Serum alkaline phosphatase is a predictor of mortality, myocardial infarction, or stent thrombosis after implantation of coronary drug-eluting stent

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Aims
The association between alkaline phosphatase (ALP) and mortality was reported in several subgroups of patients. But, the role of ALP in overall coronary artery disease (CAD) patients after percutaneous coronary intervention (PCI) remains unknown. The aim of this study was to examine the prognostic value of the ALP level in patients with CAD who underwent PCI with drug-eluting stent (DES).

Methods and results
We prospectively included CAD patients who underwent PCI with DES. After exclusion of patients with liver disease and cancer, 1636 patients were selected for the analysis of clinical outcomes (median duration of follow-up; 762 days, inter-quartile range; 494–1068 days), and were classified into tertiles by baseline measurements of ALP (≤63, 63–78, and >78 IU/L). After adjustment of potential confounders including angiographic data, the independent and dose-dependent association was observed between tertile of ALP and the adjusted hazard ratio (HR) of all-cause mortality (P for trend < 0.0001). Specifically, compared with the lowest ALP tertile, the adjusted HR of all-cause mortality in the highest tertile was 4.21 (95% confidence interval 2.03–8.71). In subgroup of patients with stable or unstable angina, a similar association was noted (P for trend < 0.0001). In terms of cardiovascular mortality, myocardial infarction, and stent thrombosis, the adjusted HRs in the highest ALP tertile were 3.92 (1.37–11.20), 1.98 (0.91–4.29), and 2.73 (1.33–5.61), respectively, compared with the lowest tertile. Furthermore, evaluation of both ALP and C-reactive protein provided better predictive value than either alone. Interesting result suggesting the mechanism was that ALP was significantly associated with the presence of angiographic coronary calcification (P for trend = 0.046).

Conclusion
Our study demonstrated that the higher serum ALP level is an independent predictor of mortality, myocardial infarction, and stent thrombosis in CAD patients after PCI with DES.

Keywords
Alkaline phosphatase • Vascular calcification • Coronary disease • Mortality • Stent thrombosis

Introduction
Intense attention has focused on the search of biomarker with an effective prognostic value in patients with coronary artery disease (CAD). Recent data demonstrated that the measurement of high-sensitive C-reactive protein, a representative inflammatory marker, could not predict 41% of stent thrombosis (ST)-elevation myocardial infarction (MI) patients, which suggested the need to identify the additional relevant markers based on the novel pathophysiological mechanisms. Since vascular calcification contributes to cardiovascular risk in various population subsets, makers of vascular calcification can be an attractive option. Several studies showed that markers of mineral metabolism such as phosphate are linked with an adverse cardiovascular outcome. Recently, it was suggested that alkaline phosphatase (ALP) plays a pivotal role in mineral...
metabolism and might be a molecular marker of vascular calcification. Indeed, ALP is a membrane-bound metallo enzyme that catalyses the hydrolysis of organic pyrophosphate, an inhibitor of vascular calcification. Accordingly, the role of ALP has been highlighted in terms of its effects on vascular disease. Recently, several studies reported a significant link between ALP and adverse outcome in patients with chronic kidney disease or those under haemodialysis. Furthermore, two recent papers showed that higher ALP levels are associated with an excess risk of death among survivors of stroke or MI.

Considering that vascular calcification contributes to atherosclerosis, vascular hardening, and ageing, serum ALP levels may also be linked with poor vascular fate in overall patients with CAD as well as MI survivors. However, whether ALP is associated with the risk of cardiovascular events in this population is unknown. Moreover, no previous study has investigated the relation between ALP and risk of ST or revascularization in patients after percutaneous coronary intervention (PCI). To evaluate the potential role of ALP as a marker in determining the prognosis of CAD patients, we assessed the hypothesis that higher ALP levels are related with poor clinical outcomes in terms of all-cause mortality, cardiovascular mortality, non-fatal MI, ST, revascularization, and composite endpoint in CAD patients treated with drug-eluting stent (DES) implantation.

Methods

Study population

The analysis was from a single-centre DES registry of CAD patients treated at Seoul National University Hospital. We prospectively enrolled a consecutive series of patients who underwent successful PCI with sirolimus-eluting stent (SES) or paclitaxel-eluting stent (PES) between February 2003 and June 2006 at our cardiovascular centre. This was an ‘all-comer analysis’ that included patients with stable angina, unstable angina, MI, and those who underwent emergent PCI. Initially, our study sample included a total of 1790 patients. We excluded patients with established liver disease and cancer, which can affect both ALP levels and adverse outcomes, prior to the procedure ($n = 106$), and then further excluded patients who lacked serum ALP levels ($n = 48$). As a result, 1636 patients were enrolled in this study. All patients received standard medical treatment during PCI procedure and hospitalization. Our study protocol was approved by the institutional review board in our hospital (No. H-1108-099-374), and is in accordance with the Declaration of Helsinki. All patients gave written informed consent for the enrolment in the registry, and the use of their clinical data in future retrospective analysis at the time of admission.

Demographic and laboratory data

We recorded demographic data, cardiovascular risk factors, and laboratory data for all patients. Cardiovascular risk factors included smoking, diabetes mellitus, hypertension, hypercholesterolaemia, peripheral vascular disease, stroke, and history of prior MI, PCI, and coronary artery bypass graft (CABG) surgery. Diabetes was diagnosed if patients exhibited fasting plasma glucose $>$7.0 mmol/L on two separate days or had a history of diabetes or using anti-diabetic agents. Hypertension was diagnosed if subjects exhibited systolic blood pressure $>$140 mmHg or diastolic blood pressure $>$90 mmHg, or had a history of hypertension or using anti-hypertensive agents. A diagnosis of hypercholesterolaemia was made in patients with a history of using cholesterol-lowering medications or who had fasting serum total cholesterol $>$5.2 mmol/L. Data on other risk factors were reported by the patients themselves or according to medical records. Fasting blood samples were drawn within 24 h before index PCI, and serum ALP levels were analysed by an automated enzymatic method (Boehringer Mannheim, Mannheim, Germany). Blood samples were also used in the standard battery of haematological and biochemical tests. The estimated glomerular filtration rate (GFR) was calculated using 4-variable Modification of Diet in the renal disease formula. We reviewed index coronary angiograms in the blind fashion, and assessed the presence and severity of coronary calcification according to the previous study. Briefly, calcification at the lesion treated with PCI was graded by semi-quantitative scores ($0 =$ no calcification, $1 =$ calcification barely visible on close examination, $2 =$ readily visible but mild degree of calcification, and $3 =$ obvious, heavy calcification).

Percutaneous coronary intervention procedure and follow-up

All procedures were performed according to the current standard guidelines. The choice of the specific DES (SES; Cypher, Cordis, Miami Lakes, FL/PES; TAXUS, Boston Scientific Corporation, Boston, MA, USA) was up to the operator. American College of Cardiology/American Heart Association lesion types were assessed by the operator. After the procedure, aspirin was scheduled to be continued indefinitely, and clopidogrel was prescribed for at least 6 months. All patients were planned to be followed clinically at 1 month, 6 months, and 1 year after the index procedure. For asymptomatic patients, routine angiographic follow-up was not mandatory.

Study objectives and definitions

The primary objective of the present study was to evaluate the association between ALP and mortality. Secondary objectives were to evaluate whether higher ALP levels would be associated with non-fatal MI, ST, target lesion revascularization (TLR), target vessel revascularization (TVR), and major adverse cardiac event (MACE). All medical records were thoroughly reviewed by an independent research nurse. Telephone interviews were done to check the development of adverse events after PCI. The date of death and cause of death according to the International Classification of Diseases, 10th Revision, were recorded. Mortality data were classified into cardiovascular and non-cardiovascular death. Cardiovascular death was defined as death caused by CAD, heart failure, arrhythmia, stroke, pulmonary embolism, or other definite vascular causes. Non-cardiovascular death was defined as death caused by accidents, cancer, pulmonary disease, and other miscellaneous causes. To verify the accuracy of mortality information, we matched our data to the nationwide official data on death certification offered by the National Statistical Office, which have been considered as reliable data in previous studies. Stent thrombosis was defined and classified by the Academic Research Consortium definition. Target lesion revascularization was defined as any repeated intervention caused by restenosis within the stented segment including the 5 mm proximal and distal margins. Target vessel revascularization was defined as any repeat intervention performed on the culprit vessel or its major branches after index PCI. Major adverse cardiac event was defined as the composite of all-cause mortality, MI, ST, TLR, and TVR.

Statistical analysis

We prespecified that the patients would be divided into three groups based on tertiles of baseline ALP levels (T1, <63; T2, 63–78; T3, >78 IU/L), and we used these tertile categories in the following analyses. To test for differences in categorical or continuous variables
among tertiles of ALP, we used the $\chi^2$ test, the one-way analysis of variance test, or the Kruskal–Wallis test. Survival rates after PCI were estimated using the Kaplan–Meier product-limit estimation method, and the survival rates of subjects by ALP tertile were compared using log-rank tests.

After checking for the violation of proportional hazard assumption, the Cox proportional hazard model was used to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) for adverse outcomes among tertiles of ALP levels. The lowest tertile of the ALP level was used as a reference. Analyses were also computed by including ALP as a continuous variable or predefined cut-off value of ALP based on the receiver operating characteristic (ROC) curve. We selected covariates known to be related with ALP levels or cardiovascular outcomes after PCI in previous papers. Analyses for linear trend across tertiles were computed in the Cox regression models. Then, we examined the Cox model using restricted cubic spline regression to obtain additional insights into the linearity of the link between ALP levels and mortality. To explore whether ALP or C-reactive protein provides additional information in predicting adverse outcomes after PCI, we computed ROC curves and tested for equality of the areas under the curves (AUC). We performed Cox regression analysis after dividing the patients into four groups according to cut-off values of ALP and C-reactive protein by ROC curves. In sensitivity analyses, we entered additional variables that might be related with adverse outcomes, but were not significantly associated in bivariate analyses, into the adjusted models. Logistic regression was used for the analysis of association between the ALP level and coronary calcification. The AUC was calculated to assess the usefulness of ALP in predicting the presence of coronary calcification. We also calculated the category-free net reclassification index (NRI) and integrated discrimination improvement (IDI). Correlation between the levels of tissue-non-specific and bone-specific ALP was assessed with Pearson’s correlation coefficient. All statistical analyses were performed with SAS version 9.1.3 (SAS Institute, Cary, NC, USA), and R (version 2.15.1). A two-sided $P$-value < 0.05 was considered as significant.

Results

Baseline characteristics

Alkaline phosphatase ranged from 4 to 246 IU/L (median 70 IU/L, inter-quartile range 58–84 IU/L, mean 72.8 ± 21.4 IU/L). Alkaline phosphatase levels outside the normal range were observed in 66 patients (4.0%) including five patients (0.3%) having ALP < 30 IU/L and 61 patients (3.7%) having ALP >115 IU/L (Figure 1). The demographic, clinical, and angiographic characteristics of patients by ALP tertiles are summarized in Table 1. Briefly, the patients in the highest ALP tertile tended to experience a higher incidence of acute coronary syndrome as the index event, to have higher C-reactive protein levels, lower estimated GFR, and more left main coronary disease at the time of the index procedure. Baseline characteristics according to gender are shown in Supplementary material online, Table S1. In this study, 87.9% (493 of 561) of women were in post-menopausal stage at the time of index PCI.

Adverse clinical events according to alkaline phosphatase levels

The median duration of the follow-up was 762 days (inter-quartile range 494–1068 days). The Kaplan–Meier survival curves (Supplementary material online, Figure S1) showed that the highest ALP tertile showed significantly worse hard outcomes than the lowest one as determined by the log-rank test; all-cause mortality ($P < 0.0001$), cardiovascular mortality ($P < 0.0001$), non-fatal MI ($P = 0.003$), ST ($P < 0.001$), and MACE ($P < 0.0001$). The majority of MACE was attributed to TLR and TVR (34.3 and 21.5%, respectively). But soft outcomes, such as individual events of TLR and TVR, were not different among three groups.

Association between alkaline phosphatase and mortality

Hazard ratio (HR) of all-cause mortality increases as the level of ALP gets higher among three tertiles (Table 2). After adjustment for confounders, a significant association remained between ALP and the risk of all-cause death [adjusted HR (95% CI), T1: reference, T2: 1.75 (0.76–4.02), T3: 4.21 (2.03–8.71), $P$ for trend $< 0.0001$]. When restricted cubic spline regression was used to explore the adjusted association between ALP and all-cause mortality, we observed an approximately linear increase in hazard of mortality as ALP levels get higher (Figure 2). The adjusted HR (95% CI) of cardiovascular mortality was 3.92 (1.37–11.20) in the highest tertile compared with the lowest one. Similar results were produced when analyses were computed by including ALP as a continuous variable or cut-off value according to the ROC curve; cut-off values are summarized in Supplementary material online, Table S2. We also performed a separate analysis of all-cause or cardiovascular mortality between men and women (Supplementary material online, Table S3). In this analysis, the association of ALP with all-cause mortality was more prominent in women, but that with cardiovascular mortality was less clear in women.

Statistical interaction was negative between the primary diagnosis as MI at admission and the mortality rate that are related with higher ALP levels ($P = 0.973$), which suggests that the association between ALP and mortality was not restricted to the MI survivors. We further analysed ALP–mortality relationships in the various subgroups. In the subgroup of angina, the adjusted HR (95% CI) of mortality in the highest tertile was 4.44 (1.84–10.72) compared with the lowest one ($P$ for interaction $= 0.932$). Similar results were observed with regard to the diagnosis of acute coronary syndrome at admission ($P$ for interaction $= 0.467$), prior stroke ($P = 0.606$), emergent PCI ($P = 0.960$), body mass index ($>\ or\ \leq 25\ kg/m^2$) ($P = 0.689$), serum albumin level ($>\ or\ \leq 35\ g/L$) (3.5 g/dL) ($P = 0.626$), or the presence of diabetes ($P = 0.638$), hypertension ($P = 0.832$), hypercholesterolaemia ($P = 0.471$), decreased renal function (estimated GFR $< 60\ mL/min/1.73\ m^2$) ($P = 0.932$), or elevated level of high-sensitivity C-reactive protein ($\geq 30\ mg/L$) (3.0 mg/dL) ($P = 0.584$).

Association between alkaline phosphatase and stent thrombosis or myocardial infarction

The incidence of ST by ALP levels is summarized in Supplementary material online, Table S4. Data regarding compliance to dual anti-platelet therapy (DAT) was limited, because compliance was determined within follow-up intervals rather than with actual dates of discontinuance. Among patients with late or very-late ST, the majority actually took DAT; five of seven patients
(71.4%) in the lowest tertile and 15 of 21 patients (71.4%) in the highest tertile. Among patients with acute or subacute ST also, majority had been taking both aspirin and clopidogrel until the events occurred except 2 patients [1 out of 9 patients (11.1%) in T1, and 1 out of 29 patients (3.4%) in T3]. A trend towards an increase in the rate of definite ST was observed as the ALP level gets higher (P for trend = 0.010). The trend was more prominent when we analyse the composite of definite and probable ST among three tertiles (P for trend, 0.0001).

In the Cox proportional hazard models (Table 3), the adjusted HR (95% CI) of MI in the highest tertile compared with lowest one was 1.98 (0.91–4.29) and there was a dose–response relationship between MI and ALP levels (P for trend = 0.019). Similar results were observed with the composite endpoint of MACE. The adjusted HR (95% CI) of any ST (definite + probable) and definite ST in the highest tertile was 2.73 (1.33–5.61) and 3.98 (1.13–14.10), respectively, compared with the lowest. The associations between the ALP level and these outcomes according to gender were also shown in Supplementary material online, Table S5. In women, the level of ALP was still marginally associated with the risk of subsequent ST, but not with that of MI. There were several factors that were independently associated with the risk of ST; age, primary diagnosis as MI at admission, and, stent diameter (Figure 3). A statistical interaction was all negative between these variables and any ST that are related with higher ALP levels: age (P for interaction = 0.237), primary diagnosis as MI (P = 0.468), and stent diameter (0.725). Similar results were observed with regard to the use of DAT (P = 0.230), hypercholesterolaemia (0.154), and emergent PCI (P = 0.616).

**Predictive models based on alkaline phosphatase and C-reactive protein**

Receiver operating characteristic curve analyses demonstrated significant associations between ALP and hard outcomes, but with slightly different cut-off values providing the best sensitivity and specificity (Supplementary material online, Table S2). Additional inclusion of these cut-off values of ALP improved the predictive power of the models based on clinical variables including age, gender, smoking, diabetes, hypertension, hypercholesterolaemia, and the use of DAT. Specifically, the AUC for predicting all-cause mortality and ST increased from 0.732 to 0.773 (P for difference = 0.018), and from 0.626 to 0.701 (P = 0.017), respectively. These improvements of discrimination by ALP were similar to that by C-reactive protein, a representative marker of inflammation (Supplementary material online, Table S6). In addition, ALP and C-reactive protein levels were minimally correlated (r = 0.095), suggesting that each marker could detect different high-risk groups. We therefore constructed multivariate-adjusted time-to-event curves after dividing the patients into four groups on the basis of whether they were above or below the cut-off values of ALP and C-reactive protein. High ALP remained an independent predictor of all-cause mortality, cardiovascular mortality, MI, ST, and MACE in patients with high baseline C-reactive protein levels (Figure 4A–E). In patients with low C-reactive protein, ALP was also an independent predictor of MI, ST, and MACE. The adjusted HRs for each of the endpoints were summarized in Supplementary material online, Table S7. Gender-specific analysis was also conducted as subanalysis (Supplementary material online, Table S8).

**Association between alkaline phosphatase and coronary calcification**

After adjustment for conventional risk factors including age, gender, smoking, diabetes, hypertension, hypercholesterolaemia, and estimated GFR, the independent and dose-dependent association was observed between tertile of ALP and the adjusted odds ratio (OR) of coronary calcification (P for trend = 0.046) (Table 4). Specifically, compared with the lowest ALP tertile, the...
**Table 1** Baseline demographic, clinical, and angiographic data according to tertiles of alkaline phosphatase levels

<table>
<thead>
<tr>
<th>ALP tertile</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Lowest (n = 541) ALP &lt; 63 IU/L</td>
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<tr>
<td>(mean = 52 IU/L)</td>
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<tr>
<td>Middle (n = 563) ALP 63–78 IU/L</td>
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<tr>
<td>(mean = 70 IU/L)</td>
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<tr>
<td>Highest (n = 532) ALP &gt; 78 IU/L</td>
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<tr>
<td>(mean = 97 IU/L)</td>
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</table>

**Demographics**

- Age, years: 63.3 ± 9.4, 63.3 ± 10.0, 64.4 ± 10.1, P = 0.078
- Female gender: 171 (31.6), 177 (31.4), 213 (40.0), P = 0.003

**Risk factors**

- Body mass index, kg/m²: 25.0 ± 3.1, 24.6 ± 3.1, 24.5 ± 3.3, P = 0.089
- Smoking: 98 (18.1), 115 (20.4), 118 (22.2), P = 0.251
- Diabetes mellitus: 205 (37.9), 191 (33.9), 214 (40.2), P = 0.092
- Hypertension: 345 (63.8), 343 (60.9), 356 (66.9), P = 0.119
- Hypercholesterolaemia: 281 (51.9), 290 (51.5), 272 (51.1), P = 0.965
- PVD: 15 (2.8), 14 (2.5), 25 (4.7), P = 0.117
- Prior myocardial infarction: 68 (12.6), 75 (13.3), 61 (11.5), P = 0.647
- Prior PCI: 9 (18.1), 115 (20.4), 118 (22.2), P = 0.251
- Prior CABG: 41 (7.6), 34 (6.0), 27 (5.1), P = 0.231

**Primary diagnosis at admission**

- Stable angina: 207 (38.3), 212 (37.7), 159 (29.9), P = 0.039
- Unstable angina: 197 (36.4), 210 (37.3), 217 (40.8)
- Myocardial infarction: 122 (22.6), 137 (24.3), 143 (26.9)

**Medication at the time of index PCI**

- Aspirin: 520 (96.3), 533 (96.2), 500 (95.4), P = 0.725
- Clopidogrel: 519 (96.1), 536 (96.8), 504 (96.4), P = 0.849
- Beta-blocker: 343 (63.9), 349 (64.4), 335 (66.5), P = 0.653
- ACE-I: 139 (25.7), 155 (27.5), 153 (28.8), P = 0.525
- Statin: 358 (66.5), 357 (66.2), 328 (65.2), P = 0.894

**Discharge medication**

- Aspirin: 518 (96.3), 524 (96.1), 485 (95.3), P = 0.678
- Clopidogrel: 517 (96.1), 528 (96.7), 486 (96.2), P = 0.856
- Beta-blocker: 343 (63.9), 349 (64.4), 335 (66.5), P = 0.653
- ACE-I: 206 (38.4), 244 (45.1), 204 (40.7), P = 0.074
- Statin: 358 (66.3), 357 (66.2), 328 (65.2), P = 0.894

**Laboratory variables**

- LVEF, %: 57 ± 11, 58 ± 11, 56 ± 12, P = 0.277
- eGFR, mL/min/1.73 m²: 70.3 ± 18.3, 69.1 ± 19.7, 65.7 ± 22.8, P = 0.001
- hs-C-reactive protein, mg/L (mg/dL): 1.6 (0.1–3.9) [0.16 (0.01–0.59)], 2.1 (0.6–7.8) [0.21 (0.06–0.78)], 3.2 (1.0–9.9) [0.32 (0.10–0.99)], P < 0.0001
- Haemoglobin, g/L (g/dL): 132.5 ± 17.6 (13.2 ± 1.8), 132.7 ± 18.1 (13.3 ± 1.8), 130.8 ± 18.7 (13.1 ± 1.9), P = 0.175
- HDL, mmol/L (mg/dL): 1.1 ± 0.3 (42.5 ± 11.6), 1.1 ± 0.3 (42.4 ± 10.6), 1.1 ± 0.3 (41.7 ± 9.9), P = 0.422
- Calcium, mmol/L (mg/dL): 2.2 ± 0.1 (8.9 ± 0.4), 2.2 ± 0.1 (8.9 ± 0.5), 2.2 ± 0.2 (8.9 ± 0.6), P = 0.859
- Phosphate, mmol/L (mg/dL): 1.1 ± 0.2 (3.5 ± 0.5), 1.1 ± 0.2 (3.5 ± 0.6), 1.1 ± 0.2 (3.5 ± 0.6), P = 0.112
- Albumin, g/L (g/dL): 39.8 ± 3.7 (4.0 ± 0.4), 39.9 ± 4.1 (4.0 ± 0.4), 38.9 ± 4.9 (3.9 ± 0.5), P < 0.0001
- Bilirubin, μmol/L (mg/dL): 15.4 ± 6.8 (0.9 ± 0.4), 13.7 ± 6.8 (0.8 ± 0.4), 13.7 ± 5.1 (0.8 ± 0.3), P = 0.028
- SGOT, IU/L: 240 (197–340), 250 (200–355), 250 (198–380), P = 0.412
- SGPT, IU/L: 23.0 (16.0–34.0), 23.5 (16.8–34.5), 24.0 (16.2–35.8), P = 0.726

**Angiographic data**

- Number of diseased vessels: 1 184 (34.0), 185 (32.9), 165 (31.0), P = 0.667
- 2 198 (32.9), 194 (34.5), 184 (32.7)
- 3 165 (31.0), 197 (37.0), 170 (32.0)
CI: 1.01–1.71: Ptertile was 1.34 (95% CI: 1.04–1.73; adjusted OR of coronary calcification in the middle and highest tertile was 1.34 (95% CI: 1.04–1.73; P = 0.036) and 1.32 (95% CI: 1.01–1.71; P = 0.010), respectively. Furthermore, the inclusion of the ALP level marginally increased the AUC (from 0.629 to 0.638; P = 0.053) for the prediction of coronary calcification when it was added on traditional risk factors mentioned above.
The NRI and IDI showed significant improvement in discrimination (NRI = 0.1596; \( P = 0.002 \), IDI = 0.0052; \( P = 0.005 \), respectively) (Supplementary material online, Table S9). In addition, we assessed whether the level of ALP was associated with the severity of coronary calcification; higher ALP level was correlated with obvious and heavy calcification (Table 4). The dose-dependent association was more prominent in this analysis (\( P \) for trend = 0.013). The levels of tissue-non-specific ALP was also correlated with that of bone-specific ALP (Pearson’s \( r = 0.64 \), \( P < 0.0001 \)), which has been reported to be a useful marker of vascular calcification (Supplementary material online, Figure S2).

Sensitivity analyses
We repeated analyses after adjusting additional variables, including a history of prior MI, stroke, PCI and CABG, and stent type; the association between ALP and the risk of all-cause mortality, cardiovascular mortality, MI, ST, and MACE remained significant (\( P \) for trend < 0.05 in all models).

Discussion
The principal findings of our study are the following: (i) the correlation between ALP and subsequent mortality was observed in overall CAD patients after PCI; (ii) the level of ALP was an independent predictor of the risks of MI and ST, as well as mortality after PCI; and (iii) the predictive value of ALP not only remained significant regardless of the C-reactive protein level, but also was additive to the prognostic information of C-reactive protein.

Possible mechanism for adverse effects of alkaline phosphatase in coronary artery disease patients
These effects of serum ALP levels on a poor cardiovascular outcome may originate from deteriorated vascular system, perhaps mediated by vascular calcification, in which ALP has been noticed to be a regulator. Vascular calcification is now thought to be an active process potentially orchestrated by vascular cells and osteogenic factors such as ALP, and indeed, ALP levels are up-regulated in vessels with medial calcification. Furthermore, it was suggested that the mechanism by which ALP regulates vascular calcification is through its catalytic effect on organic pyrophosphate, a potent inhibitor of vascular calcification. Animal studies showed that some genetic ablations or experimental inhibitors of ALP led to accumulation of organic pyrophosphate, and consequently, to amelioration of calcification. One study showed the independent association between ALP and coronary artery calcification assessed by computed tomography in haemodialysis patients. Considering that vascular calcification is a ubiquitous feature of ageing and is associated with common pathology including atherosclerosis, the putative link between ALP and vascular calcification may not be restricted to dialysis patients. In this regard, we sought to investigate the association of the ALP level with coronary calcification, a possible mechanism mediating the link between ALP and adverse clinical outcomes. Our finding showed that the level of ALP might be useful for improving the risk prediction of coronary calcification.

Several different mechanisms for the adverse effects of ALP on a clinical outcome may be considered. Since we measured the
serum level of tissue-non-specific ALP which is mainly concentrated in the bone, liver, and kidney, one possibility is that the link between ALP and cardiovascular events represents a confounder such as liver disease. In this analysis, we excluded patients with established liver disease according to medical records, laboratory and imaging tests, and also adjusted for other liver enzymes. Thus, we believe that our findings are unlikely to be limited by significant residual confounding by liver disease. A second possibility is that ALP levels may be linked to an inflammatory status. It has been known that patients with sepsis can have elevated ALP and normal bilirubin levels. Thus, we obtained data on high-sensitivity C-reactive protein, and adjusted it to reduce the risk of confounding by an inflammatory status; our results were independent from C-reactive protein levels. Moreover, our data suggested that ALP and C-reactive protein levels were minimally correlated, and thus, the combined evaluation of both ALP and C-reactive protein might be more effective in predicting adverse outcomes than the measurement of either marker alone. Finally, in our analysis of subgroup whose frozen sera were available for checking the bone-specific ALP level, we confirmed the high correlation between tissue-non-specific ALP and bone-specific one.

### Table 3  Association between the alkaline phosphatase level and myocardial infarction, stent thrombosis, or major adverse cardiac event

<table>
<thead>
<tr>
<th></th>
<th>Model 1a</th>
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<th>Model 2b</th>
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<tbody>
<tr>
<td></td>
<td>Events, n (%)</td>
<td>HR</td>
<td>95% CI</td>
<td>P-value</td>
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<tr>
<td>Non-fatal MI</td>
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<tr>
<td>Lowest tertile</td>
<td>10 (1.8)</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Middle tertile</td>
<td>10 (1.8)</td>
<td>0.94</td>
<td>0.39–2.27</td>
<td>0.898</td>
</tr>
<tr>
<td>Highest tertile</td>
<td>27 (5.1)</td>
<td>2.77</td>
<td>1.34–5.72</td>
<td>0.006</td>
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<td>P for trend*</td>
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<td>0.002</td>
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<tr>
<td>ALP (cont. variable)c</td>
<td>1.17</td>
<td>1.06–1.29</td>
<td>0.003</td>
<td>1.12</td>
</tr>
<tr>
<td>ALP (cut-off value)d</td>
<td>2.89</td>
<td>1.61–5.17</td>
<td>&lt;0.0001</td>
<td>2.73</td>
</tr>
<tr>
<td>ST (definite)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest tertile</td>
<td>5 (0.9)</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Middle tertile</td>
<td>6 (1.1)</td>
<td>1.18</td>
<td>0.36–3.86</td>
<td>0.788</td>
</tr>
<tr>
<td>Highest tertile</td>
<td>17 (3.2)</td>
<td>3.42</td>
<td>1.26–9.28</td>
<td>0.016</td>
</tr>
<tr>
<td>P for trend*</td>
<td>0.006</td>
<td>0.013</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALP (cont. variable)c</td>
<td>1.22</td>
<td>1.08–1.39</td>
<td>0.002</td>
<td>11.16</td>
</tr>
<tr>
<td>ALP (cut-off value)d</td>
<td>3.14</td>
<td>1.47–6.72</td>
<td>0.003</td>
<td>4.35</td>
</tr>
<tr>
<td>ST (definite+probable)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest tertile</td>
<td>16 (3.0)</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Middle tertile</td>
<td>21 (3.7)</td>
<td>1.22</td>
<td>0.64–2.34</td>
<td>0.544</td>
</tr>
<tr>
<td>Highest tertile</td>
<td>50 (9.4)</td>
<td>2.97</td>
<td>1.69–5.22</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P for trend*</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALP (cont. variable)c</td>
<td>1.22</td>
<td>1.13–1.31</td>
<td>&lt;0.0001</td>
<td>1.21</td>
</tr>
<tr>
<td>ALP (cut-off value)d</td>
<td>2.66</td>
<td>1.74–4.07</td>
<td>&lt;0.0001</td>
<td>3.60</td>
</tr>
<tr>
<td>MACE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest tertile</td>
<td>101 (18.7)</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Middle tertile</td>
<td>100 (17.8)</td>
<td>0.96</td>
<td>0.73–1.27</td>
<td>0.769</td>
</tr>
<tr>
<td>Highest tertile</td>
<td>152 (28.6)</td>
<td>1.64</td>
<td>1.27–2.11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P for trend*</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALP (cont. variable)c</td>
<td>1.11</td>
<td>1.06–1.15</td>
<td>&lt;0.0001</td>
<td>1.05</td>
</tr>
<tr>
<td>ALP (cut-off value)d</td>
<td>1.79</td>
<td>1.44–2.23</td>
<td>&lt;0.0001</td>
<td>1.56</td>
</tr>
</tbody>
</table>

*aModel 1: HRs have been adjusted for age, gender, diabetes mellitus, hypertension, and primary diagnosis as MI at admission.

*bModel 2: HRs have been adjusted for Model 1 variables and additional covariates as follow: (i) traditional risk factors including smoking status, hypercholesterolaemia, use of dual antiplatelet therapy and statins at the time of index PCI, and LVEF, (ii) angiographic data including multi-vessel disease, left main disease, ACC/AHA lesion type B2/C, stent length, stent diameter, and number of implanted stents, and (iii) laboratory findings including eGFR, high-sensitivity C-reactive protein, haemoglobin, HDL cholesterol, calcium, phosphatase, albumin, bilirubin, SGOT, and SGPT.

cAnalyses were computed by including ALP as a continuous variable.

dAnalyses were computed by including the predefined cut-off value of ALP according to the ROC curve; cut-off values of MI, stent thrombosis, and MACE were 77.5, 78.5, and 84.5 IU/L, respectively.

*P for trend refers to linear trend across lowest to highest tertile.
Impact of alkaline phosphatase on first-generation drug-eluting stent

Tonelli et al.\textsuperscript{9} reported independent relation between higher ALP levels and adverse clinical outcomes among survivors of MI. They speculated that this association was mediated by vascular calcification. However, their study did not demonstrate a significant link between ALP and MI. In our study, a trend towards an increase in MI risk was observed as the ALP level gets higher for 2 years’ follow-up after PCI. Furthermore, higher ALP levels were significantly associated with the risk of ST. In this regard, a novel finding of our study is the observation that a higher level of ALP was an independent predictor of ST or MI after DES implantation. To the best of our knowledge, this has not been reported previously. The mechanisms underlying the association between ALP and ST or MI are unclear and require further study.

One possible mechanism is that coronary calcification may hamper the process of re-endothelialization. Coronary calcification seems to be related to the number and functions of endothelial progenitor cells (EPCs). Indeed, one study demonstrated that patients with coronary calcification have significantly lower levels of EPC.\textsuperscript{21} Another paper reported that patients with early coronary atherosclerosis are characterized by retention of osteogenic EPCs, potentially leading to progressive calcification rather than normal repair.\textsuperscript{22} Because we do not have information on re-endothelialization, further studies are needed to assess this speculation.

The effect of coronary calcification on stent restenosis is controversial. Theoretically, calcified vessels have few viable smooth muscle cells capable of proliferating, and consequently, may exhibit less restenosis. However, more-severe trauma, under-expansion, and mal-apposition of stents, which are risk factors for restenosis, may happen during PCI frequently in calcified arteries. Previous study demonstrated that coronary calcification do not seem to affect restenosis rates.\textsuperscript{23} Another study reported that patients with calcified vessel had significantly higher rates of revascularization.\textsuperscript{24} The role of ALP in restenosis is also poorly understood. One retrospective study showed that ALP levels were significantly lower in patients with stent restenosis.\textsuperscript{25} Our study demonstrated no correlation between ALP and restenosis. However, our results should be interpreted with caution, since, besides its observational design, we examined the rate of ischaemia-driven revascularization rather than the quantitative assessment of angiographic restenosis.

Study limitations

The present study has several caveats that should be considered. First, we cannot rule out the possibility of residual confounders by unmeasured characteristics. However, we adjusted multiple potential confounding factors, including variables known to be associated with ALP levels and adverse cardiovascular outcomes. We also adjusted angiographic data to reduce the risk of confounding. Secondly, we did not obtain data on parathyroid hormone or vitamin D status, which has been suggested to affect ALP levels. Although previous studies showed that the association between ALP and mortality was independent of parathyroid hormone or vitamin D,\textsuperscript{7,9} the lack of this information may hamper clinical interpretation. Thirdly, it is possible that the nutritional status has an influence on ALP levels and adverse outcomes. It has been suggested that higher ALP levels were linked to lower albumin levels, implying the poor nutritional status.\textsuperscript{8} Although our finding showed that higher ALP levels were related with increased risks of adverse events independently of baseline albumin levels, potential

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**Figure 3** Forest plots of Cox proportional hazard ratios with 95% CI for stent thrombosis. Hazard ratios are adjusted for covariates including age, gender, diabetes mellitus, hypertension, primary diagnosis as myocardial infarction at admission, use of dual antiplatelet therapy at the time of index percutaneous coronary intervention, stent diameter, and stent length.
Figure 4 Multivariate-adjusted time-to-event curves showing the rates of (A) all-cause mortality, (B) cardiovascular mortality, (C) myocardial infarction, (D) stent thrombosis, and (E) major adverse cardiac event by alkaline phosphatase and C-reactive protein levels above or below the predefined cut-off values. Hazard ratios are adjusted for age, gender, smoking, diabetes, hypertension, hypercholesterolaemia, primary diagnosis as myocardial infarction, use of aspirin, clopidogrel, beta-blockers, angiotensin-converting enzyme inhibitors, and statins at the time of index percutaneous coronary intervention. In analyses regarding myocardial infarction and stent thrombosis, use of dual antiplatelet therapy and statins at the time of index percutaneous coronary intervention have been included instead of above-mentioned medications.
the tissue-non-specific ALP levels. When we examined the association between ALP and coronary calcification, we found that the level of tissue-non-specific ALP measured in our study (Pearson's $r = 0.64$, $P < 0.0001$) (Supplementary material online, Figure S2). Fifthly, coronary angiography is not a sensitive technique to detect coronary calcification compared with other modalities such as intravascular ultrasound (IVUS) or coronary artery calcium score (CACS). However, Tuzcu et al. suggested that the presence of angiographically visible calcification implied a large burden of calcification on IVUS. Moreover, the patients without apparent calcification on angiography might have a relatively low likelihood of significant calcification on IVUS. Future study using IVUS or CACS may provide additional insight into the link between ALP and cardiovascular events. Sixthly, we could not clearly explain the gender-specific difference observed in this analysis. Given that most women were post-menopausal status, and women tended to experience more frequent fracture (5.7 vs. 2.1% in the present study), fracture-associated mortalities could be more prevalent in women than in men. However, the relation between ALP and the risk of ST was still marginally significant, suggesting the possible contribution of ALP to poor vascular fate also in women patients. Future study with larger number of women can pave the way to validate the usefulness of ALP as a marker of adverse clinical outcomes in women. Finally, as for all observational studies, our results do not conclude the association between ALP and adverse clinical events is causal.

In conclusion, our findings demonstrated that increased levels of ALP were independently associated with mortality, MI, and ST among CAD patients after PCI using DES. Given that vascular calcification is prevalent in association with CAD, and it also is an emerging target in the treatment of atherosclerosis, ALP might be useful as a marker of disease severity or treatment response. However, further studies are needed to evaluate the possibility of a causal link to vascular calcification. To consider an ALP-targeting strategy could be the next step.

### Supplementary material

Supplementary material is available at European Heart Journal online.
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References