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Increased Serum Alkaline Phosphatase and Functional Outcome in Patients with Acute Ischemic Stroke Presenting a Low Ankle–Brachial Index

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Aims: Elevated serum alkaline phosphatase (ALP) levels are associated with an increased risk of cerebrocardiovascular diseases. However, the associations of ALP with peripheral arterial disease (PAD) and outcomes in patients with acute ischemic stroke (AIS) are not well-known.

Methods: We examined the association between ALP levels and the ankle–brachial index (ABI) in 2111 consecutive patients with AIS. A poor functional outcome was defined as a modified Rankin Scale (mRS) score of 3–6 at 3 months after stroke. A low ABI was defined as a value of ≤ 0.9.

Results: Of the total cohort, 482 patients (22.8%) had a low ABI. ALP levels were higher in patients with a low ABI than in those without ($p<0.001$). The multivariable logistic analysis revealed that quartiles of ALP levels were significantly associated with a low ABI (odds ratio [OR]: 1.20, 95% confidence interval [CI]: 1.08–1.33). Of the 1322 patients with a premorbid mRS score of 0–2, 434 patients (32.8%) had a poor outcome. The multivariable analysis revealed that elevated serum ALP levels and a low ABI were independently associated with poor stroke outcomes after adjustment for baseline characteristics (OR: 1.21, 95% CI: 1.07–1.38, and OR: 2.00, 95% CI: 1.40–2.84, respectively).

Conclusions: Increased serum ALP levels are significantly associated with a low ABI. These indicators are independent prognostic factors for poor stroke outcomes at 3 months.

Key words: Acute ischemic stroke, Serum alkaline phosphatase, Ankle–brachial index, Atherosclerosis

Introduction

Stroke is the fourth leading cause of death and a major cause of long-term disability. Thus, the risk factors for stroke must be recognized to prevent stroke onset. Peripheral arterial disease (PAD) is a well-recognized risk factor for stroke. Ischemic stroke and transient ischemic attack occur frequently in patients with symptomatic PAD[1)]. In addition, patients with acute ischemic stroke (AIS) presenting with PAD also have an increased cardiovascular risk and a worse prognosis than have those without PAD[2)]. The ankle–brachial index (ABI) is the gold standard diagnostic tool for PAD. An ABI of ≤ 0.9 predicts cardiovascular risk and mortality in patients with AIS[3)]. Because PAD is a chronic arterial occlusive disease of the lower

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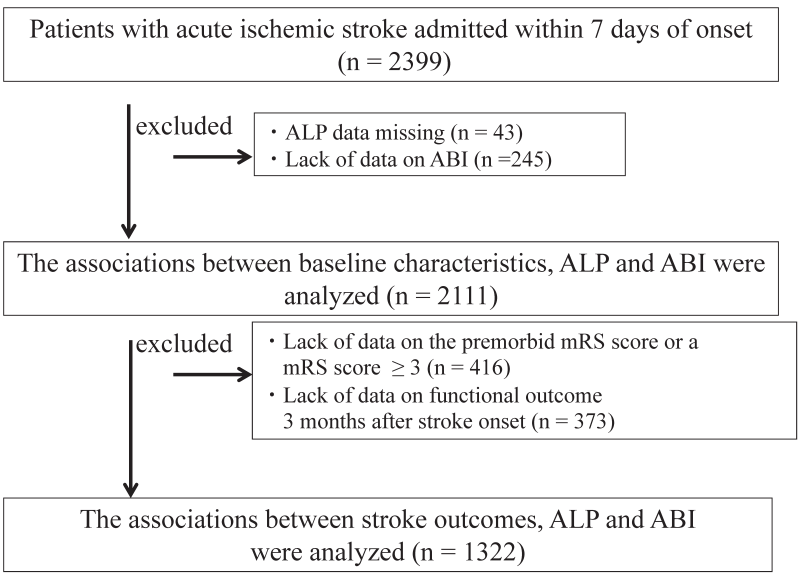

Fig. 1. Flow chart of the patient selection process

ABI, ankle–brachial index; ALP, alkaline phosphatase; mRS, modified Rankin Scale.

extremities caused by atherosclerosis, the prevalence of PAD increases with age[4]. In addition, PAD is highly prevalent among persons with chronic kidney disease (CKD)[5].

Serum alkaline phosphatase (ALP) testing is a well-known method to evaluate liver and bone diseases in clinical settings. ALP has also been reported to be a molecular marker of vascular calcification[6]. Vascular calcification plays an important role in the process of atherosclerosis. Serum ALP levels are associated with carotid intima media thickness and arterial stiffness estimated using pulse wave velocity[7, 8]. Thus, ALP is thought to be a marker of atherosclerosis. Several clinical studies have documented that elevated serum ALP levels are associated with increased risks of coronary heart disease and stroke[9]. In addition, serum ALP levels are a good predictor of all-cause mortality and functional outcomes of acute stroke[10, 11]. We previously reported that serum ALP levels extracted using a neural network analysis were independently associated with poor stroke outcomes at 3 months[12]. Although the association between increased ALP levels and stroke outcomes is not completely clear, we speculated that the effect of PAD is mediated by vascular calcification. In the present study, we investigated whether serum ALP levels are associated with a low ABI among patients with AIS and evaluated the effects of both on functional outcomes at 3 months after stroke onset.

Methods

Study Population

This double-center, hospital-based retrospective study was performed at the Hiroshima University Hospital and Chikamori Hospital and involved consecutive patients with AIS who were hospitalized within 7 days of onset between October 2009 and September 2018. A total of 2399 patients were admitted to our hospital, and 288 of these patients were excluded because of missing ALP levels (n=43) or a lack of data on ABI (n=245). We evaluated the association between ALP levels and ABI. In addition, we excluded 416 patients because of a lack of data on the premorbid modified Rankin Scale (mRS) score or having a score of ≥ 3. We also excluded 373 patients because of a lack of data on functional outcomes 3 months after stroke onset **(Fig. 1)**. Several significant differences in baseline characteristics were observed between the patients included in the analysis and those excluded **(Supplemental Table 1)**. This study complies with the Declaration of Helsinki guidelines for investigations involving humans, and the study protocol was approved by the Ethics Committees of Hiroshima University (#E-856) and Chikamori Hospital (#269). This study was retrospectively performed under the opt-out method using clinical records. Informed consent for participation was not obtained from each participant.

Assessment of Clinical Characteristics

Ischemic stroke was defined as the sudden onset of acute neurological deficits and confirmed by acute infarction on brain computed tomography or magnetic resonance imaging. The following clinical characteristics were recorded at admission: age; sex; body mass index (BMI); malignancy; and the classical vascular risk factors hypertension, diabetes mellitus, dyslipidemia, CKD, atrial fibrillation, daily alcohol intake (>40 g), smoking habit (current smokers or noncurrent smokers), and history of stroke and ischemic heart disease. The criteria for hypertension, diabetes mellitus, dyslipidemia, and atrial fibrillation have been defined previously[13]. CKD was evaluated by calculating the estimated glomerular filtration rate (eGFR) using the following equation from the Japanese Society of Nephrology: $eGFR = 194 \times Cr^{-1.094} \times age^{-0.287}$ (mL/min/1.73 m^2). For women, the eGFR was multiplied by a correction factor of 0.739[14]. CKD was defined as an eGFR level less than 60 mL/min/1.73 m^2. Most blood samples were collected at admission, and ALP, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (γ-GTP) levels were evaluated. Neurological symptom severity at admission was assessed according to the National Institutes of Health Stroke Scale (NIHSS) score. Stroke subtypes were classified according to the TOAST classification as follows: small-vessel occlusion, large-artery atherosclerosis, cardioembolic stroke, and stroke of other etiology (determined and undetermined etiologies)[15].

The primary outcome was evaluated on the basis of the 3-month functional status: a poor outcome was defined as an mRS score of 3 to 6, and a good outcome was defined as an mRS score of 0 to 2. Briefly, attending physicians evaluated the mRS score at 3 months after stroke onset by examining the patients. When physicians were unable to examine the patients, the mRS score was assessed by the attending physicians on the basis of a review of the medical records or contacting the caregivers of patients.

Measurement of the ABI

The ABI was measured using a noninvasive automatic pulse wave analyzer (model BP-203RPE-III; Nihon Colin, Tokyo, Japan, and Fukuda Denshi Vasera VS-1000 or VS-1500, Tokyo, Japan) after the patient rested for 5 minutes in the supine position. According to the recommendations of the American Heart Association[16], the ABI was calculated as the ratio of the systolic pressure in the posterior tibial artery to the highest systolic pressure in the two brachial arteries. ABI values ≤ 0.9 were defined as low according to published guidelines[17].

Statistical Analyses

Categorical variables are presented as numbers and percentages, and continuous variables are presented as the means with standard deviations (SDs) or medians (interquartile ranges). The statistical significance of intergroup differences was assessed using the χ^2 test for categorical variables and Student's *t*-test or the Mann–Whitney *U* test for continuous variables. The enrolled patients were grouped into quartiles based on serum ALP levels to evaluate the associations of various factors with the serum ALP levels. Trends in the prevalence of a low ABI according to the quarters of ALP in patients with and without CKD were tested using the Cochran–Armitage test. Factors associated with a low ABI, except for stroke subtype, were selected by a backward selection procedure using $p > 0.10$ of the likelihood ratio as the exclusion criterion. Next, a multivariable logistic analysis was performed for quartiles of ALP levels and other baseline factors that remained considerably different after the previously mentioned stepwise procedure. In addition, a multivariable logistic analysis was performed for a low ABI, quartiles of ALP levels, and other baseline factors that remained predictors of a poor stroke outcome after the previously mentioned stepwise procedure. The patients who were assessed for the 3-month functional outcome after stroke onset were classified into eight groups according to the ABI (≤ 0.9 or >0.9) and quartiles of ALP levels. The odds ratio (OR) for a poor outcome in each group was also calculated using the multivariate logistic regression analysis and compared with the reference (lowest quartiles of ALP for patients with an ABI of >0.9). In all analyses, $p < 0.05$ was used to indicate statistical significance. All analyses were performed using JMP 14.0 software (SAS Institute, Inc., Cary, NC).

Results

Associations between the Baseline Characteristics and a Low ABI

The clinical and demographic data are shown in Table 1. Of the 2111 patients, 482 patients (22.8%) had a low ABI. The patients with a low ABI were older and less frequently male and had a lower BMI and frequency of dyslipidemia, and a higher percentage had hypertension, diabetes mellitus, CKD, atrial fibrillation, previous stroke and ischemic heart disease than had patients with a normal ABI. The patients with a low ABI also exhibited a lower frequency of daily alcohol intake and current smoking status than did those with a normal ABI. The patients

Table 1. Indicators associated with ABI assessed with univariable analyses

	Total (n=2111)	Normal ABI (n=1629)	Low ABI (n=482)	p
Age, years	75.4 ± 11.6	73.7 ± 11.5	81.3 ± 9.8	<0.001
Sex, male	1227 (58.1)	1001 (61.5)	226 (46.9)	<0.001
Body mass index, kg/m^2	23.1 ± 3.7	23.4 ± 3.7	22.0 ± 3.5	<0.001
Daily alcohol intake	548 (26.1) (n=2101)	486 (30.0) (n=1620)	62 (12.9) (n=481)	<0.001
Current smoking status	410 (19.5) (n=2102)	336 (20.7) (n=1621)	74 (15.4) (n=481)	0.009
Hypertension	1469 (69.6) (n=2110)	1114 (68.4) (n=1628)	355 (73.7)	0.029
Diabetes mellitus	696 (33.0)	503 (30.9)	193 (40.0)	<0.001
Dyslipidemia	1006 (47.7)	799 (49.1)	207 (43.0)	0.019
Chronic kidney disease	835 (40.0)	559 (34.3)	276 (57.3)	<0.001
Atrial fibrillation	426 (20.2) (n=2110)	277 (17.0) (n=1628)	149 (30.9)	<0.001
Malignancy	314 (14.9)	240 (14.7)	74 (15.4)	0.737
History of stroke	708 (33.6) (n=2110)	495 (30.4) (n=1628)	213 (44.2)	<0.001
History of ischemic heart disease	266 (12.6)	181 (11.1)	85 (17.6)	<0.001
ALT, U/L, median (IQR)	22 (19–28)	23 (19–28)	22 (18–29)	0.873
AST, U/L, median (IQR)	17 (13–25)	18 (13–25)	15 (11–22)	0.795
ALP, U/L, median (IQR)	244 (198–301)	239 (196–295)	262 (211–320)	0.001
γ-GTP, median (IQR)	25 (16–42)	26 (17–43)	23 (15–41)	0.004
NIHSS score at admission	3 (1–8)	3 (1–6)	6 (2–16)	<0.001
Stroke subtype				<0.001
Small-vessel occlusion	601 (28.5)	496 (30.5)	105 (21.8)	
Large-artery atherosclerosis	517 (24.5)	412 (25.3)	105 (21.8)	
Cardioembolic stroke	534 (25.3)	357 (21.9)	177 (36.7)	
Other etiology	459 (21.7)	364 (22.3)	95 (19.7)	

Data are presented as the means ± standard deviations for age and body mass index; as the medians (interquartile ranges) for each laboratory parameter and baseline NIHSS score; and as the number of patients (%) for other measures.
ABI, ankle-brachial index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; γ-GTP, γ-glutamyl transferase; NIHSS, National Institute Health Stroke Scale.

with a low ABI exhibited severe neurological deficits at admission. Higher serum ALP levels were observed in the patients with a low ABI than in those with a normal ABI (median [interquartile range], 262 U/L [211–320] vs. 239 U/L [196–295], p=0.001).

Associations between Baseline Characteristics and Serum ALP Levels

All patients were divided into four groups (quartiles of ALP levels). Among patients with higher quartiles of ALP levels, patients in the fourth quartile were older and more likely to be female, had a lower BMI, were more likely to be a nondrinker, and had a higher frequency of previous stroke and low ABI than those in the first quartile **(Supplemental Table 2)**. The patients with the highest quartile of ALP levels had the highest frequency of a low ABI (30.4%, p< 0.001, **Supplemental Fig. 1A**). We also evaluated trends in the prevalence of a low ABI according to the quartiles of ALP levels in the patients with or without CKD. The prevalence of a low ABI was significantly associated with the higher quartile of ALP levels in the patients with and without CKD (p<0.001 and p< 0.001, respectively) **(Supplemental Fig. 1B and 1C)**. The multivariable logistic regression analysis revealed that quartiles of ALP levels were independently associated with a low ABI (OR: 1.20, 95% confidence interval [CI]: 1.08–1.33, p=0.001) **(Table 2)**. Higher quartiles of ALP levels were also associated with a low ABI among patients with CKD (OR: 1.26, 95% CI: 1.09–1.45, p=0.001), although the associations between ALP levels and a low ABI were not significant for patients without CKD (OR: 1.16, 95% CI: 0.99–1.35, p=0.068).

Stroke Outcome

Of the 1322 patients, 434 patients (32.8%) had a poor stroke outcome at 3 months **(Supplemental Table 3)**. These patients were significantly older; were more likely to be female; had a lower BMI; and exhibited a higher frequency of CKD, atrial fibrillation, history of stroke and a lower frequency of daily alcohol intake, smoking, and dyslipidemia. The patients with a poor outcome had a higher frequency

Table 2. Results of the multivariable analysis to determine factors associated with a low ABI

	Total (n=2111)		eGFR <60 (n=830)		eGFR ≥ 60 (n=1276)	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Age, years	1.05 (1.03–1.06)	<0.001	1.04 (1.02–1.06)	<0.001	1.05 (1.03–1.07)	<0.001
Body mass index, kg/m^2	0.91 (0.88–0.95)	<0.001	0.97 (0.94–0.99)	0.016	0.90 (0.86–0.95)	<0.001
Daily alcohol intake	0.59 (0.43–0.82)	0.001	–	–	0.59 (0.37–0.94)	0.023
Current smoking status	1.40 (1.02–1.94)	0.041	–	–	1.62 (1.03–2.54)	0.038
Diabetes mellitus	2.07 (1.62–2.66)	<0.001	1.67 (1.19–2.33)	0.003	2.64 (1.82–3.82)	<0.001
Dyslipidemia	–	–	–	–	–	–
Chronic kidney disease	1.88 (1.48–2.38)	<0.001	–	–	–	–
Atrial fibrillation	1.56 (1.19–2.05)	0.002	1.42 (1.00–2.03)	0.052	1.71 (1.12–2.59)	0.014
History of stroke	1.37 (1.08–1.73)	0.010	1.30 (0.94–1.78)	0.109	1.41 (0.99–2.02)	0.058
History of ischemic heart disease	1.48 (1.08–2.04)	0.017	–	–	1.70 (1.04–2.77)	0.037
NIHSS score at admission	1.05 (1.03–1.06)	<0.001	1.02 (1.00–1.04)	0.019	1.08 (1.06–1.10)	<0.001
ALP, quartiles	1.20 (1.08–1.33)	0.001	1.26 (1.09–1.45)	0.001	1.16 (0.99–1.35)	0.068

The factors listed in Table 1, except for stroke subtypes, were selected for a low ABI using a backward selection procedure, with a p value >0.10 used as the exclusion criterion for the likelihood ratio test. Next, a multivariable logistic regression analysis was performed for quartiles of ALP levels and other baseline factors associated with a low ABI after the aforementioned stepwise procedure.
ABI, ankle-brachial index; eGFR, estimated glomerular filtration rate; OR, odds ratio; CI, confidence interval; NIHSS, National Institute Health Stroke Scale; ALP, alkaline phosphatase.

of a low ABI (32.0% vs. 12.3%, p<0.001) and exhibited severe neurological deficits at admission. Serum AST and ALP levels were higher in patients with a poor outcome than in those with a good outcome. In the multivariable analysis adjusted for confounding factors, serum ALP levels and a low ABI were independently associated with poor functional outcome at 3 months (OR: 1.21, 95% CI: 1.07–1.38, p=0.003, and OR: 2.00, 95% CI: 1.40–2.84, p< 0.001, respectively; **Table 3**). Regardless of renal function, a low ABI and increased serum ALP levels were independently associated with a poor outcome at 3 months among patients with and without CKD **(Table 3)**. The adjusted ORs for a poor outcome in each group stratified according to the ABI and quartiles of ALP levels are shown in **Fig. 2**. The highest quartiles of ALP levels for patients with a low ABI showed a remarkable association with a poor outcome, which was not observed with the lowest quartiles of ALP levels for patients with a normal ABI (OR: 3.75, 95% CI: 1.96–7.20) **(Fig. 2 and Supplemental Table 4)**.

Discussion

In the present study, higher ALP levels were independently associated with a low ABI in patients with AIS. In addition, both increased ALP levels and a low ABI were independently associated with a poor stroke outcome after adjustment for baseline characteristics.

Several explanations for the increased ALP levels in patients with AIS presenting with PAD have been proposed. First, ALP plays a role in vascular calcification, promoting the process of atherosclerosis[6]. Vascular calcification, a pathway involved in the initiation and progression of atherosclerosis[18], promotes vascular aging and atherosclerosis and may result in PAD. Vascular calcification occurs particularly frequently in patients with CKD, possibly because interactions between ALP, phosphate, and vitamin D are associated with calcium and bone metabolism. In this study, the prevalence of a low ABI was significantly associated with a higher quartile of ALP levels among both patients with and without CKD. The multivariable analysis revealed that increased ALP levels were independently associated with PAD among patients with CKD, although those associations were not significant among patients without CKD. Second, the systemic inflammatory status may be associated with serum ALP levels. ALP correlates with C-reactive protein, which suggests an association between ALP and cardiovascular disease through inflammation[19]. Chronic inflammation plays an important role in initiating and promoting the atherosclerotic process. PAD is a common manifestation of atherosclerosis associated with systemic inflammation. Recent studies from Asian countries have shown the association between serum ALP levels and features of carotid atherosclerosis, such as carotid intima media thickness and extracranial carotid artery stenosis, or arterial stiffness evaluated by measuring

Table 3. Results of the multivariable analysis to determine the factors associated with a poor outcome at 3 months

	Total (n=1322)		eGFR <60 (n=516)		eGFR ≥ 60 (n=801)	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Age, years	1.06 (1.04–1.08)	<0.001	1.07 (1.04–1.10)	<0.001	1.06 (1.04–1.08)	<0.001
Sex, male	–	–	–	–	0.71 (0.47–1.09)	0.119
Body mass index, kg/m^2	0.97 (0.93–1.01)	0.134	0.89 (0.83–0.96)	0.002	–	–
Daily alcohol intake	–	–	–	–	1.44 (0.93–2.23)	0.104
Diabetes mellitus	–	–	–	–	–	–
History of stroke	1.79 (1.32–2.43)	<0.001	1.55 (0.98–2.45)	0.063	2.00 (1.33–3.01)	0.001
AST, U/L	1.01 (0.99–1.02)	0.133	–	–	–	–
NIHSS score at admission	1.21 (1.17–1.25)	<0.001	1.17 (1.12–1.22)	<0.001	1.27 (1.21–1.33)	<0.001
Low ABI	2.00 (1.40–2.84)	<0.001	1.83 (1.12–2.98)	0.016	2.00 (1.17–3.42)	0.013
ALP, quartiles	1.21 (1.07–1.38)	0.003	1.26 (1.03–1.54)	0.025	1.21 (1.02–1.43)	0.030

The factors that were associated with poor stroke outcome, except for stroke subtypes, were selected by a backward selection procedure using $p>0.10$ of the likelihood ratio as the exclusion criterion. Next, a multivariable logistic regression analysis was performed for a low ABI, quartiles of ALP levels and other baseline factors that remained predictors of a poor stroke outcome after the previously mentioned stepwise procedure. eGFR, estimated glomerular filtration rate; OR, odds ratio; CI, confidence interval; AST, aspartate aminotransferase; NIHSS, National Institute Health Stroke Scale; ABI, ankle-brachial index; ALP, alkaline phosphatase.

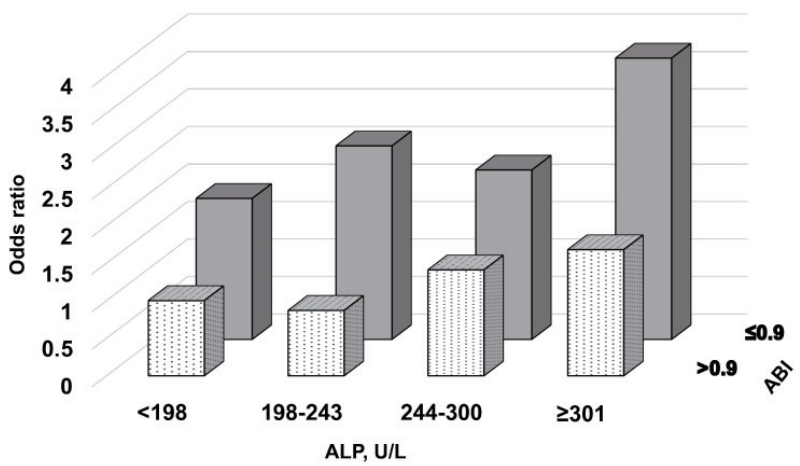

Fig. 2.

Odds ratio for a poor functional outcome in each group according to the ABI and quartiles of ALP levels adjusted for related indicators of a poor outcome (age, body mass index, history of stroke, aspartate aminotransferase levels, and National Institute Health Stroke Scale score at admission).

the brachial-ankle pulse wave velocity[7, 8]. Uehara *et al.* previously reported that a high serum ALP level at admission was a predictor of subsequent ischemic stroke events in patients with transient ischemic attack attributable to intracranial atherosclerosis[20] and that increased serum ALP levels at admission predicted the development of early neurological deterioration in patients with symptomatic intracranial atherosclerosis[21]. In addition, Cheung BM, *et al.* reported that elevated serum ALP levels correlated with PAD, independent of other cardiovascular risk factors, in the general Chinese population aged ≥ 40 years, but a low ABI was not significantly associated with the levels of other liver enzymes[22]. Interestingly, the relationship between serum ALP levels and a low ABI was significant among patients with AIS, especially those with CKD. Serum ALP levels may be a useful blood biological marker to detect the presence of PAD in patients with AIS.

PAD is a well-established prognostic factor for poor stroke outcomes in patients with AIS[3]. Increased ALP levels are also a risk factor for cardiovascular disease and stroke. In a community-based prospective cohort study of a Middle Eastern population,

Kabootari M, *et al.* showed that higher serum ALP levels were an independent risk factor for coronary heart disease, stroke, and all-cause mortality[9]. Numerous studies have documented that elevated serum ALP levels are associated with increased mortality in patients with CKD[23, 24]. A positive association between increased ALP levels and total mortality was also observed among patients with preserved renal function[25]. Zong *et al.* reported associations of high serum ALP levels with all-cause mortality, stroke recurrence, and poor functional outcomes in patients with stroke and preserved renal function (eGFR ≥ 60 mL/min/1.73 m^2)[26]. In the present study, increased ALP levels were associated with poor stroke outcomes, regardless of renal dysfunction. Kim *et al.* showed that a higher ALP level was an independent prognostic factor for poor functional outcomes at 3 months in patients with AIS[10], consistent with our results. Although these authors evaluated cerebral atherosclerosis, an association between ALP levels and cerebral atherosclerosis was not observed. In the present study, increased ALP levels and a low ABI were closely associated in patients with AIS, especially those with CKD. We found independent associations of high ALP levels and a low ABI with poor stroke outcome. High ALP levels are associated with increased mortality and cardiovascular events via mechanisms that involve vascular calcification, inflammation, and endothelial dysfunction[27]. A strong relationship between serum ALP levels and endothelial dysfunction, an independent predictor of cardiovascular events, was shown in a large cohort of hypertensive patients[28]. In the central nervous system, tissue-nonspecific alkaline phosphatase (TNAP) is expressed at high levels in brain endothelial cells. TNAP may play a role in neuroinflammation and vascular endothelial dysfunction in patients with stroke[29]. Endothelial dysfunction in PAD is also related to increases in plasma markers of inflammation[30]. Therefore, indicators such as elevated serum ALP levels and a low ABI might influence poor stroke outcomes through inflammation or endothelial dysfunction.

The present study had several limitations. First, because of our retrospective design, we excluded 1077 patients for various reasons, such as a lack of data on ALP levels, ABI, and functional outcomes 3 months after stroke onset. Because we focused on functional outcomes of stroke in our study, we excluded the participants with an mRS score of ≥ 3 before stroke onset. Therefore, our study may have exhibited selection bias. Second, serum ALP levels are potentially affected by age, medication use, liver function, smoking status, alcohol consumption, and liver and metabolic diseases[31]. Although we collected data on risk factors for ischemic stroke and liver enzymes, including AST, ALT, and γ-GTP, no data on liver or bone diseases were collected. In addition, we were unable to evaluate serum phosphate or 25-hydroxyvitamin D levels, which have been suggested to influence serum ALP levels. Third, because our study was a cross-sectional study, a causal relationship between ABI and ALP levels could not be proven. Third, we measured the total ALP activity, and thus, we were unable to evaluate which type of ALP was associated with a low ABI. However, most previous studies investigating the association between serum ALP levels and vascular diseases, including stroke and cardiovascular disease, have measured total ALP activity. Therefore, the results from our study in a large cohort of patients with AIS are clinically relevant.

Conclusions

In conclusion, increased serum ALP levels were significantly associated with a low ABI. In addition, these indicators were independent prognostic factors for a poor stroke outcome at 3 months. Therefore, increased serum ALP levels may be a useful tool for clinicians to determine the possibility of PAD and a predictor of a poor stroke outcome.

Conflict of Interest

Hirofumi Maruyama received grants from Daiichi Sankyo Co., Ltd.; these grants are unrelated to the submitted work.

All other authors have no conflicts of interest to declare.

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Supplemental Table 1. Comparison of included and excluded participants

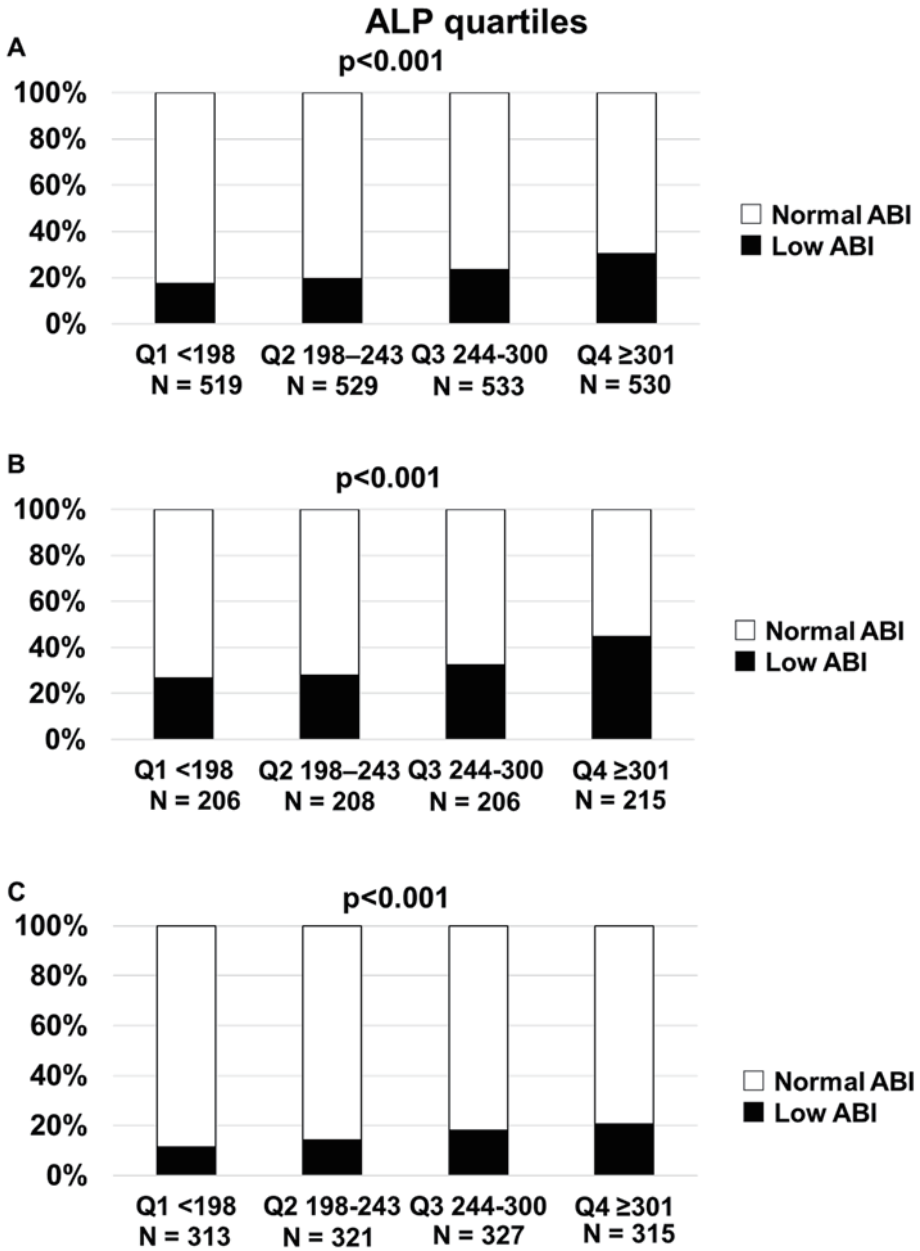
	Included (n=1322)	Excluded (n=1077)	p
Age, years	73.7 ± 11.2	76.3 ± 12.6	<0.001
Sex, male	836 (63.2)	553 (51.4)	<0.001
Body mass index, kg/m^2	23.3 ± 3.8	22.6 ± 3.6	<0.001
Daily alcohol intake	402 (30.6) (n=1313)	208 (19.8) (n=1050)	<0.001
Current smoking status	290 (22.1) (n=1314)	165 (15.7) (n=1050)	<0.001
Hypertension	930 (70.4)	725 (67.4) (n=1076)	0.118
Diabetes mellitus	467 (35.3)	325 (30.2) (n=1075)	0.008
Dyslipidemia	661 (50.0)	472 (44.0) (n=1073)	0.003
Chronic kidney disease	521 (39.4)	444 (41.2) (n=1075)	0.367
Atrial fibrillation	270 (20.4)	251 (23.4)	0.084
Malignancy	202 (15.3)	214 (19.9)	0.277
History of stroke	373 (28.2) (n=1321)	408 (37.9)	<0.001
History of ischemic heart disease	185 (14.0)	120 (11.1)	0.037
NIHSS score at admission	3 (1–6)	4 (2–14)	<0.001
Stroke subtype			0.004
Small-vessel occlusion	369 (27.9)	267 (24.8)	
Large-artery atherosclerosis	341 (25.8)	211 (19.6)	
Cardioembolic stroke	333 (25.2)	312 (29.0)	
Other etiology	279 (21.1)	287 (26.7)	

Data are presented as the means ± standard deviations for age and body mass index; as the medians (interquartile ranges) for each laboratory parameter and baseline NIHSS score; and as the number of patients (%) for other measures.
NIHSS, National Institute Health Stroke Scale.

Supplemental Table 2. Indicators associated with quartiles of ALP levels

	Q1 <198 (n=519)	Q2 198–243 (n=529)	Q3 244–300 (n=533)	Q4 ≥ 301 (n=530)	p
Age, years	73.5 ± 12.2	75.3 ± 12.1	75.8 ± 11.1	77.0 ± 10.7	<0.001
Sex, male	330 (63.6)	322 (60.9)	297 (55.7)	278 (52.5)	0.001
Body mass index, kg/m^2	23.4 ± 3.5	23.1 ± 3.8	23.0 ± 3.8	22.7 ± 3.7	0.025
Daily alcohol intake	167 (32.3) (n=517)	150 (28.6) (n=525)	127 (24.0) (n=530)	104 (19.7) (n=529)	<0.001
Current smoking status	98 (19.0) (n=517)	114 (21.7) (n=526)	106 (20.0) (n=530)	92 (17.4) (n=529)	0.353
Hypertension	365 (70.3)	355 (67.1)	374 (70.3) (n=532)	375 (70.8)	0.544
Diabetes mellitus	172 (33.1)	181 (34.2)	167 (31.3)	176 (33.2)	0.792
Dyslipidemia	260 (50.1)	244 (46.1)	272 (51.0)	230 (43.4)	0.046
Chronic kidney disease	206 (39.7)	208 (39.3)	206 (38.7)	215 (40.6)	0.935
Atrial fibrillation	104 (20.0)	102 (19.3)	108 (20.3) (n=532)	112 (21.1)	0.902
Malignancy	78 (15.0)	77 (14.6)	73 (13.7)	86 (16.2)	0.705
History of stroke	148 (28.6) (n=518)	160 (30.3)	198 (37.2)	202 (38.1)	<0.001
History of ischemic heart disease	69 (13.3)	61 (11.5)	67 (12.6)	69 (13.0)	0.835
Low ABI (≤ 0.9)	91 (17.5)	104 (19.7)	126 (23.6)	161 (30.4)	<0.001
ALT, U/L, median (IQR)	22 (18–27)	23 (18–28)	22 (19–28)	23 (19–31)	<0.001
AST, U/L, median (IQR)	16 (12–24)	17 (13–25)	17 (13–23)	18 (13–28)	0.004
γ-GTP, median (IQR)	24 (16–36)	23 (16–39)	25 (17–43)	27 (17–52)	<0.001
NIHSS score at admission	3 (1–6)	3 (2–7)	3 (1–9)	3 (1–9)	0.007
Stroke subtype					0.375
Small-vessel occlusion	150 (28.9)	152 (28.7)	157 (29.5)	142 (26.8)	
Large-artery atherosclerosis	115 (22.2)	147 (27.8)	117 (22.0)	138 (26.0)	
Cardioembolic stroke	132 (25.4)	121 (22.9)	147 (27.6)	134 (25.3)	
Other etiology	122 (23.5)	109 (20.6)	112 (21.0)	116 (21.9)	

Data are presented as the means ± standard deviations for age and body mass index; as the medians (interquartile ranges) for each laboratory parameter and baseline NIHSS score; and as the number of patients (%) for other measures.
ALP, alkaline phosphatase; ABI, ankle-brachial index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyl transferase; NIHSS, National Institute Health Stroke Scale.



Supplemental Fig. 1. Distribution of low ABI values according to the quartiles of serum ALP levels in the total cohort of patients (A) and patients with (B) and without (C) CKD

Significant differences in ABI and ALP distributions were observed between patients with and without CKD; open columns, normal ABI; black columns, low ABI.

Supplemental Table 3. Results of the univariable analysis to determine the factors associated with 3-month functional outcomes

	mRS 0–2 (n=888)	mRS 3–6 (n=434)	p
Age, years	71.3 ± 11.3	78.8 ± 9.2	<0.001
Sex, male	595 (67.0)	241 (55.5)	<0.001
Body mass index, kg/m^2	23.7 ± 3.7	22.6 ± 3.9	<0.001
Daily alcohol intake	291 (33.0) (n=882)	111 (25.8) (n=431)	0.008
Current smoking status	224 (25.4) (n=883)	66 (15.3) (n=431)	<0.001
Hypertension	617 (69.5)	313 (72.1)	0.324
Diabetes mellitus	319 (35.9)	148 (34.1)	0.515
Dyslipidemia	461 (51.9)	200 (46.1)	0.046
Chronic kidney disease	317 (35.7)	204 (47.0)	<0.001
Atrial fibrillation	142 (16.0)	128 (29.5)	<0.001
Malignancy	129 (14.5)	73 (16.8)	0.277
History of stroke	224 (25.3) (n=887)	149 (34.3)	<0.001
History of ischemic heart disease	116 (13.1)	69 (15.9)	0.163
Low ABI	109 (12.3)	139 (32.0)	<0.001
ALT, U/L, median (IQR)	18 (14–26)	16 (12–25)	0.425
AST, U/L, median (IQR)	22 (19–28)	24 (20–31)	<0.001
ALP, U/L, median (IQR)	233 (190–286)	256 (207–307)	<0.001
γ-GTP, median (IQR)	28 (18–46)	24 (17–42)	0.346
NIHSS score at admission	2 (1–4)	6 (3–14)	<0.001
Stroke subtype			<0.001
Small-vessel occlusion	268 (30.2)	101 (23.3)	
Large-artery atherosclerosis	237 (26.7)	104 (24.0)	
Cardioembolic stroke	186 (21.0)	147 (33.9)	
Other etiology	197 (22.2)	82 (18.9)	

Data are presented as the means ± standard deviations for age and body mass index; as the medians (interquartile ranges) for each laboratory parameter and baseline NIHSS score; and as the number of patients (%) for other measures.
mRS, modified Rankin Scale; ABI, ankle-brachial index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; γ-GTP, γ-glutamyl transferase; NIHSS, National Institute Health Stroke Scale.

Supplemental Table 4. Odds ratios for a poor outcome in groups categorized by ABI and quartiles of ALP levels

ALP (U/L)	ABI >0.9	ABI ≤ 0.9
<198	Group 1 (n=272) 1.0 (reference)	Group 5 (n=54) 1.88 (0.90–3.91)
198–243	Group 2 (n=289) 0.87 (0.54–1.40)	Group 6 (n=45) 2.58 (1.11–5.97)
244–300	Group 3 (n=262) 1.41 (0.88–2.25)	Group 7 (n=64) 2.26 (1.06–4.83)
≥ 301	Group 4 (n=251) 1.68 (1.04–2.69)	Group 8 (n=85) 3.75 (1.96–7.20)

Odds ratios (95% CIs) were calculated with a multivariable logistic regression analysis adjusted for related indicators of poor outcomes (age, body mass index, history of stroke, aspartate aminotransferase levels, and National Institute Health Stroke Scale score at admission).
ABI, ankle-brachial index; ALP, alkaline phosphatase.