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Original Article

Clinical Application of the Ratio of Serum Bone Isoform to Total Alkaline Phosphatase in General Practice

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Alkaline phosphatase (ALP) is an enzyme that is expressed in a variety of tissues. Among the isoforms of ALP, bone-specific alkaline phosphatase (BAP) is used as a marker for evaluating bone metabolism. We investigated the clinical usefulness of the ratio of serum BAP to total ALP for the diagnosis of various disorders in general practice. We retrospectively analyzed the cases of 107 Japanese patients whose serum BAP levels were examined, focusing on clinical characteristics. We observed that the BAP/ALP ratios of the patients with fever and those with inflammatory diseases were significantly lower than the ratios of other patient groups. The BAP/ALP ratios of the patients with osteoporosis and those with metabolic bone diseases were higher than those of the patients with other conditions. The BAP/ALP ratio was found to be negatively correlated with age, a correlation that has not been found in other ethnicities. The serum BAP/ALP ratio was inversely correlated with serum CRP levels but was positively correlated with serum albumin levels and hemoglobin concentrations. Collectively, our results suggest that the BAP/ALP ratio could be a useful predictor for important geriatric conditions seen in general practice.

Key words: alkaline phosphatase, BAP, CRP, inflammation, osteoporosis

Alkaline phosphatase (ALP) is an enzyme that catalyzes the hydrolysis of monophosphate esters under alkaline pH conditions [1]. ALP is expressed in various tissues. The concentrations of ALP are highest in the bone and liver, and they are also high in tissues of the intestines, kidney, testes, and syncytiotrophoblasts [2]. Human ALP is classified into four isozymes: tissue-nonspecific isozyme ALP (TNALP, *i.e.*, bone-, liver- and kidney-specific ALP), intestinal-type ALP (IALP), placental-type ALP, and placental-like ALP [2,3].

Serum ALP levels are affected by various diseases, but in some cases, the sources of ALP can be characterized by their isozymes. In clinical practice, the serum level of ALP is used mostly as a marker for bone and hepatic diseases [4]. Serum ALP levels are increased not only in hepatic diseases such as cirrhosis and chronic hepatitis but also in the bilious disorders such as choledocholithiasis and sclerosing cholangitis [3]. Serum ALP levels also tend to be increased in embryonic and testicular diseases such as ovarian and testicular cancers [2].

Bone-specific alkaline phosphatase (BAP) levels are greatly affected by humoral factors that have effects on bone formation and resorption [5,6]. The turnover of

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bone metabolism is regulated by various factors including parathyroid hormone (PTH), vitamin D, and sex steroids [7]. Recent studies have further suggested an association between the serum ALP levels and the risk of cardiovascular or renal damage [8, 9].

There have been several studies in which the relationships between serum levels of ALP and diseases of tissues related to the isozymes have been analyzed. However, there has been no study evaluating the relevance of serum ALP levels to a variety of chief complaints, general conditions, and/or co-existing complications. In the present study, we investigated the comparative levels of the bone isoform of serum ALP to determine the clinical usefulness of BAP and its application for the differential diagnoses of various disorders seen in general practice.

Patients and Methods

Patients. We retrospectively analyzed the medical records of 107 patients whose serum BAP and ALP levels were examined at the Department of General Medicine, Okayama University Hospital during the period from January 2016 to September 2019. The patients were 45 males and 62 females with a mean±SD age of 58.5 ± 17.5 years (range, 18-94 years).

We classified the patients into the following six groups based on their chief complaints: (1) the symptom-free group, (2) the pain group (including patients with somatic pain such as headache, myalgia, lumbago, arthralgia, and various types of visceral pain), (3) the psychological group (including patients with depression, sleep disorder, mood disorder, or anorexia), (4) the fever group (patients with body temperature >37.5°C), (5) the weight gain group (including obese patients and patients with edema), and (6) the urinary symptom group (including patients with pollakisuria, urinary discomfort, or incontinence). The patients were also classified into 6 groups based on their primary diseases: (1) metabolic bone diseases, (2) nephrological and endocrine diseases, (3) primary osteoporosis, (4) inflammatory diseases, (5) hematologic and oncologic diseases, and (6) nervous system and psychological diseases. The patients' chief complaints and the primary diseases were assessed from electronic medical records by 2 or 3 of the study's authors.

In another study, all of the patients were classified into 3 groups depending on their febrile symptoms as follows: fever, fever-free, and symptom-free groups. They were also classified into 2 groups based on the levels of inflammatory markers: high erythrocyte sedimentation rate (ESR; >15 mm) versus low ESR groups (≤15 mm) and high C-reactive protein (CRP; >0.15 mg/dl) versus low CRP groups (≤0.15 mg/dl). The patients were also divided into groups according to whether they had the following complications: hypertension, dyslipidemia, diabetes mellitus, and osteoporosis.

The study protocol was approved by the Institutional Review Board (IRB) of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences (#K1907-018).

Laboratory examinations. Each patient's ESR and serum levels of BAP, ALP, CRP, albumin, and hemoglobin had been determined by an auto-analyzer system in the Central Laboratory of Okayama University Hospital. Serum ALP levels (U/l) were determined according to the standard method of the Japan Society of Clinical Chemistry (JSCC), and serum BAP levels (μg/l) were measured using the chemiluminescent enzyme immunoassay (CLEIA) method. Serum BAP levels (μg/l) measured by the CLEIA method were converted to enzyme units (U/l) using the following formula with a correlation coefficient of 0.962 [10]: BAP (U/l) = (BAP (μg/l) + 2.265)/0.733. The serum CRP levels (mg/dl) were determined by a latex-agglutination method using latex particles conjugated with anti-CRP antiserum, and the normal range was ≤0.15 mg/dl. The femoral young adult mean (YAM) was calculated using the patient's femoral bone mineral density, which was measured by a DXA system called Horizon-type A (140/100 kVp, 2.5 mA, 82 sec; Hologic, Marlborough, MA, USA) at Okayama University Hospital [11].

Statistical analysis. Results are shown as the mean±SEM of data. The Steel-Dwass test, one-way analysis of variance (ANOVA), and Mann-Whitney *U*-test were used to determine significant differences between pairs of groups. If differences were detected by the one-way ANOVA, the Tukey post-hoc test was used to determine which means differed. Spearman's rank correlation coefficients were also used to determine inter-relationships between parameters. *P*-values < 0.05 were accepted as significant. All statistical analyses were performed with EZR, which is a graphical user interface for R [12].

Results

Patients' characteristics. All of the patients were first categorized by their symptoms (one chief complaint per patient). The number of patients was largest in the symptom-free group (n = 33, 30.8%) followed by the pain group (n = 32, 29.9%), the psychological group (n = 20, 18.7%), the symptoms related to fever group (n = 8, 7.5%), the weight gain group (n = 7, 6.5%), and the urinary symptom group (n = 7, 6.5%). The percentages of females in the six symptom-based groups (53.1-70.0%) were slightly higher than the percentages of males.

The patients were also classified by disease-based categories (one major disease per patient). The number of patients with metabolic bone diseases (n = 27, 25.2%) was the largest, followed by patients with nephrological and endocrine diseases (n = 24, 22.4%), primary osteoporosis (n = 17, 15.9%), inflammatory diseases (n = 15, 15.0%), hematologic or oncologic diseases (n = 14, 13.1%), and nervous system or psychological diseases (n = 10, 9.3%). The percentages of female patients were higher than the percentages of male patients in the categories of primary osteoporosis (82.4%), nervous system and psychological diseases (80.0%), and metabolic bone diseases (66.7%). The demographic data for the symptom-based and disease-based categories are shown in Table 1.

Gender-dependent distribution of serum BAP and BAP/ALP levels. The age and gender distributions of the patients are shown in Fig. 1A. As stated above, the patients were 45 males (42.1%) and 62 females (57.9%). In both the males and females, the largest numbers of patients were in the age range of 51-70 years (males: n = 20, 44.4%; females: n = 21, 33.9%), and the proportions of patients under 30 years of age were small (males: n = 5, 11.1%; females: n = 2, 3.2%). Female patients were dominant in the age ranges of 31-50 years and > 71 years (Fig. 1A). As shown in Fig. 1B, the serum levels of BAP ($p = 0.729$) and ALP ($p = 0.532$) were not significantly different between the male and female patients, although the ratios of BAP/ALP in the female patients tended to be high ($p = 0.257$) compared to those in the male patients.

Category-based distributions of serum BAP and ALP levels and BAP/ALP ratios. The serum BAP and ALP levels and the BAP/ALP ratios were examined by symptom-based categories as shown in Fig. 2A. The serum levels of BAP and ALP were not significantly different among the 6 groups of symptom-based categories. However, the BAP/ALP ratio in the fever group ($p = 0.013$) was significantly lower than that in the

Table 1 Demographic data for symptom-based and disease-based categories

	Age	Male (%)	Female (%)	Number
Symptom-baed categories				
Symptom-free group	58.4 ± 17.0	15 (45.5)	18 (54.5)	33
Pain group	59.9 ± 16.1	15 (46.9)	17 (53.1)	32
Psychologic group	49.7 ± 18.4	6 (30.0)	14 (70.0)	20
Fever group	60.6 ± 19.0	3 (37.5)	5 (62.5)	8
Weight gain group	66.4 ± 23.0	3 (42.9)	4 (57.1)	7
Urinary symptom group	67.6 ± 7.3	3 (42.9)	4 (57.1)	7
Total	58.5 ± 17.5	45 (42.1)	62 (57.9)	107
	Age	Male (%)	Female (%)	Number
Disease-based categories				
Metabolic bone diseases	59.4 ± 11.9	9 (33.3)	18 (66.7)	27
Nephrological and endocrine diseases	53.7 ± 18.5	14 (58.3)	10 (41.7)	24
Primary osteoporosis	61.0 ± 19.7	3 (17.6)	14 (82.4)	17
Inflammatory diseases	65.2 ± 13.3	9 (60.0)	6 (40.0)	15
Hematologic and oncologic diseases	66.0 ± 16.2	8 (57.1)	6 (42.9)	14
Nervous system and psychological diseases	42.8 ± 21.5	2 (20.0)	8 (80.0)	10
Total	58.5 ± 17.5	45 (42.1)	62 (57.9)	107

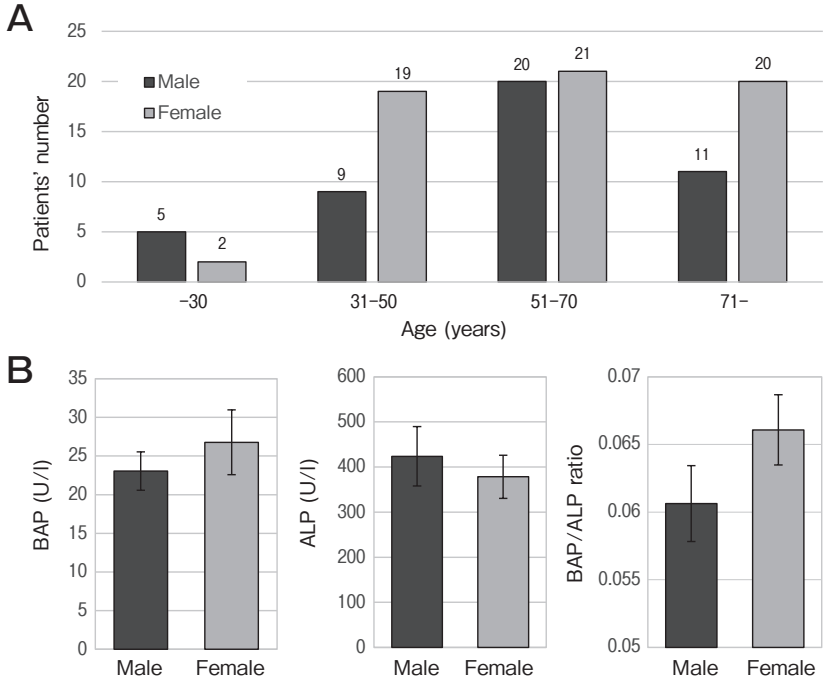

Fig. 1 Serum BAP and ALP levels, BAP/ALP ratios, and gender differences. **A**, Age distributions of the male and female patients; **B**, Comparison of the BAP and ALP levels and BAP/ALP ratios by gender. Bars: mean±SEM. The data were analyzed by the Mann-Whitney *U*-test.

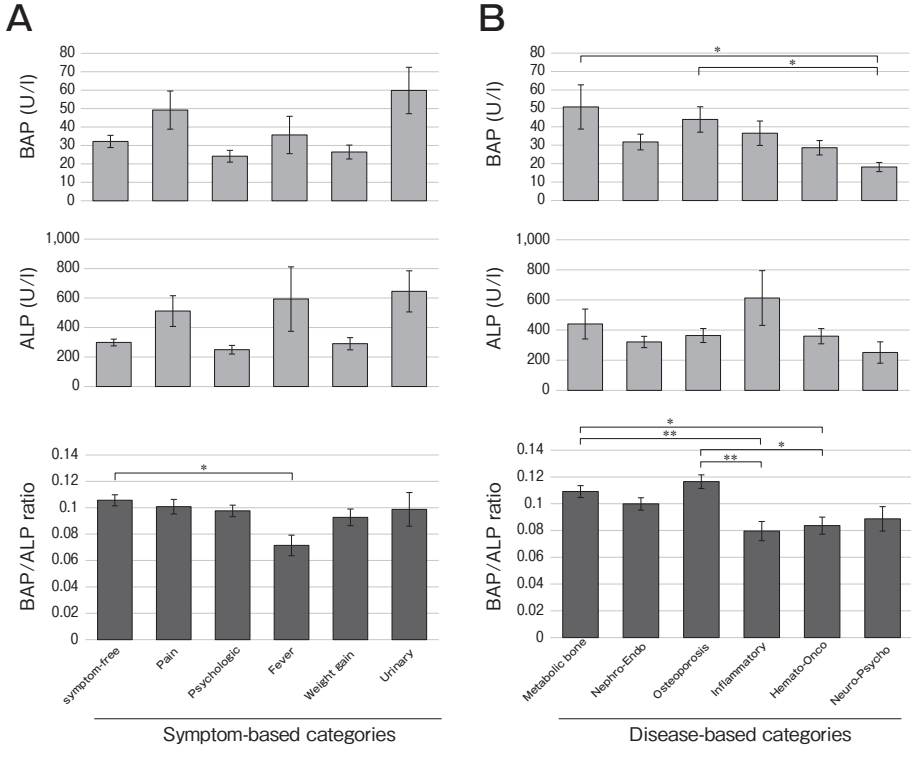

Fig. 2 Serum BAP and ALP levels and BAP/ALP ratios in symptom-based and disease-based categories. **A**, Comparison of the BAP and ALP levels and BAP/ALP ratios among the six symptom-based groups; **B**, BAP and ALP levels and the BAP/ALP ratios among the six disease-based groups. Bars: mean± SEM. The data were analyzed by the Steel-Dwass test (for BAP and ALP) and ANOVA (for BAP/ALP ratio). $*p<0.05$, $**p<0.01$ between the indicated groups.

symptom-free group (Fig. 2A). Figure 2B provides the distributions of serum BAP and ALP levels and BAP/ALP ratios in the disease-based categories. The serum BAP levels in the metabolic bone disease group ($p=0.034$) and the primary osteoporosis group ($p=0.018$) were significantly higher than the level in the group of neurologic and psychological diseases. The serum levels of ALP were not significantly different among the six disease-based categories. The serum BAP/ALP ratios in the groups of metabolic bone disease ($p=0.0033$) and primary osteoporosis ($p=0.0031$) were significantly higher than those in the group of inflammatory diseases, and the serum BAP/ALP ratios in the groups of metabolic bone disease ($p=0.020$) and primary osteoporosis ($p=0.018$) were also significantly higher than the ratio in the group of hematologic and oncologic diseases (Fig. 2B).

The relationships of the BAP/ALP ratio with inflammatory markers and complications. As shown in Fig. 3A, the serum BAP/ALP ratio in the fever group was significantly lower than the ratios in the symptom-free ($p=0.00037$) and fever-free ($p=0.0011$) groups. Figure 3B illustrates the relationships between the BAP/ALP ratio and inflammatory markers. The BAP/ALP ratios were not significantly different ($p=0.21$) between the high ESR group (>15 mm) and the low ESR group (≤15 mm), whereas the BAP/ALP ratio in the high CRP group (>0.15 mg/dl) was significantly lower ($p=0.00038$) than that in the low CRP group (≤0.15 mg/dl). Figure 3C shows the differences in BAP/ALP ratios with respect to major complications. There were no significant differences in BAP/ALP ratios among the patients with hypertension ($p=0.057$), dyslipidemia ($p=0.85$), and diabetes mellitus ($p=0.79$), but the BAP/ALP ratio of the patients with osteoporosis was significantly higher ($p=0.028$) than that of the patients without osteoporosis.

Inter-relationships between the BAP/ALP ratio and the related factors. The inter-relationships between the BAP/ALP ratio and age, serum levels of CRP and

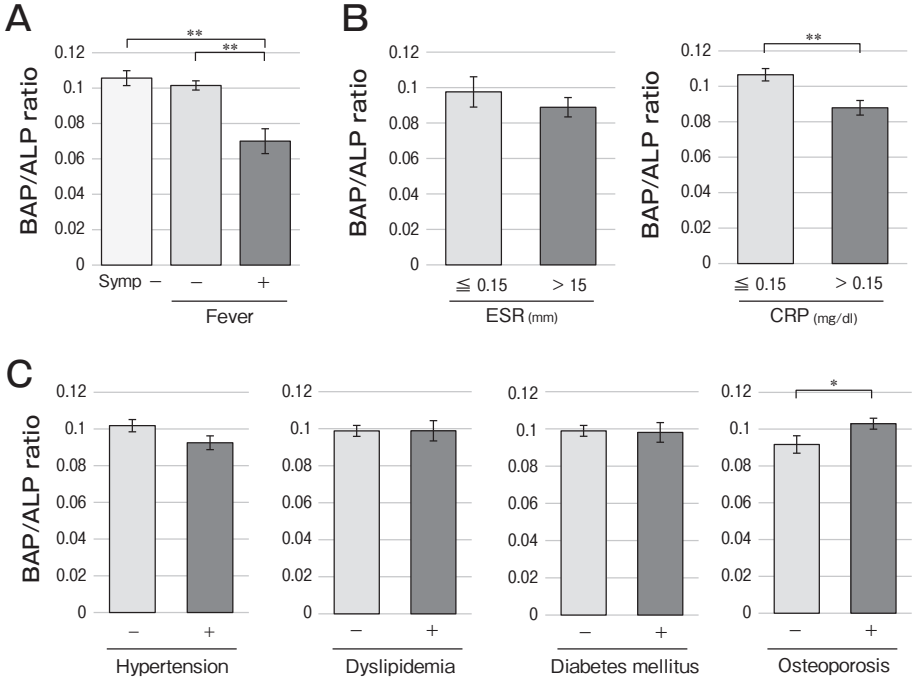

Fig. 3 Comparison of BAP/ALP ratios in the presence and absence of inflammation and complications. **A**, Comparison of BAP/ALP ratios by fever among the fever group (n=9), the fever-free group (n=98), and the symptom-free group (n=33); **B**, The BAP/ALP ratios based on the levels of inflammatory markers among the high ESR group (>15 mm, n=21), low ESR group (≤15 mm, n=14), high CRP group (>0.15 mg/dl, n=42) and low CRP group (≤0.15 mg/dl, n=56); **C**, BAP/ALP ratios based on complications including hypertension (−: n=73 and +: n=34), dyslipidemia (−: n=85 and +: n=22), diabetes mellitus (−: n=90 and +: n=17), and osteoporosis (−: n=39 and +: n=68). The data were analyzed by ANOVA (**A**) or the Mann-Whitney *U*-test (**B**, **C**). *$p<0.05$, **$p<0.01$ between the indicated groups.

albumin, and hemoglobin concentration are shown in Fig. 4. The serum BAP/ALP ratio was negatively correlated with age ($R = -0.285$, $p = 0.00294$, n = 107) and the serum CRP level ($R = -0.453$, $p = 0.0000027$, n = 98). On the other hand, the serum BAP/ALP ratio was positively correlated with the serum albumin level ($R = 0.429$, $p = 0.0000061$, n = 103) and the hemoglobin concentration ($R = 0.336$, $p = 0.00043$, n = 107).

Discussion

As noted above, BAP is a marker of bone formation [6]. The results of our present study, in which the data of patients who visited a general medicine outpatient clinic were investigated, provide a new perspective on the potential roles of BAP. First, the serum BAP/ALP ratios of the patients with fever and those with inflammatory diseases were significantly lower than those of the other groups of patients. Second, the BAP/ALP ratios of the patients with primary osteoporosis and those with metabolic bone diseases were significantly higher than those of the patients with other disorders. Third, the BAP/ALP ratio had significant correlations with aging and with anabolic and inflammatory factors.

It was notable that the BAP/ALP ratios of the patients with fever and the patients with inflammatory diseases were lower than those of the patients with other disorders. Lower serum BAP/ALP ratios indicate that the contribution of the activity of BAP to the total ALP activities is relatively small. We observed an inverse correlation between the serum BAP/ALP ratio and the serum CRP level, in addition to the relationships of the BAP/ALP ratio with fever and inflammation. The results of several experimental studies suggested an inter-relationship between systemic inflammation and ALP activity. An *in vitro* study using mouse osteoblastic cells revealed that elevated CRP suppressed the activity of runt-related transcription factor 2 (Runx2) as a transcription factor of TNALP mRNA, leading to a decrease of BAP activity [13]. It was also demonstrated

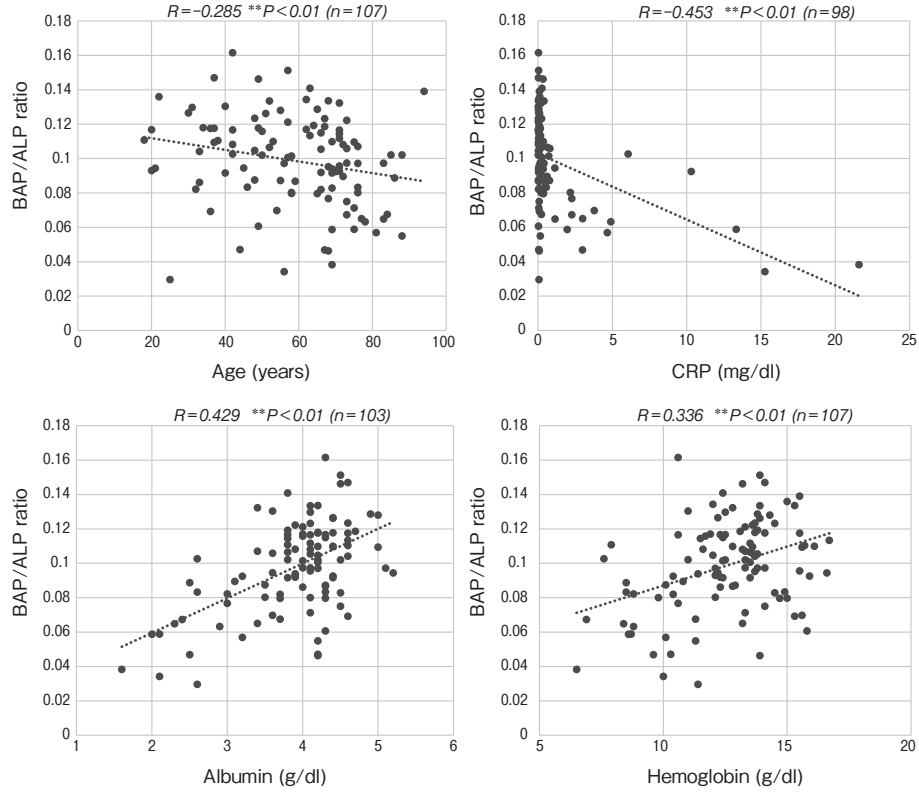

Fig. 4 Inter-relationships between the serum BAP/ALP ratio and changes in related markers. Correlations between the serum BAP/ALP ratio and the patients' age (years, n = 107), serum CRP level (mg/dl, n = 98), albumin level (g/dl, n = 103), and hemoglobin concentration (g/dl, n = 107) were analyzed by Spearman's rank correlation coefficients. $^{**}p < 0.01$ between the indicated factors.

that *in vitro*, tumor necrosis factor stimulates BAP activity [14], leading to vascular calcification.

In a mouse model of sepsis or acute inflammation, it was shown that the levels of IALP and liver-specific TNALP are elevated [15], and it was reported that IALP may mitigate inflammatory responses through the dephosphorylation of proinflammatory ATP [16]. Thus, various types of ALP isozymes, as total ALP activity, are increased in inflammatory conditions, and these increases may lead to a decreased BAP/ALP ratio in patients with inflammatory diseases. The BAP/ALP ratio may thus be a useful indicator of systemic inflammation.

Regarding the relationship between the serum BAP/ALP ratio and aging, it is known that the serum BAP level does not necessarily decrease with aging, whereas the bone turnover tends to be low in elderly people [17]. Several investigations have shown variations in the BAP level depending on ethnicity. According to a report by Zhao *et al.*, the BAP levels were highest in the age group of 20-29 years and reached a plateau in the age group of 50-59 years [18]. An Italian study suggested that BAP levels do not differ depending on age [19]. According to a recent Japanese study, a high BAP level might be an effective indicator of future cardiovascular events and the extent of vascular calcification [20].

Although it has been suggested in previous reports that the BAP level might be stable in people past their 60s, no report has described the age-related patterns of BAP in a Japanese population. In our present study of Japanese patients, a negative correlation was found between age and the BAP/ALP ratio. The present results suggest that the BAP levels in Japanese people decrease with aging, which may be different from other ethnicities.

On the other hand, our analyses revealed that the BAP/ALP ratios of the patients with primary osteoporosis and those with other metabolic bone diseases tended to be higher than those of the patients with other conditions. High-turnover osteoporosis, such as postmenopausal osteoporosis, has been shown to not only activate bone formation but also increase bone resorption [21], which might have led to an increase in the serum BAP level. As for metabolic bone disorders, it was shown that high serum PTH levels abnormally stimulate born turnover in patients with primary hyperparathyroidism, resulting in elevated serum BAP levels [22]. Osteomalacia can also result in the development of secondary hyperparathyroidism, which also contributes to a high serum BAP level [23]. In the present study, the patients complicated with osteoporosis (Fig. 3C) included not only patients with primary osteoporosis but also patients with secondary osteoporosis due to metabolic bone disorders, such as primary hyperparathyroidism, osteomalacia, and Paget's disease of the bone and multiple myeloma. Thus, the BAP/ALP ratios of the present patients with osteoporosis might have been relatively high.

Taking the present findings together, we propose that the BAP/ALP ratio is also a useful marker for estimating generalized bone metabolism. The serum albumin level is considered a critical nutritional index [24] and has been shown to decrease with aging [25]. The hemoglobin concentration also decreases with age [26]. Considering these points, we speculate that the BAP/ALP ratio could be one of the parameters indicating geriatric disorders, which include a wide range of conditions representing multiple organ impairment in older people.

Collectively, our results indicate the clinical relevance of the serum BAP and ALP levels to various chief complaints and diseases seen in general practice. However, our study has several limitations that should be considered. First, the proportion of patients with metabolic and endocrine diseases was relatively high, which might reduce the generalizability of the results. Second, since our results were obtained using retrospective data from a single center in Japan, a multicenter study with a larger population is necessary. Third, the ALP measurements were performed according to the JSCC standard, not according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). This could have affected the data for serum ALP, since intestinal and placental isozymes and blood types are known to modify the ALP levels standardized by the JSCC method [27]. Fourth, since the patients' chief complaints and primary diseases were obtained from electronic medical records by a limited number of researchers, the possibility of sampling bias cannot be completely excluded. Despite these limitations, considering that the patients' serum BAP/ALP ratios were correlated with aging and nutrition factors, we propose that the BAP/ALP ratio can also be a useful predictor of important geriatric conditions. Further cohort studies are needed to reveal in-depth relationships between the BAP/ALP ratio and systemic

disorders in general medicine.

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