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Associations between alkaline phosphatase and hypertension in relation to circulating CD34-positive cell levels pertaining to elderly Japanese men

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Serum alkaline phosphatase (ALP) could be an indicator of osteoblastic activity, which initiates hematopoietic stem cell (CD34-positive cell) production in bone marrow. Since chronic inflammation, which is a known risk factor for hypertension and endothelium dysfunction, stimulates bone marrow activity, ALP could be positively associated with hypertension. To clarify those associations, we conducted a cross-sectional study of 479 elderly Japanese men aged 60-69. Circulating CD34-positive cell levels could influence associations between serum ALP and hypertension because CD34-positive cell production is also a factor known to contribute to endothelial repair. Therefore, participants were stratified by the median value of circulating CD34-positive cell levels (1.00 cells/μL). A low level of circulating CD34-positive cells was identified in 240 members of the study population. A significantly positive association of ALP with hypertension was detected among participants with low circulating CD34-positive cell levels (multivariable-OR (odds ratio) for hypertension resulting from a 1 standard deviation (SD) increment in serum ALP (58.3 IU/L) = 1.44 (1.06, 1.95)) but not among those with high CD34-positive cell levels (multivariable-OR=0.91 (0.67, 1.23)). We also observed a significant effect of the interaction of circulating CD34-positive cell levels on the association between serum ALP and hypertension (multivariable p=0.011). Serum ALP was found to be positively associated with hypertension among elderly participants with low but not with high circulating CD34-positive cell levels. Since CD34-positive cells are a factor known to contribute to endothelial repair, capability for endothelial repair can be expected to have an effect on the association between serum ALP and hypertension.

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Introduction

Approximately 95% of the alkaline phosphatase (ALP) in serum from healthy adults is derived from bone and liver equally [1, 2]. Because ALP is primarily secreted by the bone and the liver [2], ALP is generally recognized as a marker of bony or hepatic disease even if only a small amount is secreted by intestine, kidneys, and leukocytes. ALP could therefore act as an early differentiation marker of osteoblast and osteoblastic activity [3].

Since osteoblasts regulate the production of hematopoietic stem cells in bone marrow [4,5] and chronic inflammation also should stimulate such bone marrow activity [6-8], ALP could be expected to be associated with a chronic inflammatory

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condition, which constitutes an important risk factor for hypertension and endothelium dysfunction.

Furthermore, bone marrow-derived endothelial progenitor cells such as CD34-positive cells have been reported to play an important role in maintaining the vascular endothelium [9,10]. Therefore, serum ALP levels may correlate with vascular homeostatic activity which is stimulated by endothelial injury. This indicates that participants with higher levels of circulating CD34-positive cells might be more capable of maintaining the vascular endothelium than those with lower levels. In addition, it is well known that hypertension and endothelial dysfunction (atherosclerosis) are subject to a vicious cycle, in which hypertension induces endothelial dysfunction and vice versa [11-13]. Therefore, higher CD34-positive cell levels might act as an influential factor on the association between ALP and hypertension.

To clarify this and other relevant associations, we conducted a cross-sectional study of 479 elderly Japanese community dwelling men aged 60-69 who underwent an annual health check-up in one of 2013-2015.

Methods

Study population

All procedures involving human participants were performed in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the Ethics Committee of Nagasaki University Graduate School of Biomedical Sciences (project registration number: 14051404). Written consent forms were available in Japanese to ensure a comprehensive understanding of the study objectives, and informed consent was provided by all the participants.

The source population comprised 617 male residents 60 to 69 years old of Goto City and Saza Town, rural communities in western Japan, who participated in this study between 2013 and 2015. Participants for whom laboratory data were not available (n=33) were excluded. To avoid the influence of acute inflammatory disease, participants with a high white blood cell count (WBC) (≥10,000 cells/μL) (n=8) were excluded. Since chronic kidney disease (CKD) might act as a strong confounding factor on the analyses [14], participants with CKD (Glomerular Filtration Rate (GFR) <60 ml/min/1.73m^2) (n=92) were also excluded. To avoid the effect of bone disorders such as osteoporosis, bone fracture, and bone tumor, we further excluded persons with a high serum ALP level (>400 IU/L) (n=5) as in a previous study of ours [15]. The remaining 479 men with a mean age of 65.4 years (standard deviation (SD): ±2.6; range: 60-69) were enrolled in the study.

Data Collection and Laboratory Measurements

Trained interviewers obtained information on medical history, as well as use of antihypertensive agents. Body weight and height were measured with an automatic body composition analyzer (BF-220; Tanita, Tokyo, Japan). Systolic and diastolic blood pressures were recorded at rest. Hypertension was defined as systolic blood pressure ≥140mmHg and/or diastolic blood pressure ≥90mmHg and/or use of antihypertensive medication as in a previous study which dealt with the association between ALP and hypertension [16].

Fasting blood samples were collected in an EDTA-2K tube, a heparin sodium tube and a siliconized tube. The number of WBC (cell/μL) in samples from the EDTA-2K tube were measured at SRL, Inc. (Tokyo, Japan) with an automated procedure.

Fresh samples (obtained within 24 hours of collection from the heparin sodium tube) were used to determine the number of CD34-positive cells. BD Trucount™ (Beckton Dickinson Biosciences, San Jose, CA, USA) technology, an accurate and reproducible single platform assay which conformed with the International Society of Hematotherapy and Graft Engineering (ISHAGE) guidelines [17,18] and supported by automated software for the BD FACSCanto™ II system, was used to measure the number of circulating CD34-positive cells.

Serum samples were separated to measure the concentration of aspartate aminotransferase (AST) and γ-glutamyltranspeptidase (γ-GTP) by using the Japanese Society of Clinical Chemistry (JSCC) standardization method. Triglycerides (TG) and creatinine were measured enzymatically. HDL-cholesterol (HDLc) was measured by using the direct method, and hemoglobin A1c (HbA1c) by using the latex coagulation method. Glomerular filtration rate (GFR) was estimated by means of an established method using three adaptations which were recently proposed by the working group of the Japanese Chronic Kidney Disease Initiative [19]. According to this adapted version, GFR (mL/min/1.73m^2) = 194 × (serum creatinine (enzyme method))$^{-1.094}$ × (age)$^{-0.287}$.

Measurement of carotid intima media thickness (CIMT) was determined by ultrasonography of the left and right carotid arteries by an experienced vascular technician using a LOGIQ Book XP with a 10-MHz transducer (GE Healthcare, Milwaukee, WI, USA). Mean values for the left and right CIMT were calculated using automated digital edge-detection software (Intimascope; MediaCross, Tokyo, Japan) and a

protocol that has been described in detail elsewhere [20].

Brachial-ankle pulse wave velocity (PWV) is generally used to evaluate arterial stiffness. However, PWV measurements can be strongly affected by blood pressure [21], so that the Cardio Ankle Vascular Index (CAVI) was recently developed in Japan to avoid the susceptibility of PWV measurements to blood pressure [22]. CAVI was recorded with a Vasera VS-1000 vascular screening system (Fukuda Denshi, Tokyo, Japan) with the subject resting in a supine position.

Statistical Analysis

Characteristics of the study population stratified by circulating CD34-levels divided by median value (1.00 cells/μL) were expressed as mean ± standard deviation. A χ^2 test was performed to calculate the p value of each variable based on circulating CD34-positive cell levels.

Simple correlation analysis of ALP with relevant factors was performed to calculate circulating CD34-positive cell levels. Because TG showed a skewed distribution, logarithmic transformation was performed.

Logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (CI) of hypertension associated with ALP levels. Two different approaches were used to make adjustments for confounding factors. The first adjustment was only for age, and the second consisted of other possible confounding factors, that is, BMI (kg/m^2), HDLc (mg/dL), TG (mg/dL), HbA1c (%), AST (U/L), γ-GTP (U/L), GFR (mL/min/1.73m^2) and WBC (cell/μL).

Data for CAVI were available for 212 participants. Since ALP levels might affect endothelial activation [16], we used simple correlation analysis to calculate circulating CD34-positive cell level-specific associations between ALP and CAVI.

For sensitivity analysis, we rerun models without excluding CKD participants.

All statistical analyses were performed with the SAS system for Windows (version 9.4: SAS Inc., Cary, NC). Values of $p<0.05$ were regarded as being statistically significant.

Results

Of the total study population of 479, 240 showed low CD34-positive cell levels (<1.00 cells/μL).

Table 1 shows characteristics of the study population stratified by CD34-positive cell levels. Those with high CD34-positive cell levels showed significantly higher BMI, HbA1c and WBC than those with low levels.

Results of a circulating CD34-positive cell level-specific simple correlation analysis of ALP and other variables are shown in Table 2. For subjects with low CD34-positive cell levels, ALP was significantly positively associated with systolic blood pressure, diastolic blood pressure, anti-hypertensive medication use and WBC, and significantly inversely associated with HDLc. For those with high CD34-positive cell levels, ALP was significantly positively associated with TG and WBC, and significantly inversely associated with BMI.

Table 3 shows ORs and 95%CIs for hypertension in

Table 1. Characteristics of study population by circulating CD34-positive cell levels

	Low CD34-positive cell levels (<1.00 cells/μL)	High CD34-positive cell levels (≥1.00 cells/μL)	p
No. at risk	240	239	
Age, years	65.5 ± 2.5	65.3 ± 2.7	0.337
Systolic blood pressure, mmHg	134 ± 18	135 ± 17	0.643
Diastolic blood pressure, mmHg	81 ± 12	81 ± 11	0.845
Antihypertensive medication use, %	38.3	46.4	0.073
Body mass index (BMI), kg/m^2	22.7 ± 3.0	23.9 ± 2.8	<0.001
Serum HDL-cholesterol (HDLc), mg/dL	58 ± 13	57 ± 14	0.556
Serum triglycerides (TG), mg/dL	109 ± 88	125 ± 96	0.064
Hemoglobin A1c (HbA1c), %	5.6 ± 0.5	5.8 ± 0.7	<0.001
Serum aspartate aminotransferase (AST), U/L	25 ± 9	25 ± 8	0.422
Serum γ-glutamyltranspeptidase (γ-GTP), U/L	46 ± 40	49 ± 53	0.424
Serum creatinine, mg/dL	0.88 ± 0.11	0.79 ± 0.10	0.464
Glomerular Filtration Rate (GFR), mL/min/1.73m^2	76.5 ± 13.0	76.9 ± 11.1	0.726
White blood cell (WBC), cell/μL	5003 ± 1255	6068 ± 1289	<0.001
Serum alkaline phosphatase (ALP), U/L	224 ± 59	217 ± 58	0.204
Mean carotid intima-media thickness (CIMT), mm	0.67 ± 0.11	0.68 ± 0.11	0.246

Values: mean ± standard deviation.

Table 2. Simple correlation analysis of alkaline phosphatase (ALP) and other variables in relation to circulating CD34-positive cell levels

	Low CD34-positive cell levels (<1.00 cells/μL)		High CD34-positive cell levels (≥1.00 cells/μL)	
	r	p	r	p
No. at risk	240		239	
Age	0.03	0.689	-0.003	0.965
Systolic blood pressure	0.13	0.040	0.02	0.755
Diastolic blood pressure	0.15	0.019	0.03	0.634
Antihypertensive medication use	0.14	0.033	-0.03	0.633
Body mass index (BMI)	-0.04	0.517	-0.14	0.027
Serum HDL-cholesterol (HDLc)	-0.15	0.019	-0.04	0.583
Serum triglycerides (TG)	0.04	0.563	0.26	<0.001
Hemoglobin A1c (HbA1c)	0.01	0.855	0.08	0.197
Serum aspartate aminotransferase (AST)	0.12	0.055	0.12	0.067
Serum γ-glutamyltranspeptidase (γ-GTP)	0.07	0.272	0.12	0.067
Serum creatinine	-0.08	0.190	-0.04	0.536
Glomerular Filtration Rate (GFR)	0.09	0.167	0.05	0.471
White blood cell (WBC)	0.25	<0.001	0.15	0.023
Mean carotid intima-media thickness (CIMT)	0.02	0.808	0.005	0.938

TG was calculated as logarithmic values.

Table 3. Odds ratios (ORs) and 95% confidence intervals (CIs) for hypertension in relation to alkaline phosphatase (ALP) stratified by circulating CD34-positive cell levels

	Alkaline phosphatase (ALP) quartiles				p for trend	1 SD increment in ALP (58.3 U/L)
	Q1 (low)	Q2	Q3	Q4 (high)		
Low CD34-positive cell levels (<1.00 cells/μL)						
No. of participants	58	55	64	63		
No. of cases (%)	29 (50.0)	30 (54.5)	38 (59.4)	46 (73.0)		
Age-adjusted ORs	1.00	1.23 (0.59, 2.60)	1.43 (0.70, 2.95)	2.65 (1.24, 5.68)	0.013	1.41 (1.07, 1.85)
Multivariable ORs	1.00	1.05 (0.47, 2.33)	1.35 (0.61, 2.97)	2.66 (1.15, 6.16)	0.021	1.44 (1.06, 1.95)
High CD34-positive cell levels (≥1.00 cells/μL)						
No. of participants	63	62	56	58		
No. of cases (%)	41 (65.1)	45 (72.6)	33 (58.9)	36 (62.1)		
Age-adjusted ORs	1.00	1.42 (0.66, 3.05)	0.75 (0.35, 1.58)	0.86 (0.41, 1.82)	0.384	0.86 (0.66, 1.13)
Multivariable ORs	1.00	1.33 (0.59, 2.97)	0.78 (0.36, 1.70)	0.96 (0.42, 2.20)	0.623	0.91 (0.67,1.23)

Hypertension was defined as systolic blood pressure ≥140mmHg, and/or diastolic blood pressure ≥90mmHg, and/or antihypertensive medication use. Multivariable ORs: adjusted further for age and BMI, HDLc, TG, HbA1C, AST, γ-GTP, GFR and WBC. BMI: body mass index. HDLc: HDL-cholesterol. TG: triglycerides. HbA1c: hemoglobin A1c. AST: aspartate aminotransferase. γ-GTP: γ-glutamyltranspeptidase. WBC: white blood cells. ALP quartiles : <179 U/L for Q1, 179-214 U/L for Q2, 215-255 U/L for Q3, and ≥256 U/L for Q4

relation to ALP stratified by CD34-positive cell levels. For low levels, ALP was significantly positively associated with hypertension whereas no significant such association was observed for high levels.

We also evaluated the effect of the interaction between ALP and CD34-positive cell levels (both low and high) on hypertension. Significant interaction between ALP and CD34-positive cell levels was observed, with a p value for the effect of this fully adjusted interaction on hypertension of p=0.011.

Since the same kinds of anti-hypertensive medication might influence the osteoblastic stem cells and hematopoietic stem cells, which in turn might influence the circulating CD34-positive cell level-specific association between ALP and hypertension [23,24], we performed further analysis limited to the participants not taking anti-hypertensive medication. Essentially the same associations were observed even though the statistical power of these associations did not reach significant values. The multivariable ORs of hypertension for an increment of 1 standard deviation (SD) were 1.44 (0.97, 2.14) for subjects with low circulating CD34-positive cell levels (n=148) and 0.80 (0.53, 1.23) for

those with high levels (n=128). As for the interaction, the multivariable model showed a significant value (p=0.028).

A further analysis was performed to evaluate the influence of ALP on endothelial function of the 212 participants (116 for low and 96 for high circulating CD34-positive cell levels) for whom CAVI data were available. The results of a simple correlation analysis showed that, for participants with low circulating CD34-positive cell levels, ALP was significantly positively associated with CAVI (r=0.25, p=0.007), but not for those with high circulating CD34-positive cell levels (r=0.04, p=0.730).

Sensitivity analysis showed similar associations to the main results.

Discussion

The major findings of the present study of elderly Japanese men are that serum ALP was associated with hypertension for participants with low circulating CD34-positive cell levels, but not for those with high levels.

Previously, we reported that serum ALP is associated with hypertension for both male and female non-drinkers, but not for drinkers [16]. This finding indicates drinker status might be a strong confounding factor on those associations because serum ALP levels are influenced by alcohol consumption [25,26], which has been positively associated with hypertension [27], while alcohol consumption also influences endothelial function [28,29]. In the current study we found further evidence that the positive association between ALP and hypertension is observed only for subjects with low circulating CD34-positive cell levels.

Since ALP shares common biological pathways with C-reactive protein (CRP), a marker of inflammation [30-32], ALP could also function as an inflammatory mediator. And since CRP is known to be positively associated with hypertension [33], ALP could be positively associated with hypertension by indicating the activity of inflammation. WBC, which is also well known as an inflammatory maker, is also reported to be positively associated with hypertension [34]. In addition, our present study found that ALP is significantly positively associated with WBC in participants both with low and with high levels of CD34-positive cells, a finding which partly supports the notion of a mechanism showing that inflammation could be attributed to the positive association between ALP and hypertension. However, our study also found that the significant positive association between ALP and hypertension remained significant even after further adjustment for WBC.

Alkaline phosphatase (ALP) is a membrane homo-dimeric enzyme that catalyzes the hydrolysis of organic pyrophosphate [35]. ALP has been found to contribute to vascular calcification, which is one aspect of atherosclerosis, by catalyzing the hydrolysis of organic pyrophosphate, an inhibitor of vascular calcification [36]. In addition, it is well known that hypertension and endothelial dysfunction (atherosclerosis) are subject to a vicious cycle [11-13]. Therefore, ALP could be positively associated with hypertension thus indicating the presence of atherosclerosis. Serum ALP is reported to be adversely associated with levels of arterial structure and function in hypertensive African men [37]. Another study found a strong and significant inverse relationship between alkaline phosphatase and endothelium-dependent vasodilation among naïve hypertensive patients [38]. The findings of those studies support our results presented here. However, we found no significant associations between ALP and CIMT among participants with either low or high levels of CD34-positive cells, even though a positive association between ALP and CAVI was observed only for participants with low CD34-positive cell levels.

Triglycerides are positively associated with intestinal ALP [39] and ALP is also reported to be positively associated with triglyceride [16]. The positive association between ALP and hypertension could also be attributed to triglycerides since they have been reported to be positively associated with blood pressure (both systolic and diastolic) for subjects with low but not high CD34-positive cell levels [40]. However, in our study a significant positive association between ALP and triglycerides was observed only for participants with high CD34-positive cell levels.

Finally, ALP is an early differentiation marker of osteoblast and osteoblastic activity [3]. Since osteoblasts regulate the production of hematopoietic stem cells in bone marrow [4,5] and bone marrow-derived endothelial progenitor cells such as CD34-positive cells have been reported to play an important role in maintaining the vascular endothelium [9,10], serum ALP levels may correlate with vascular homeostatic activity including inflammatory activity. Therefore, an increase in the level of circulating CD34-positive cells should have a beneficial effect by preventing endothelial dysfunction in cases without a significant association between ALP and hypertension because of the aforementioned vicious cycle between hypertension and endothelial dysfunction [11-13].

This study has certain potential limitations, which warrant consideration. First, because ALP isoenzymes were not measured [41], we could not assess which type of ALP was associated with the risk of hypertension. Although the positive association between ALP levels and hypertension

was shown to be independent of the traditional risk factors, we did not adjust for other potential confounders whose values have been associated with ALP, such as calorie, protein, vitamin C, magnesium, and zinc deficiencies [42], and alcohol consumption status. Additionally, because this was a cross-sectional study, causal relationships could not be established.

Conclusion

In conclusion, our findings show that serum ALP is associated with hypertension for participants with low circulating CD34-positive cell levels, but not for those with high levels. For analyses of associations between ALP and hypertension, circulating CD34-positive cell levels, which might influence the capability of endothelium maintenance, should thus be considered a potential influencer.

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References

1. Magnusson P, Degerblad M, Sääf M, et al: Different responses of bone alkaline phosphatase isoforms during recombinant insulin-like growth factor-I (IGF-I) and during growth hormone therapy in adults with growth hormone deficiency. J Bone Miner Res 1997;12:210-220.
2. Magnusson P, Sharp CA, Magnusson M, et al: Effect of chronic renal failure on bone turnover and bone alkaline phosphatase isoforms. Kidney Int 2001;60:257-265.
3. Bronckers AL, Gay S, Finkelman RD, et al: Developmental appearance of Gla proteins (osteocalcin) and alkaline phosphatase in tooth germs and bones of the rat. Bone Miner 1987:361-373.
4. Calvi L.M, Adams G.B, Weibrecht K.W, et al: Osteoblastic cells regulate the haematopoietic stem cell niche. Nature 2003;425:841-846.
5. Zhang J, Niu C, Ye L, et al: Identification of the haematopoietic stem cell niche and control of the niche size. Nature 2003;425:836-841.
6. Ueda Y, Kondo M, Kelsoe G: Inflammation and the reciprocal production of granulocytes and lymphocytes in bone marrow. J Exp Med 2005;201: 1771-1780.
7. Nagai Y, Garrett K.P, Ohta S, et al: Toll-like receptors on hematopoietic progenitor cells stimulate innate immune system replenishment. Immunity 2006;24:801-812.
8. Omatsu Y, Seike M, Sugiyama T, et al: Foxc1 is a critical regulator of haematopoietic stem/progenitor cell niche formation. Nature 2014; 508:536-540.
9. Asahara T, Murohara T, Sullivan A, et al: Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964-967.
10. Takahashi T, Kalka C, Masuda H, et al: Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. Nat Med 1999;5:434-438.
11. Dohi Y: Hypertension and Oxidative stress. Nagoya Med J 2010;51:153-158. [Article in Japanese]
12. Higashi Y, Sueda T, Yoshizumi M: Role of Angiotensin II-related oxidative stress and nitric oxide in endothelial function in hypertension. J Jpn Coll Angiol 2003;43:47-52. [Article in Japanese]
13. Nakamura S, Moriguchi A, Morishita R, et al: A novel vascular modulator, hepatocyte growth factor (HGF), as a potential index of the severity of hypertension. Biochem Biophys Res Commun 1998; 242: 238-243.
14. Beddhu S, Ma X, Baird B, et al: Serum alkaline phosphatase and mortality in African Americans with chronic kidney disease. Clin J Am Soc Nephrol 2009;4:1805-1810.
15. Shimizu Y, Sato S, Koyamatsu J, et al: Association between circulating CD34-positive cells and serum alkaline phosphatase in relation to body mass index for elderly Japanese men. J Physiol Anthropol 2016;35:2.
16. Shimizu Y, Nakazato M, Sekita T, et al: Association between alkaline phosphatase and hypertension in a rural Japanese population: the Nagasaki Islands study. J Physiol Anthropol 2013;32:10.
17. Sutherland DR, Anderson L, Keeney M, et al: The ISHAGE guidelines for CD34+ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. J Hematother 1996;5:213-226.
18. Barnett D, Janossy G, Lubenko A, et al: Guideline for the flow cytometric enumeration of CD34+ haematopoietic stem cells. Prepared by the CD34+ haematopoietic stem cell working party. General Haematology Task Force of the British Committee for Standards in Haematology. Clin Lab Haematol 1999;21:301-308.
19. Imai E, Horio M, Watanabe T, et al: Prevalence of chronic kidney disease in the Japanese general population. Clin Exp Nephrol 2009; 13:621-630.
20. Hara T, Takamura N, Akashi S, et al: Evaluation of clinical markers of atherosclerosis in young and elderly Japanese adults. Clin Chem Lab Med 2006;44:824-829.
21. Shirai K, Utino J, Otsuka K, et al: A novel blood pressure-independent arterial wall stiffness parameter; cardio-ankle vascular index (CAVI). J Atheroscler Thromb 2006;13:101-107.
22. Yambe T, Yoshizawa M, Saijo Y, et al: Brachio-ankle pulse wave velocity and cardio-ankle vascular index (CAVI). Biomed Pharmacother 2004;58:S95-98.
23. Choi Y.J, Lee J.Y, Lee S.J, et al: Alpha-adrenergic blocker mediated osteoblastic stem cell differentiation. Biochem Biophys Res Commun 2011;416:232-238.
24. Azizi M, Rousseau A, Ezan E, et al: Acute angiotensin-converting enzyme inhibition increases the plasma level of the natural stem cell regulator N-acetyl-seryl-aspartyl-lysyl-proline. J clin Invest 1996;97: 839-844.
25. Tolstrup J.S, Grønbaek M, Tybjaerg H.A, et al: Alcohol intake, alcohol dehydrogenase genotypes, and liver damage and disease in the Danish general population. Am J Gastroenterol 2009;104:2182-2188.
26. Osaretin A.T.E, Chioma L.A: Gender and alcohol consumption affect human serum enzymes, protein and bilirubin. Asian J Biochem 2007; 2:330-336.
27. Clark L.T. Alcohol-induced hypertension: mechanisms, complications, and clinical implications. J Natl Med Assoc 1985;77:385-389.
28. van Bussel B.C.T, Henry R.M.A, Schalkwijk C.G, et al: Alcohol and red wine consumption, but not fruit, vegetables, fish or dairy products, are associated with less endothelial dysfunction and less low-grade inflammation: the Hoorn Study. Eur J Nutr 2018;57:1409-1419.
29. Oda N, Kajikawa M, Maruhashi T, et al: Endothelial function is impaired in relation to alcohol intake even in the case of light alcohol consumption in Asian men; Flow-mediated Dilation Japan (FMD-J) Study. Int J Cardiol 2017;230:523-528.
30. Webber M, Krishnan A, Thomas N.G, et al: Association between serum

alkaline phosphatase and C-reactive protein in the United States National Health and Nutrition Examination Survey 2005-2006. Clin Chem Lab Med 2010;48:167-173.
31. Cheung B.M.Y, Ong K.L, Cheung R.V, et al: Association between plasma alkaline phosphatase and C-reactive protein in Hong Kong Chinese. Clin Chem Lab Med 2008;46:523-527.
32. Kerner A, Avizohar O, Sella R, et al: Association between elevated liver enzymes and C-reactive protein: possible hepatic contribution to systemic inflammation in the metabolic syndrome. Arterioscler Thromb Vasc Biol 2005;25:193-197.
33. Hage F.G: C-reactive protein and hypertension. J Hum Hypertens 2014;28:410-415.
34. Shimizu Y, Yoshimine H, Nagayoshi M, et al: Short stature is an inflammatory disadvantage among middle-aged Japanese men. Environ Health Prev Med 2016;21:361-367.
35. Lomashvili K.A, Garg P, Narisawa S, et al: Upregulation of alkaline phosphatase and pyrophosphate hydrolysis: potential mechanism for uremic vascular calcification. Kidney Int 2008;73:1024-1030.
36. Harmey D, Hessle L, Narisawa S, et al: Concerted regulation of inorganic pyrophosphate and osteopontin by akp2, enpp1, and ank: an integrated model of the pathogenesis of mineralization disorders. Am J Pathol 2004;164:1199-1209.
37. Schutte R, Huisman H.W, Malan L, et al: Alkaline phosphatase and arterial structure and function in hypertensive African men: The SABPA study. Int J Cardiol 2013;167:1995-2001.
38. Perticone F, Perticone M, Maio R, et al: Serum alkaline phosphatase negatively affects endothelium-dependent vasodilation in naïve hypertensive patients. Hypertension 2015;66:874-880.
39. Kikuno A, Nakano T, Matsushita M, et al: Elevated post-prandial triglyceride concentrations accord to the activity of intestinal alkaline phosphatase isozyme in human-concerning with the ABO blood group secretor and their non-secretor-. J Saitama Medical University 2010; 36:119-124. [Article in Japanese]
40. Shimizu Y, Sato S, Noguchi Y, et al: Triglycerides and blood pressure in relation to circulating CD34-positive cell levels among community-dwelling elderly Japanese men: a cross-sectinal study. Environ Health Prev Med 2017;22:77.
41. Wilkinson J.H: Clinical applications of isoenzymes. Clin Chem 1970; 16:733-739.
42. Lum G: Significance of low serum alkaline phosphatase activity in a predominantly adult male population. Clin Chem 1995;41:515-518.