagrelor versus clopidogrel in acute myocardial infarction patients with multivessel disease; From Korea Acute Myocardial Infarction Registry-National Institute of Health. J Cardiol 2020; 75: 2231-45.
7. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. J Am Coll Cardiol 2007; 49: 1505-16.
8. Sofi F, Marcucci R, Gori AM, Giusti B, Abbate R, Gensini GF. Clopidogrel non-responsiveness and risk of cardiovascular morbidity. An updated meta-analysis. Thromb Haemost 2010; 103: 841-8.
9. Stone GW, Witzenbichler B, Weisz G, et al. Platelet reactivity and clinical outcomes after coronary artery implantation of drug-eluting stents (ADAPT-DES): a prospective multicentre registry study. Lancet 2013; 382: 614-23.
10. Targher G, Byrne CD. Circulating markers of liver function and cardiovascular disease risk. Arterioscler Thromb Vasc Biol 2015; 35: 2290-6.
11. Kunutsor SK, Apekey TA, Khan H. Liver enzymes and risk of cardiovascular disease in the general population: a meta-analysis of prospective cohort studies. Atherosclerosis 2014; 236: 7-17.
12. Park JB, Kang DY, Yang HM, et al. Serum alkaline phosphatase is a predictor of mortality, myocardial infarction, or stent thrombosis after implantation of coronary drug-eluting stent. Eur Heart J 2013; 34: 920-31.
13. Gurbel PA, Blien KP, Guer Y, et al. Platelet reactivity in patients and recurrent events post-stenting: results of the PREPARE POST-STENTING Study. J Am Coll Cardiol 2005; 46: 1820-6.
14. Craft RM, Chavez JJ, Bressee SJ, Wortham DC, Cohen E, Carroll RC. A novel modification of the Thrombelastograph assay, isolating platelet function, correlates with optical platelet aggregation. J Lab Clin Med 2004; 143: 301-9.
15. Sambu N, Radhakrishnan A, Dent H, et al. Personalised anti-platelet therapy in stent thrombosis: observations from the Clopidogrel Resistance in Stent Thrombosis (CREST) registry. Heart 2012; 98: 706-11.
16. Tang YD, Wang W, Yang M, et al. Randomized comparisons of double-dose clopidogrel or adjunctive cilostazol versus standard dual antiplatelet in patients with high posttreatment platelet reactivity: results of the CREATIVE Trial. Circulation 2018; 137: 2231-45.
17. Tonelli M, Curhan G, Pfeffer M, et al. Relation between alka-
line phosphatase, serum phosphate, and all-cause or cardiovascular mortality. Circulation 2009; 120: 1784-92.

18. Ryu WS, Lee SH, Kim CK, Kim BJ, Yoon BW. Increased serum alkaline phosphatase as a predictor of long-term mortality after stroke. Neurology 2010; 75: 1995-2002.

19. Gopalakrishnan M, Lotfi AS. Stent thrombosis. Semin Thromb Hemost 2018; 44: 46-51.

20. Shantouf R, Kovesdy CP, Kim Y, et al. Association of serum alkaline phosphatase with coronary artery calcification in maintenance hemodialysis patients. Clin J Am Soc Nephrol 2009; 4: 1106-14.

21. Nairisawa S, Harmey D, Yadav MC, O’Neill WC, Hoylaerts MF, Millán JL. Novel inhibitors of alkaline phosphatase suppress vascular smooth muscle cell calcification. J Bone Miner Res 2007; 22: 1700-10.

22. Park DW, Yun SC, Lee JY, et al. C-reactive protein and the risk of stent thrombosis and cardiovascular events after drug-eluting stent implantation. Circulation 2009; 120: 1987-95.

23. Montone RA, Ferrante G, Bacà M, Niccoli G. Predictive value of C-reactive protein after drug-eluting stent implantation. Future Cardiol 2010; 6: 167-79.

24. Hatmi M, Haye B, Gavaret JM, Vargaftig BB, Jacquin C. Alkaline phosphatase prevents platelet stimulation by thromboxane-mimetics. Br J Pharmacol 1991; 104: 554-8.

25. Margolin N, Truc TA, Saussy DL Jr, Mais DE. Effect of alkaline phosphatase on thromboxane mimetic induced platelet activation. Prostaglandins 1994; 48: 235-46.

26. Weitberg AB. The effect of alkaline phosphatase on platelet aggregation. Haematologica (Budap) 1989; 22: 65-8.

27. Zhou YJ, Zou H, Zheng JN, et al. Serum alkaline phosphatase, a risk factor for non-alcoholic fatty liver, but only for women in their 30s and 40s: evidence from a large cohort study. Expert Rev Gastroenterol Hepatol 2017; 11: 269-76.

28. Malehmir M, Pfister D, Gallage S, et al. Platelet GP Ibalpha is a mediator and potential interventional target for NASH and subsequent liver cancer. Nat Med 2019; 25: 641-55.

29. Gremmel T, Mueller M, Koppensteiner R, Panzer S. Liver function is associated with response to clopidogrel therapy in patients undergoing angioplasty and stenting. Angiology 2016; 67: 835-9.

30. Haarhaus M, Brandenburg V, Kalantar-Zadeh K, Stenvinkel P, Magnusson P. Alkaline phosphatase: a novel treatment target for cardiovascular disease in CKD. Nat Rev Nephrol 2017; 13: 429-42.

31. Haarhaus M, Ruy KK, Nicholls SJ, et al. Apabetalone lowers serum alkaline phosphatase and improves cardiovascular risk in patients with cardiovascular disease. Atherosclerosis 2019; 290: 59-65.

**Supplemental Files**

Supplemental Figure
Please see supplemental files; https://doi.org/10.1536/ihj.20-285