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Biological sources of variations of tartrate-resistant acid phosphatase 5b in a healthy Japanese population

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Abstract

Background: Tartrate-resistant acid phosphatase 5b (TRACP5b) is a bone resorption marker that is mainly used in clinical management of osteoporosis. For proper interpretations of test results for serum TRACP5b, we explored their biological sources of variation, esp. age-related changes, and associations with other bone-related markers in healthy Japanese adults.

Methods: During the 2009 East-Southeast Asian multicentre study for determination of reference intervals, 72 major laboratory tests were measured by centralized assays in 3541 well-defined healthy volunteers. The current study included 1980 test results in Japanese subjects for five bone-related markers: TRACP5b, bone alkaline phosphatase, intact parathyroid hormone, calcium and inorganic phosphate. Information on sources of variation, including body mass index, smoking habits and ABO-blood group, were obtained from a health status questionnaire.

Results: Gender-specific profiles of age-related changes were observed for each parameter. Increased values starting from 40 years of age in females were most prominent for TRACP5b, followed by bone alkaline phosphatase and inorganic phosphate. TRACP5b in males decreased with body mass index, bone alkaline phosphatase and TRACP5b were higher in blood type-O subjects, especially in males. TRACPT5b was closely correlated with bone alkaline phosphatase, and moderately correlated with adjusted calcium and inorganic phosphate, especially in females aged $\geqslant$45 years. Reference intervals for each analyte were determined parametrically based on gender and age.

Conclusions: This study elucidated sources of variation of TRACP5b and related bone markers in healthy Japanese subjects and demonstrated a specific age profile for each marker. These results are of relevance for better clinical usage and interpretations of serum levels of bone markers.

Keywords

Reference interval, BMI-related changes, calcium, inorganic phosphate, bone alkaline phosphatase, intact parathyroid hormone, ABO blood group

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Introduction

Bone turnover markers (BTMs) are proteins or protein-derived biomarkers that are released in the process of bone metabolism. BTMs are functionally categorized into markers of bone resorption and bone formation.[1,2] Serum concentrations of BTMs are

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widely used for monitoring the therapeutic efficacy of bisphosphonates in patients with osteoporosis and for detection of bone metastasis of cancer.[3,4]

Tartrate-resistant acid phosphatase 5b (TRACP5b) is a commonly measured bone resorption marker, together with CTX and NTX.[2] TRACP5b is produced in osteoclasts and released into the blood.[5] TRACP5b is suitable for primary use in evaluating the activity of osteoclast because its serum level is not affected by diurnal changes, food intake, or renal dysfunction, in contrast to CTX and NTX.[6–8]

Bone-specific alkaline phosphatase (BAP) is a bone formation marker[2] that is produced in osteoblasts and attached to the outer surface of these cells.[9] BAP is required for mineralization of bone matrix by controlling inorganic pyrophosphate, a potent calcification inhibitor.[10] Soluble BAP is released into serum from osteoblasts, and its serum concentration reflects the activity of osteoblasts and bone formation, and thus is measured in therapeutic monitoring of osteoporosis.[11]

In 2009, we collaborated in a large-scale multicentre study that was conducted in East and Southeast Asia for derivation of reference intervals (RIs), coordinated by the International Federation of Clinical Chemistry (IFCC) and the Asian Pacific Federation of Clinical Biochemistry (APFCB).[12,13] The study recruited 3541 healthy volunteers from eight countries and targeted 72 major laboratory tests, including five bone-related markers: TRACP5b, BAP, intact parathyroid hormone (PTH), calcium (Ca) and inorganic phosphate (IP). Serum TRACP5b showed some ethnic differences, with higher levels in Japanese and Vietnamese populations.[12] The study also found age dependency of TRACP5b, IP and BAP only in females but did not show an age range at which changes occur for each analyte. Potential sources of variation other than sex and age, such as body mass index (BMI), exercise levels, smoking, blood group and associations of TRACP5b reference values (RVs) with other bone-related markers, were not evaluated in that study.

With this background, we conducted the current study as a comprehensive analysis of sources of variations of bone-related markers and associations among these markers, with the goal of better clinical use and interpretation of TRACP5b and related markers. To avoid the influence of ethnic differences in RVs of TRACP5b, the analysis was limited to Japanese subjects ($n = 1980$).

Materials and methods

Source data

Source data were obtained in the Asian multicentre reference interval study conducted from 2008 to 2010 in eight Asian countries for derivation of common RIs.[11,12] The study was coordinated by the IFCC: the Committee on Plasma Proteins (C-PP), the Committee on Reference Intervals and Decision Limits (C-RIDL), the Science Committee of the APFCB and the Japan Society of Clinical Chemistry (JSCC). The volunteers considered themselves to be healthy. Volunteers with any of the following conditions were excluded: (1) BMI $\geqslant 28\,kg/m^2$, (2) excessive daily consumption of alcohol ($\geqslant 75$ g ethanol), (3) smoking >20 cigarettes/day, (4) regular medication for chronic diseases, (5) hospitalization for any acute disease or surgery within the past two weeks and (6) known carrier status for HBV, HCV or HIV.

A total of 72 common analytes, including lipids, electrolytes, enzymes, inflammatory markers, hormones, tumour markers and vitamins, were measured in all subjects using centralized assays in Tokyo, as described elsewhere.[11,12] A health status questionnaire was conducted to screen for eligibility for the study, which provided information on BMI, ABO blood type, levels of alcohol consumption, smoking habits, regular exercise, daily activities (hours of standing and working per day).

In the current study, we used the test results for serum concentrations of five bone-related markers: TRACP5b, BAP, PTH, Ca and IP. Among the 2082 Japanese volunteers, test results for 1980 subjects were used after secondary exclusion based on the presence of any extremely abnormal value among the 72 analytes. This study was approved by the Ethical Committee of Yamaguchi University Graduate School of Medicine, Faculty of Health Sciences (#2008–04) and by each institutional research board of the collaborating laboratories. All volunteers signed informed consent forms that allowed anonymized use of test results.

Measurements

TRACP5b was measured manually with an enzyme immunoassay (N-Test TRACP-5b Nittobo, Nittobo Medical Co., Ltd, Tokyo). It is of note that there are two commercial assay reagents for TRACP5b,[14,15] both of which have comparable analytical performance. However, the above one used in this study is supposed to be unaffected by TRACP5a or an isoform of TRACP derived from macrophages.[15]

BAP and PTH were measured using a chemiluminescent enzyme immunoassay with a DXI autoanalyzer (Beckman-Coulter, Tokyo). Calcium was measured by indirect potentiometry and IP by the timed endpoint molybdate UV method with a DXC autoanalyzer (Beckman-Coulter). Blood was collected into a vacuum blood collection tube containing a serum-separating agent. After the blood was centrifuged for 10 min at room temperature, serum was aliquoted and

cryopreserved at −80°C. All samples were transferred to the Ariake Research Laboratory (Beckman Coulter, Inc., Tokyo, Japan) and measured collectively. Detailed information on the measurements is described elsewhere.[12,13] Since serum concentrations of calcium (Ca) is influenced by serum albumin, values of adjusted calcium (aCa: mmol/L) were calculated as total calcium (mmol/L) + 0.02 (40–albumin [g/L]).[16]

Statistical analyses

Sources of variation analyses. Biological sources of variation were explored by multivariate regression analysis (MRA) for each of the five analytes related to bone metabolism. MRA was performed separately for each gender. The RV of each analyte was used as the objective variable and the following six factors were used as fixed explanatory variables: age, BMI, ABO blood group (BG) B and O, and levels of smoking and alcohol intake. Dummy variables BG-B and BG-O were used by setting BG-A and BG-AB combined as a reference category. Smoking (SmkLvl) was ranked into four levels using the smoking index (SI: the number of cigarettes per day multiplied by duration of smoking in year): 0: none; 1: $SI \leqslant 150$; 2: $150 \leqslant SI < 300$; 3: $300 \leqslant SI$, i.e. those who smoked >20 cigarettes a day were excluded. Alcohol intake was ranked in five grades by ethanol intake in g/day: 0: none; 1: ⩽12.5; 2: 12.5 to ⩽25; 3:25 to ⩽50; 4: >50 g/day. The significance of explanatory variables was judged by the standardized partial regression coefficient (r_p), which corresponds to the partial correlation coefficient, taking values between −1.0 and 1.0. In analyses and graphical presentations, RVs for TRACP5b, BAP and PTH were logarithmically transformed to give a distribution closer to a Gaussian shape. Correlations among the parameters were examined by Spearman correlation analysis. The magnitude of between-subgroup differences after partition of RVs by sex and age was expressed as the standard deviation ratio (SDR), which was computed using a two-level nested ANOVA.[17,18] SDR ⩾0.3 was regarded as a notable between-subgroup difference. All analyses and graphing were performed using general purpose statistical software (StatFlex ver. 7, Artech Co., Ltd, Osaka, Japan).

Determination of reference intervals. RIs were derived by the parametric method after normalizing RVs using a modified Box-Cox power transformation method.[17] The appropriateness of the transformation was confirmed from the linearity of the cumulative distribution of RVs after the transformation on a probability paper plot and by use of a Kolmogorov-Smirnov test of normality. The 90% confidence interval (CI) of the lower limit (LL) and upper limit (UL) of the RI were calculated by the bootstrap method through random resampling of the same data-set 50 times. The final LL and UL of the RI by the parametric method was chosen as the average of the iteratively derived LLs and ULs.

Graphical delineation of age profiles. Age-related change profiles of the five parameters were examined using a two-dimensional scattergram for each gender by plotting age on the X-axis and the test result for each parameter on the Y-axis. Curves representing the median and 90% CIs were drawn using the following smoothing procedure:

1. A paired data-set with data size N composed of age (Xi) and test result (Yi) (i = 1−N) was multiplied by R times to expand the size to N×R. At the time of duplication, uniform random values between −2.0 and 2.0 were added to Xi, and those between $-1.0\times\Delta$ and $1.0\times\Delta$ were added to Yi, where Δ is a unit of reporting: for TRACP5b, $\Delta = 0.1$ U/L; for BAP, $\Delta = 1.0$ μg/L. R was set to 10 in this study.
2. The expanded data-sets for TRACP5b, BAP and PTH were logarithmically transformed to approximate a Gaussian distribution.
3. The data-set for each parameter was stratified by an age block of every five years, and block-wise summary statistics of mean and 90% CIs were calculated. For logarithmically transformed values, the statistics were reverse transformed to the original scale.
4. The five-year data block was moved upwards by one year of age for calculating the summary statistics in a stepwise fashion.
5. Curves connecting the summary statistics were smoothed using the B-spline function.

Results

Demography of the healthy subjects

The demographic profile of 1980 healthy Japanese volunteers comprised of 878 males and 1102 females was as tabulated in Table 1. Notable features for males and females were, respectively, as follows. Age in median and range was 38 (18–64):36 (20–63) years with balanced distribution for the age range of 18 to 64; BMI in mean ± SD was 22.4 ± 2.5:20.5 ± 2.4 kg/m^2 with only 8.4:2.9% of them exceeding general obesity level of 26 kg/m^2. Habits of smoking was 'yes' in 28.5:6.4%; habits of regular exercise was self-reported in 46.9:26.1%; hours in standing posture per day was self-reported as 5.0 ± 3.2:6.5 ± 3.4 h. The profiles in the last two items indicate relatively high physical activities of the participants. The ABO blood group

Table 1. The demography of healthy Japanese volunteers.

Parameters			Male	Female
Sample size (*n*)			878	1102
Age (year)	Median (range)		38 (18–64)	36 (20–63)
	n (%)	<30	239 (27.2)	339 (30.6)
		30–40	245 (27.9)	296 (26.7)
		40–50	203 (23.1)	242 (21.9)
		50⩽	192 (21.8)	230 (20.8)
ABO blood type	*n* (%)	A	302 (38.8)	395 (39.2)
		AB	79 (10.2)	90 (8.9)
		B	174 (22.4)	211 (20.9)
		O	223 (28.7)	312 (31.0)
BH (cm)	Mean ± SD		170.5 ± 5.6	157.7 ± 5.1
BW (kg)	Mean ± SD		65.10 ± 8.32	51.10 ± 6.52
BMI (kg/m^2)	Mean ± SD		22.39 ± 2.50	20.54 ± 2.37
	n (%)	<18	27 (3.1)	122 (11.2)
		18–22	361 (41.6)	700 (64.2)
		22–26	407 (46.9)	237 (21.7)
		26⩽	73 (8.4)	32 (2.9)
Level of smoking[a]	*n* (%)	0	624 (71.5)	1025 (93.6)
		1	81 (9.3)	34 (3.1)
		2	93 (10.7)	26 (2.4)
		3	75 (8.6)	10 (0.9)
Level of alcohol consumption/day[b]	*n* (%)	0	128 (14.6)	342 (31.2)
		1–2	302 (34.6)	512 (46.6)
		3–5	444 (50.8)	245 (22.3)
Level of regular exercise/week[c]	*n* (%)	0	468 (54.1)	803 (73.9)
		1–3	306 (35.4)	232 (21.4)
		4–7	91 (10.5)	51 (4.7)
Hours of standing/day	Mean ± SD		5.0 ± 3.2	6.5 ± 3.4
Hours of working/day	Mean ± SD		9.5 ± 1.8	8.5 ± 1.5

[a]smoking index (SI) = cigarette pieces/day ×year.
0: SI=; 1 = SI < 100, 2: SI < 300, 3: SI ⩾ 300.
[b]Average alcohol consumption (EtOH g)/day.
0: none; 1: <12.5 g; 2: <25 g; 3: <50 g; 4:<100 g; 5:⩽100 g.
[c]Regular exercise: days/week × intensity (0.5 [low], 1.0 [high]).

compositions (both sexes combined) were as expected for Japanese: A 39.0%; AB 9.5%; B 21.6%; O 30.0%.

Sources of variations

Sources of variation for the five bone metabolic parameters were analysed using MRA. The analysis was performed separately for each sex with a fixed set of explanatory variables: age, BMI, BG-B, BG-O, SmkLvl and DrkLvl. The results are listed in Table 2. The level of significance of r_p was defined as weak, $P < 0.01$; slight, $P < 0.001$ and moderate, $P < 0.0001$.

For TRACP5b, the profile of associated parameters differed greatly between the sexes. In males, there was a moderate negative association with BMI ($r_p = -0.225$) and slight positive associations with BG-O (0.141) and SmkLvl (0.126). In contrast, in females, TRACP5b had a moderate association with age (0.375), but only weak negative associations with BMI and DrkLvl. BAP had a weak positive association with BG-O (0.201) and weak negative associations with SmkLvl (0.133) and age (−0.113) in males, and a moderate positive association with age (0.282) and a slight association with BG-O (0.126) in females. PTH only showed a positive association with age, which was slightly stronger in males

Table 2. Results of multiple regression analyses performed for exploration of sources of variations.

			Explanatory variables											
Obj var	*n*	R	Age	*P*	BMI	*P*	BG-B	*P*	BG-O	*P*	SmkLvl	*P*	DrkLvl	*P*
Male														
TRACP5b	753	0.289	0.004		**–0.225**	***	0.042		**0.141**	**	**0.126**	**	0.017	
BAP	766	0.268	**–0.113**	*	0.002		0.065		**0.201**	***	**0.133**	**	–0.078	
PTH	766	0.238	**0.217**	***	0.046		0.022		0.001		0.004		0.012	
aCa	763	0.081	–0.019		0.045		0.019		–0.053		0.019		0.019	
IP	766	0.363	**–0.333**	***	–0.071		–0.018		–0.073		–0.024		0.011	
Female														
TRACP5b	968	0.387	**0.375**	***	**–0.096**	*	–0.0218		0.028		–0.047		**–0.091**	*
BAP	987	0.304	**0.282**	***	–0.006		0.0436		**0.126**	**	0.012		–0.027	
PTH	987	0.165	**0.136**	***	0.050		–0.0412		–0.046		–0.027		0.024	
aCa	988	0.170	**0.131**	***	0.066		–0.005		–0.008		–0.041		–0.043	
IP	987	0.191	–0.047		**–0.101**	*	–0.0235		–0.030		**–0.096**	*	**–0.099**	*

BAP: bone-specific alkaline phosphatase; PTH: parathyroid hormone; aCa: adjusted calcium; IP: inorganic phosphate; BMI: body mass index; TRACP5b: tartrate-resistant acid phosphatase 5b.

R represents multiple correlation coeffcient. Listed values are standardized partial regression coefficents (rp). The values with $P < 0.01$ were marked by bold font.

*$P < 0.01$; **$P < 0.001$; ***$P < 0.0001$.

(0.217) than in females (0.136). For aCa and IP, the associations were unremarkable except for a weak positive association of aCa with age in females (0.131), and a moderate negative association of IP with age in males (−0.333). The insignificant r_p for IP with age in females is attributable to the biphasic nature of the age-related changes for this marker, as described below.

The profiles of age-related changes are shown graphically for each parameter in Figure 1. Data points and summary curves (median and 90% CIs) are shown in blue for males and in red for females. From the shapes of the curves, the following findings were notable:

1. TRACP5b and BAP showed age-related increases in females approximately starting from 40 years old. The trend was more pronounced for TRACP5b. No age-related changes were observed in males.
2. PTH had no sex differences but showed a slight age-related increase in both sexes.
3. aCa showed a weak tendency to increase with age in females, also after 40 years old. This tendency was not apparent in males.
4. IP in females showed a biphasic age-related change: a decrease until 40 years old and a subsequent increase. In males, IP showed a monotonous decrease with age.

BMI-, SmkLvl- and BG-related changes for the parameters identified by MRA are shown graphically in Figure 2. A BMI-related decrease in RVs of TRACP5b was seen only in males. A slightly higher TRACP5b in smokers was also only apparent in males. Regarding BG-related changes, both TRACP5b and BAP showed a tendency to have higher levels in individuals with BG-O compared to those with BG-A or BG-AB, especially in males.

Associations among the five bone-related markers

Associations among the bone-related parameters were examined by making a matrix of Spearman correlation coefficients (r_S) calculated separately for each gender (Table 3). In females, the analysis was performed after partitioning subjects at 45 years of age. Among the three BTMs (TRACP5b, BAP and PTH), the most notable finding was the close association of RVs for TRACP5b and BAP (r_S of 0.446 in males and 0.422 [<45 y] and 0.571 [⩾45 y] in females). Neither TRACP5b nor BAP showed a significant association with PTH in either sex. Regarding associations of the three BTMs with aCa and IP, in females with ⩾45 year olds, TRACP5b was moderately positive correlated with aCa ($r_S = 0.394$), IP (0.414), and PTH was negatively correlated with aCa (−0.240) and IP (−0.227). In males, PTH had a weak negative association with aCa (−0.274).

Determination of reference intervals for TRACP5b, BAP and PTH

To judge the need for partitioning RVs by age, SDR for age was calculated for each gender as SDR_{ageM} and SDR_{ageF}. These values were 0.00 and 0.654 for

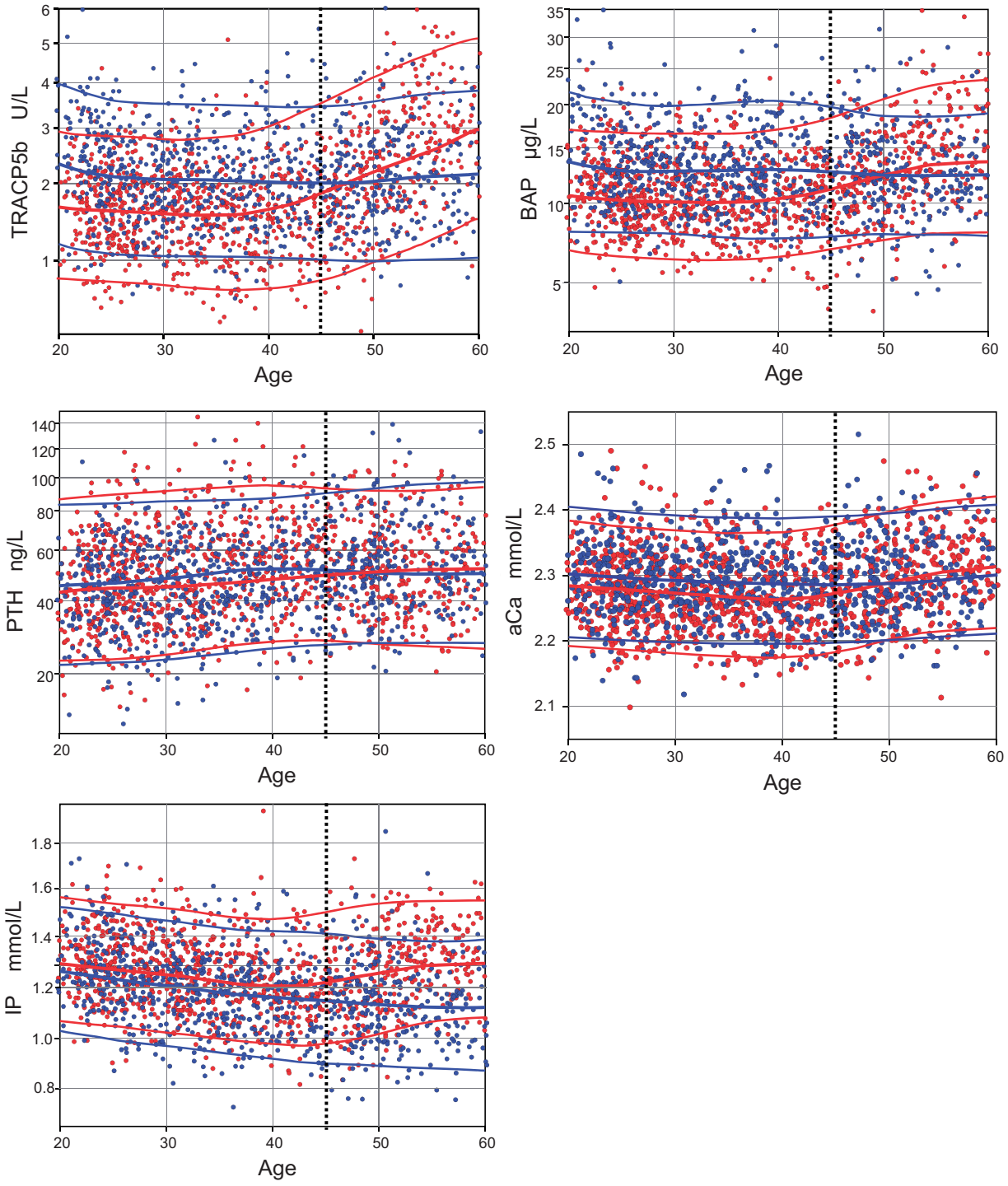

Figure 1. Sex- and age-related changes of five bone-related markers among healthy Japanese subjects. In each graph, the age and test result of each subject are plotted as blue dots for males and red dots for females. The curves representing mean and 90% CI for each gender were predicted using the algorithm described in the Methods section. The vertical broken line indicates 45 years of age, at which RVs were partitioned for females in determining RIs.

TRACP5b, 0.103 and 0.518 for BAP, and 0.275 and 0.178 for PTH, respectively. By setting SDR >0.4 as a threshold, RVs for females were partitioned at 45 years of age to form two groups: 18–44 and ⩾45 y. The cut-off value was arbitrarily set as a midpoint of the rising tendency of RVs, which started at 40 and peaked a little after 50. The derived RIs and their 90% CIs are listed in Table 4. To confirm the validity

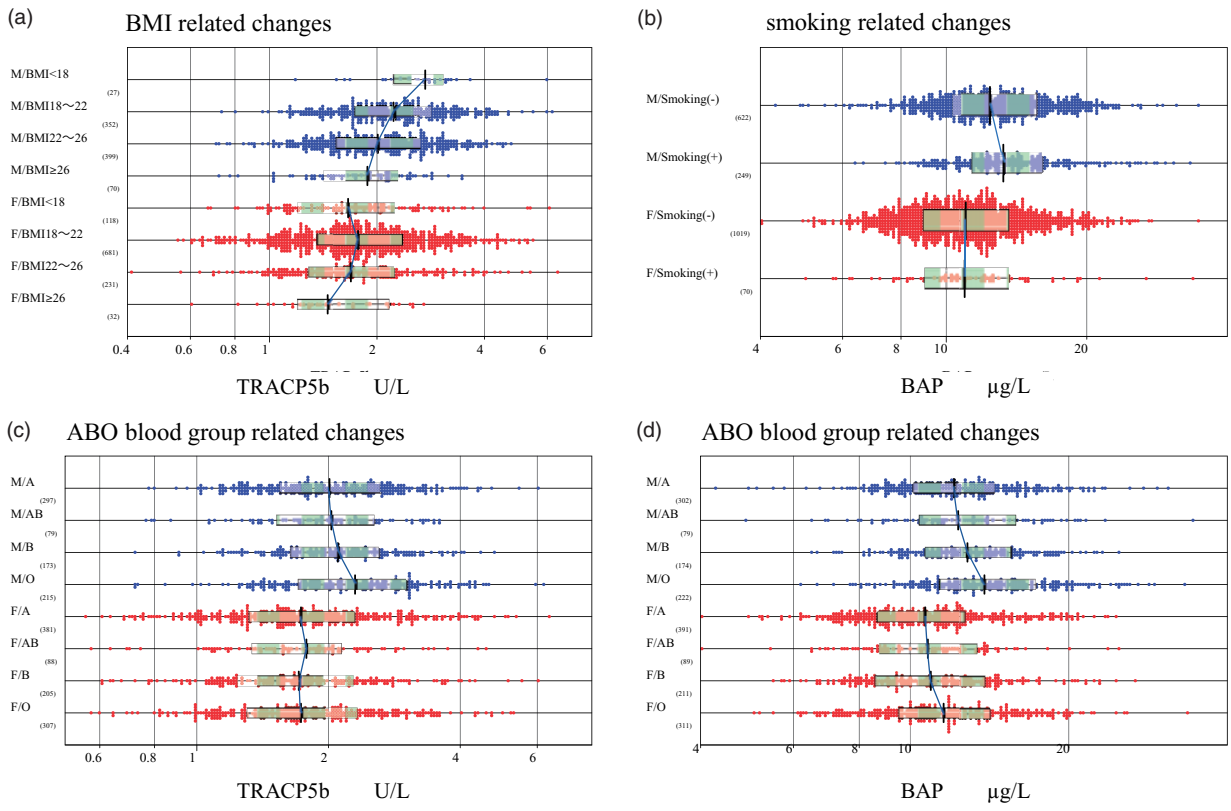

Figure 2. BMI-, blood group-, and smoking-related changes in RVs for TRACP5b and BAP. (a) RVs of TRACP5b were partitioned by gender and BMI in four strata: <18, $18-22$, $22-26$ and $\geqslant 26$ kg/m^2. (b) RVs of BAP were partitioned by gender and smoking habit. (c and d) RVs of TRAP5b or BAP were partitioned by ABO blood group. M and F represent male and female, respectively. The box in the middle of each scatter plot represents the central 50% range of RVs and the central vertical line indicates the median.

Table 3. Spearman's correlation coefficients between bone-related markers.

		TRACP5b	BAP	PTH	aCa	IP
Male	TRACP5b	1.000				
All age	BAP	**0.446**	1.000			
	PTH	0.002	0.055	1.000		
	aCa	0.156	−0.002	**−0.274**	1.000	
	IP	0.075	−0.074	−0.131	0.181	1.000
Female	TRACP5b	1.000				
<45 year olds	BAP	**0.422**	1.000			
	PTH	0.112	0.098	1.000		
	aCa	0.128	**–0.059**	**–0.207**	1.000	
	IP	0.061	**–0.079**	–0.148	0.206	1.000
Female	TRACP5b	1.000				
⩾45 year olds	BAP	**0.571**	1.000			
	PTH	**–0.087**	0.041	1.000		
	aCa	**0.394**	**0.226**	**–0.240**		
	IP	**0.414**	**0.183**	**–0.227**	**0.278**	1.000

BAP: bone-specific alkaline phosphatase; PTH: parathyroid hormone; aCa: adjusted calcium; IP: inorganic phosphate; BMI: body mass index; TRACP5b: tartrate-resistant acid phosphatase 5b.
Correlation coefficient below –0.15 or above 0.15 were regarded as practically significant and marked by bold font.

Table 4. List of RIs determined by the parametric method.

Item	Unit	Sex	Age	*n*	90%CI of LL		Reference interval			90%CI of UL	
							LL	Me	UL		
TRACP5b	U/L	M	All	855	0.97	1.13	1.05	2.09	4.22	4.04	4.39
		F	18∼44	728	0.71	0.77	0.74	1.57	2.99	2.87	3.12
		F	45∼65	340	0.91	1.08	1.00	2.34	5.06	4.76	5.35
BAP	μg/L	M	All	868	7.21	8.11	7.7	12.9	23.2	22.2	24.2
		F	18∼44	747	5.89	6.48	6.2	10.3	17.8	17.0	18.7
		F	45∼65	349	6.54	7.42	7.0	13.0	23.6	21.8	25.4
PTH	ng/L	MF	All age	1971	22.81	24.81	23.8	49.2	96.6	93.0	100.1

M: male; F: female; MF: M + F; LL: lower limit; UL: upper limit; Me: median; CI: confidence interval; BAP: bone-specific alkaline phosphatase; PTH: parathyroid hormone; TRACP5b: tartrate-resistant acid phosphatase 5b.

of the RIs, the Gaussian transformation by the parametric method was evaluated from the linearity of the probability paper plot and with a Kolmogorov-Smirnov test. As shown in Supplemental Figure 1, perfect Gaussian transformation was confirmed in deriving all the RIs.

Discussion

In this study, we explored the relationships of TRACP5b with other bone-related markers. The finding of a high correlation of TRACP5b with BAP can be explained by the biological cross-talk involving osteoclasts and osteoblasts. TRACP5b is secreted from osteoclasts, and differentiation of osteoclasts is controlled by receptor activator of NF-κB ligand (RANKL), the production of which depends on osteoblasts.[19,20] BAP is secreted from osteoblasts, and therefore, both secretion of TRACP5b from osteoclasts and production of BAP depend on the activity of osteoblasts. As a result, serum concentrations of TRACP5b and BAP have a strong positive association, although there is no direct link between the levels of the two markers.

The finding of a negative correlation between serum concentrations of PTH and aCa ($r_S = -0.274$ in males; -0.240 in females $\geqslant 45$ years) in healthy subjects was as expected.[21] However, the moderate positive correlations of TRACP5b with aCa ($rs = 0.394$) and IP (0.414) in females after 45 years of age have not been reported previously. Serum TRACP5b concentrations are correlated with the number of osteoclasts.[5] Denosumab, a human monoclonal antibody against RANKL, can induce hypocalcaemia and hypophosphatemia in patients with osteoporosis,[22] prostate cancer, small cell lung cancer and bone metastasis,[23] with the cause of hypocalcaemia thought to be suppression of bone resorption by denosumab, which reduces release of calcium from bone into the blood. Thus, the correlations of TRACP5b with aCa and IP in healthy subjects may be explained by analogy to the clinical findings. On the other hand, there was no appreciable association between BAP and aCa regardless of sex, indicating that the BAP concentration is rather independent of the serum calcium concentration.

The age-related increase of TRACP5b in females can be explained by the rapid decrease in serum estrogen at menopause. In premenopausal women, estrogen suppresses osteoclast activity by inducing apoptosis through estrogen receptor (ER), thereby preventing bone resorption and secretion of TRACP5b.[24,25] To the contrary, the activity of osteoclast is enhanced after menopause, resulting in increased bone resorption and secretion of TRACP5b. To counteract the bone resorption, osteoblast activity is also enhanced with increased release of BAP.[26,27] However, it is notable that this age-related increase was more prominent in RVs of TRACP5b than BAP, suggesting a predominance of osteoclast over osteoblast activity due to menopausal changes.

BMI, ABO blood group and smoking habits were found to be sources of variation for some bone-metabolic markers. The negative association of TRACP5b with BMI can be explained by obesity-induced suppression of osteoclasts, since obesity has a general positive effect on skeletal strength. BMI is correlated with bone mineral density, and females with low BMI have a higher risk of bone fracture than those with high BMI.[28] The correlation of BTMs with BMI may also be caused by other factors, since Thomas et al. reported that high BMI induces the cytokine-like hormone leptin, which inhibits proliferation of osteoclasts.[29] Thus, it seems that a high BMI has an overall suppressive effect on bone resorption.

The association of ABO blood type with ALP is well known. People with BG-O tend to have higher ALP activity, which is attributed to the small intestine-derived ALP-5 isozyme.[30] However, the findings of

higher BAP reflecting ALP3 of bone origin and higher TRACP5b in BG-O subjects are new, and a further study is needed to explain the possible causes.

Limitations of this study were as follows: (1) Although TRACP5b and other bone-related markers are of relevance in clinical management of osteoporosis, in this study, we did not measure other tests relevant for osteoporosis such as bone mineral density, fibroblast growth factor 23, vitamin D. In fact, this study was conducted as a part of the Asian study[12] for deriving RIs and analysing sources of variations targeting 72 major analytes, and thus those markers of osteoporosis were out of our scope. Therefore, we cannot deny inclusion of subjects with osteoporosis in our analyses, despite our deliberate efforts of excluding any volunteers who were taking regular medication. (2) We did not include a query item on daily ingestion of supplements in the health-status questionnaire. Therefore, we cannot deny possible influence of calcium and/or vitamin D supplements on serum concentrations of bone-related markers. However, in a similar study conducted four years later targeting a very similar Japanese population,[31] the proportion of individuals with regular calcium or vitamin D supplementation was only 3% [unpublished observation]. Therefore, the influences of such supplements should be negligible. (3) We limited our analyses only to the data of Japanese volunteers, and thus our findings may not be fully applicable to other ethnic groups. In fact, we noted between-country differences in serum concentrations of TRACP5b.[13] Nevertheless, we noted similar age-related change profiles of the five bone-related markers and correlations among them in other countries as well [data not shown].

The clinical implications of the findings on the five bone-related markers are as follows: (1) it is necessary to always take into consideration of age-specific profile in reading test results of female subjects, especially for TRACP5b, BAP, IP and aCa; (2) TRACP5b may be decreased in males with high BMI; (3) concentrations of BAP are slightly higher in individuals with type-O blood group; (4) there exist close positive associations of TRACP5b with BAP, aCa and IP in healthy females above 45 years of age, a deviation from which may indicates presence of pathological conditions. In short, demographic profile of a given patient, such as sex, age, BMI, and blood group, must be always considered for appropriate interpretation of bone-related marker test results and associations among them.

Conclusion

Sources of variation of serum TRAPC5b, BAP, PTH, aCa and IP and associations among these markers were explored in healthy Japanese adults. Gender-specific profiles of age-related changes were observed specific for each marker. Increased values during middle age in females were most prominent for TRACP5b, followed by BAP and IP. TRACP5b in men decreased with BMI, and BAP and TRACP5b were a little higher in blood type-O subjects, especially in men. TRACP5b was closely correlated with BAP, and moderately correlated with aCa and IP, especially in women aged $\geqslant$45. RIs for each analyte were determined parametrically. In females, RIs for TRACP5b and BAP were derived after partitioning at 45 years of age. These findings on biological sources of variations and associations among bone-related markers are of relevance in interpreting deviations of those test results commonly observed among apparently healthy subjects.

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Ethical approval

This study was approved by the Ethical Committee of Yamaguchi University Graduate School of Medicine, Faculty of Health Sciences (#2008–04) and by each institutional research board of the collaborating laboratories. All volunteers signed informed consent forms that allowed anonymized use of test results.

Guarantor

KI.

Contributorship

WK, KI, and KM conceptualized and designed the study. KI and YS acquired the source data and prepared it for use in this study. KI and WK performed a series of data analyses. WK and KI wrote the manuscript. KM and YS critically reviewed the manuscript.

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Supplemental material

Supplemental material for this article is available online.

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