Clinical Chemistry 54:2
356–365 (2008)

Sources of Variation of Commonly Measured Serum Analytes in 6 Asian Cities and Consideration of Common Reference Intervals

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BACKGROUND: In a previous study to determine the feasibility of common reference intervals in Asia, we found significant differences among populations from 6 cities. In this study, we attempted to define the sources of these differences.

METHODS: We enrolled 580 healthy volunteers (279 men, 301 women, 20–62 years old), after a selection process that was based on the Clinical and Laboratory Standards Institute guidelines, and used a lifestyle questionnaire. All sera were obtained at a basal state and frozen at −80 °C until the collective assay was done. We measured 21 basic chemical analytes and 10 serum proteins.

RESULTS: We used 3-level nested ANOVA to separate the variation (SD) into between-city (SD-city), between-sex (SD-sex), between-age (SD-age), and between-individual (SD-indiv) components. SD-indiv corresponds to one-quarter of the "pure" reference interval obtained after removing variations due to city, sex, and age. The SD-sex to SD-indiv ratio was >0.8 for creatinine, urate, retinol-binding protein, and transthyretin. We observed high SD-city to SD-indiv ratios, ranging from 0.4 to 0.7, for 11 analytes including lactate dehydrogenase (LDH), electrolytes, IgG, and complement components and SD-age to SD-indiv ratios >0.4 for LDH, alkaline phosphatase, and total cholesterol. Multiple regression analysis demonstrated several other relevant sources of variation, including body mass index, alcohol consumption, and cigarette smoking, although their contributions were generally smaller than those for sex, region, or age.

CONCLUSION: We observed unacceptably large regional differences in measured values of some analytes even after adjustment for age, sex, and lifestyle variables. Genetic and environmental factors may account for the residual differences.

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Two of the stated goals for the preparation of the international serum protein reference material CRM470[7] were to decrease between-manufacturer variance in assays and to determine the feasibility of common reference materials *(1–4)*. In the previous Asian Reference Interval Project, we used a protocol developed by the IFCC Committee on Plasma Proteins *(5)* to explore the feasibility of using common reference intervals (RIs) for these proteins in different geographical regions in Asia.

Contrary to our expectation, we found large differences in test results for individuals from 6 Asian cities, not only for major serum proteins but also for other commonly measured biochemical analytes. The analytical results were comparable because all specimens were freshly collected and measured collectively in one laboratory. Unfortunately, we did not obtain detailed demographic information from the donors and were unable to analyze factors possibly accounting for the differences.

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Received May 14, 2007; accepted October 29, 2007.
Previously published online at DOI: 10.1373/clinchem.2007.091843

[7] Nonstandard abbreviations: CRM470, certified reference material 470; RI, reference interval; BMI, body mass index; CLSI, Clinical and Laboratory Standards Institute; AST, aspartate transaminase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; GGT, γ-glutamyltransferase; CK, creatine kinase; TG, triglyceride; TCho, total cholesterol; IP, inorganic phosphate; TP, total protein; Glb, globulin; CRE, creatinine; UN, urea nitrogen; UA, urate; Tf, transferrin; TTR, transthyretin; RBP, retinol binding protein; CysC, cystatin C; CRP, C-reactive protein.

Table 1. Planned composition of healthy participants.					
	Age, years				
Sex	20–29	30–39	40–49	50–60	All
Male	5–10	10–15	10–15	5–10	30–50
Female	5–10	10–15	10–15	5–10	30–50
All	10–20	20–30	20–30	10–20	60–100

In this second study, we sought to confirm our earlier findings, using a lifestyle questionnaire to explore possible causes for the differences and evaluating possible geographical differences in other analytes. Because we did not seek to determine the RI for each analyte, the number of study participants was set to approximately 60–100 per city, adequate for analyzing between-city differences adjusted for various demographic factors.

Materials and Methods

STUDY PARTICIPANTS

The objectives of the project and the protocol were sent to the member nations of the Asian Pacific Federation of Clinical Biochemistry (APFCB). Six laboratories expressed a desire to participate in the study: the central laboratories of Prince of Wales Hospital (Shatin, Hong Kong), Yonsei University Hospital (Seoul, South Korea), Yamaguchi University Hospital (Ube, Yamaguchi, Japan), Asahikawa Medical College Hospital (Asahikawa, Japan), Yuan Ching Clinical Laboratory (Taipei, Taiwan), and Prodia Clinical Laboratory (Jakarta, Indonesia).

The target population was healthy individuals 20–60 years old. To avoid possible bias attributable to differences in physical activity and/or climatic influences depending on working environment, we limited the participants primarily to those who were working for the clinical laboratory or its affiliated hospital. However, those healthy individuals who were family members of the laboratory staff and regarded as having similar levels of daily physical activities and working indoors were included. Their proportion was <15% in all cities except Taipei, where the size of the laboratory was small. Therefore, white-collar workers from a company affiliated with the laboratory were also enrolled as equivalent to the laboratory workers. The planned number of participants was 60–100 per city, as shown in Table 1.

The following exclusion criteria were applied during recruitment: anyone who reported having any chronic disorder requiring regular medication, who had just recovered (≤14 days) from surgery or any acute illness, or who was pregnant, a heavy smoker (≥25 cigarettes/day), or overweight [body mass index (BMI) >30]. The numbers of individuals who agreed to participate are shown in Table 2.

LIFESTYLE QUESTIONNAIRE

The questionnaire survey, conducted at the time of blood collection, was designed to obtain demographic information recommended in the Clinical and Laboratory Standards Institute (CLSI) guideline *(6)* as well as additional information to explore other factors possibly associated with regional differences. These other factors included regular exercise (at least once per week for ≥1 years: yes or no), average h/day of standing, cigarette smoking, approximate alcohol consumption (quantified as g ethanol/day), and amount of various food types consumed per week in 5 grades for meat, fish, vegetables, beans/tofu, milk/dairy, eggs, fruits, sweets, fried food, and salty food.

SPECIMENS AND ASSAYS

Blood was sampled between 0800 and 1000 after an overnight fast (≥10 h). The volunteers were asked to remain seated for at least 15 min before sampling.

Table 2. Actual composition (age and sex) of the healthy participants.

	Age of men, years					Age of women, years					
City	20–29	30–39	40–49	50–62	All	20–29	30–39	40–49	50–62	All	Total
Asahikawa	8	13	13	7	41	7	12	12	8	39	80
Seoul	6	11	17	11	45	7	16	20	14	57	102
Yamaguchi	9	7	15	8	39	12	5	10	15	42	81
Taipei	12	28	18	5	63	19	10	5	3	37	100
Hong Kong	13	12	10	11	46	15	19	24	16	74	120
Jakarta	9	13	13	10	45	11	16	16	9	52	97
All	57	84	86	52	279	71	78	87	65	301	580

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Blood (10 mL) was drawn from each volunteer into evacuated serum-separator blood collection tubes. The collected samples were centrifuged at room temperature for 10 min except in Taiwan, where the sample tubes were centrifuged for 5 min at 4 °C. The sera were poured into 2 separate containers and then deep frozen at −80 °C.

The measured analytes consisted of 2 groups. Group 1 included aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ-glutamyltransferase (GGT), creatine kinase (CK), amylase, triglyceride (TG), total cholesterol (TCho), HDL cholesterol, sodium, potassium, chloride, calcium adjusted for albumin, inorganic phosphate (IP), total protein (TP), albumin, globulin (Glb, computed as TP − albumin), creatinine (CRE), urea nitrogen (UN), and urate (UA). Group 2 included 10 serum proteins: IgG, IgA, IgM, complement components (C3, C4), transferrin (Tf), transthyretin (TTR), retinol binding protein (RBP), cystatin C (CysC), and C-reactive protein (CRP).

All the specimens were sent by air freight in deep-frozen state to the Special Reference Laboratory, Inc. (Hachiohji, Tokyo) by July 2006. Measurements for the group 1 analytes were performed using a Hitachi 7170 autoanalyzer. The analytical methods employed were as follows: AST, ALT, LDH, ALP, GGT, CK, and amylase by a Japanese Society of Clinical Chemistry (JSCC) recommended method, using the Japanese certified enzyme reference material *(7)*; TP, biuret method; albumin, bromcresol green method; TCho, TG, CRE, UN, and UA, enzymatic methods; HDL, direct enzymatic method with specific inhibition of cholesterol in other fractions; Na, K, and Cl, ion-selective electrode method; Ca, *o*-cresolphthalein complexone method; and IP, molybdate method. The group 2 analytes were assayed by timed nephelometry using a Behring Nephelometer-II Analyzer (Dade Behring Ltd).

STATISTICAL ANALYSES

On the basis of a preliminary analysis, we performed logarithmic transformation of test results for AST, ALT, GGT, CK, TG, and CRP to normalize the data distributions. Values outside the mean and 4SD were deleted as outliers.

The magnitudes of the between-city (btw-city), between-sex (btw-sex), and between-age (btw-age) group components of variation were computed by use of 3-level nested ANOVA *(8)* after coding each record by city, sex (0 = male, 1 = female), and age group (1 = 20–29, 2 = 30–39, 3 = 40–49, 4 = 50+ years). Each component of variation was derived as a variance and then expressed as SD by taking the square root. These components were designated as between-city SD (SD_{city}), between-sex SD (SD_{sex}), and between-age SD (SD_{age}). The SD for the residual variance provided by the 3-level nested ANOVA is regarded as the crude between-individual SD (SD_{indiv}). The SD_{indiv} corresponds to one-quarter of "pure" RI derived after removing variations due to age group, sex, and city. Thus the magnitude of SD_{indiv} represents the sum of (*a*) between-individual variation (not attributable to age, sex, or city, but to other factors, such as smoking, level of exercise, alcohol consumption, or preferences for foods), (*b*) within-individual variation (mainly dependent on sampling conditions), and (*c*) analytical variation. For those analytes that were logarithmically transformed before performing the 3-level nested ANOVA, the back-transformed mean (*M*) and *SD* were computed as follows from the logarithmic mean and SD (M_T and SD_T):

$$M = \exp(M_T)$$

$$SD = \frac{\exp(M_T + SD_T) - \exp(M_T - SD_T)}{2}$$

Multiple regression analysis was performed to explore sources of variation other than city, sex, and age. For this purpose, the above information obtained from the lifestyle questionnaire was used for the explanatory variables. To adjust for regional differences, dummy variables representing each city were introduced by setting Yamaguchi as the reference category. A dummy variable for sex was set as male = 0, female = 1. A best-fit regression model was sought by use of a stepwise selection method; however, the dummy variables for sex and city were always included irrespective of their significance to adjust for their possible confounding influences.

Results

SEX AND REGIONAL DIFFERENCES

Table 3 shows the results of the 3-level nested ANOVA. The magnitudes of 4 components of variance, btw-city, btw-sex, btw-age, and btw-indiv, were expressed as the relative percentage of the overall variance and listed in columns 4–7 of Table 3. Contributions >10% are marked by superscript a and b. The btw-indiv variance is regarded as the residual variance obtained after extracting the variation from city, sex, and age.

Each component of variance was transformed to the SD and listed in columns 8–11, denoted btw-city (SD_{city}), btw-sex (SD_{sex}), btw-age (SD_{age}), and btw-indiv (SD_{indiv}). Because SD_{indiv} is regarded as equivalent to one-quarter of the RI, the ratio of SD_{city}, SD_{sex}, or SD_{age} to SD_{indiv} (SD ratio) was computed to com-

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Table 3. Results of the 3-level nested ANOVA.

Analyte	Unit	Grand Mean	Variance components, % Btw-city	Btw-sex	Btw-age	Btw-indiv	SD (ratio to btw-indiv SD) Btw-city	Btw-sex	Btw-age	Btw-indiv
AST[c]	U/L	20.1	0.0	10.6	6.2	83.3	0.00 (0.00)	1.85 (0.31)[b]	1.42 (0.24)[b]	5.20
ALT[c]	U/L	16.9	0.2	22.0[a]	3.9	74.0	0.33 (0.04)	3.98 (0.44)[a]	1.67 (0.18)	7.30
LDH	U/L	155.4	30.2[a]	0.0	11.8[b]	58.1	17.05 (0.72)[a]	0.00 (0.00)	10.66 (0.45)[a]	23.65
ALP	U/L	205.3	0.0	5.5	18.6[b]	75.9	0.00 (0.00)	13.83 (0.27)[b]	25.49 (0.50)[a]	51.44
GGT[c]	U/L	27.0	0.0	33.5[a]	6.3	60.2	0.00 (0.00)	8.49 (0.60)[a]	3.68 (0.26)[b]	11.38
CK[c]	U/L	103.3	0.0	29.9[a]	1.3	68.8	0.00 (0.00)	27.94 (0.54)[a]	5.88 (0.11)	42.37
Amylase	U/L	78.1	7.0	0.0	0.0	93.0	5.74 (0.27)[b]	0.00 (0.00)	0.00 (0.00)	21.01
TCho	mmol/L	5.02	0.0	0.0	19.1[b]	80.9	0.00 (0.00)	0.00 (0.00)	0.39 (0.49)[a]	0.80
HDL	mmol/L	1.60	0.0	27.7[a]	0.9	71.4	0.00 (0.00)	0.19 (0.62)[a]	0.04 (0.11)	0.31
TG[c]	mmol/L	1.07	0.0	16.6[b]	9.1	74.3	0.00 (0.00)	0.21 (0.39)[a]	0.16 (0.29)	0.45
TP	g/L	73.9	22.1[a]	0.0	4.8	73.1	2.00 (0.55)[a]	0.00 (0.00)	0.93 (0.26)[b]	3.63
Albumin	g/L	45.8	4.8	10.6[b]	10.0[b]	74.6	0.57 (0.25)[b]	0.84 (0.38)[b]	0.82 (0.37)[b]	2.24
Glb	g/L	28.1	17.7[b]	4.7	2.1	75.5	1.43 (0.48)[a]	0.74 (0.25)[b]	0.50 (0.17)	2.95
UN	mmol/L	4.60	15.0[b]	11.9[b]	1.9	71.1	0.48 (0.46)[a]	0.42 (0.41)[a]	0.17 (0.16)	1.04
CRE	μmol/L	65.6	0.0	65.0[a]	0.4	34.5	0.00 (0.00)	13.59 (1.37)[a]	1.08 (0.11)	9.91
UA	μmol/L	314	0.0	57.5[a]	0.7	41.7	0.00 (0.00)	73.18 (1.17)[a]	8.33 (0.13)	62.35
Na	mmol/L	142.5	14.3[b]	2.4	10.8[b]	72.5	0.81 (0.44)[a]	0.33 (0.18)	0.70 (0.39)	1.82
K	mmol/L	4.30	14.8[b]	2.4	0.4	82.4	0.15 (0.42)[a]	0.06 (0.17)	0.03 (0.07)	0.35
Cl	mmol/L	103.9	19.5[b]	6.7	2.8	71.0	0.95 (0.52)[a]	0.55 (0.31)[b]	0.36 (0.20)	1.81
aCa	mmol/L	2.20	6.3	0.0	4.6	89.1	0.02 (0.27)[b]	0.00 (0.00)	0.01 (0.23)	0.06
IP	mmol/L	1.34	15.3[b]	1.6	11.3[b]	71.9	0.08 (0.46)[a]	0.02 (0.15)	0.06 (0.40)[a]	0.16
IgG	g/L	13.60	15.4[b]	5.3	0.5	78.8	1.00 (0.44)[a]	0.59 (0.26)[b]	0.18 (0.08)	2.25
IgA	g/L	2.34	2.2	0.0	2.3	95.5	0.12 (0.15)	0.00 (0.00)	0.12 (0.15)	0.80
IgM	g/L	1.23	0.0	22.6[a]	8.5	68.9	0.00 (0.00)	0.24 (0.57)[a]	0.15 (0.35)[b]	0.42
C3	g/L	1.072	27.0[a]	2.0	2.8	68.1	0.11 (0.63)[a]	0.03 (0.17)	0.03 (0.20)	0.17
C4	g/L	0.228	21.3[a]	0.0	6.1	72.6	0.04 (0.54)[a]	0.00 (0.00)	0.02 (0.29)[b]	0.07
Transferrin	g/L	2.42	0.0	9.4	2.0	88.6	0.00 (0.00)	0.12 (0.33)[b]	0.06 (0.15)	0.38
TTR	mg/L	269.9	0.0	44.6[a]	1.5	53.9	0.00 (0.00)	40.70 (0.91)[a]	7.48 (0.17)	44.75
RBP	mg/L	38.9	0.0	36.8[a]	7.7	55.6	0.00 (0.00)	7.84 (0.81)[a]	3.58 (0.37)[b]	9.64
CysC	mg/L	0.713	0.9	33.9[a]	4.9	60.3	0.01 (0.12)	0.07 (0.75)[a]	0.03 (0.28)[b]	0.09
CRP[c]	mg/L	0.985	12.1[b]	0.0	3.5	84.4	0.21 (0.26)[b]	0.00 (0.00)	0.11 (0.14)	0.56

SD, square root of a given component of variance, corresponding to standard deviation attributable to the component. aCa, adjusted calcium computed using the formula: aCa = Ca + (40 − albumin)/40. For variance components, [a] value greater than 20% and [b] value between 10 to 20%; for SD ratio, [a] value ≥0.4 was regarded as significant and [b] value 0.25~0.4 as marginally significant. [c] ANOVA was performed in log-scale but SD and CV shown here were obtained by reverse transformation.

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pare the extent of influence of each SD component in deriving the RI. The ratios are shown within parentheses next to the values for SDs. We empirically set a critical value for the SD ratio as 0.4. Above this value, we usually consider partitioning reference values (see "Discussion" for reasoning).

The SD ratios for btw-sex (SD_{sex}/SD_{indiv}) >0.4 were noted for ALT, GGT, CK, HDL, TG, UN, CRE, UA, IgM, TTR, RBP, and CysC. The SD ratios for btw-city (SD_{city}/SD_{indiv}) were >0.4 for LDH, TP, Glb, UN, Na, K, Cl, IP, IgG, C3, and C4. SD ratios for btw-age (SD_{age}/SD_{indiv}) were >0.4 for LDH, ALP, TCho, and IP.

When the SD ratio is between 0.25 and 0.4, partitioning reference values may not be required, but the factors have to be considered in interpreting the test results.

Fig. 1 shows the distributions of actual test results for representative analytes, grouped by city and sex. The cities were aligned by geographical latitude from north to south. The box within each scattergram denotes the central 50% range (25th to 75th centiles), and the vertical line in the middle of the box indicates the median. The magnitudes of btw-city and btw-sex differences are shown at the top of each panel.

OTHER SOURCES OF DIFFERENCES

Table 4 shows the results of multiple regression analysis. The contributions of sex- and city-related differences were expressed in terms of overall regression model fit as the multiple correlation coefficient (R). The R for btw-sex difference shown in column 3 was obtained using a regression model that included only sex as an explanatory variable. The R in column 4 was obtained using another model including both dummy variables for sex and city together. Thus the difference of R between columns 3 and 4 represents the contribution of btw-city differences.

The R in column 5 was derived from a full model containing the relevant explanatory variables selected by the stepwise method. In this model, variables for sex and city were always included as control variables to adjust for their possible confounding influence.

The explanatory variables that exceed a significance level of $P < 0.01$ are listed in the righthand columns. The relative importance of the variables representing age, BMI, cigarette-smoking, alcohol (g ethanol/day), and regular exercise was evaluated via the statistical significance of the *t* values in the related columns. *t* Values with minus signs indicate that the test values are inversely related to the level of the factor concerned. Miscellaneous variables are listed in the last 2 columns with the variable names and associated *t* values.

Comparing the R values in columns 3–5, essentially the same information was obtained as in Table 3 regarding contribution of sex, city, and age. The contributions of the other variables are relatively small in most cases, as judged from the minor increment of R value by their inclusion.

Discussion

The international laboratory medicine community is actively pursuing the possibility of global standardization of laboratory tests, at least for commonly measured analytes. Accompanying this trend, there is growing momentum for deriving common RIs by the collaborative effort of clinical laboratories. Such an effort has already been made by the Scandinavian Society of Clinical Chemistry, which published common RIs to be used in the allied countries *(9–12)*. As a whole, the Scandinavian Society believed there were no practically significant differences among their countries in test results for any analyte. However, as discussed below, this judgment was based on where to set acceptable limits for implementing common RI.

We launched a project in 1999 *(5)* to explore the feasibility of deriving common RIs in 6 Asian cities (Hong Kong, Shanghai, Seoul, Kuala Lumpur, Taipei, and Tokyo) for 13 serum proteins (group 2 analytes plus 3) standardized by CRM470. We also measured 7 clinical chemical analytes (AST, ALT, GGT, CRE, TCho, HDL, and TG) whose results were intended to be used in excluding individuals with latent abnormalities. Contrary to our expectation, we observed large between-city variations for many analytes, although we assayed all specimens collectively at a single laboratory in Japan.

We compared the results obtained in the 2 studies for 10 proteins measured in common. Moderate to large between-city differences (SD_{city}/SD_{indiv} ratios) observed in test results for IgG (0.38), C3 (0.78), C4 (0.59), and CRP (0.39) in the first study were also observed in the second study (0.44, 0.63, 0.54, and 0.26, respectively). The lack of noticeable differences for transferrin (0.12), RBP (0.0), and CysC (0.00) in the first study was also seen in the second (0.00, 0.00, and 0.12). As a whole, we confirmed the presence of moderate regional differences in the serum proteins. However, the regional differences observed in the first study for IgA (0.33), IgM (0.33), and TTR (0.50) were not reproduced in the second (0.15, 0.00, and 0.00).

Among the group 2 analytes, IgG, C3, and C4 showed the most prominent between-city differences. These proteins are markers for chronic inflammation. Because the contributions of other factors revealed by multiple regression analysis were much less than the between-city differences, the observed differences are likely to represent a difference in the level of exposure to infectious agents. If the cities are sorted by their latitudes as in Fig. 1, progressively higher values of IgG and C3 are seen as the location approaches the tropics, suggesting higher exposure to infectious agents in cities closer to the tropics. A similar regional trend was also observed for TP and Glb.

Racial differences in serum concentrations of proteins cannot be neglected; Johnson et al. *(13)* reported that whites and Asian Indians living in the same community in England showed different concentrations of serum IgG and α_1-acid glycoprotein, and that serum concentrations of α_1-antitrypsin varied according to genetic phenotype. Therefore, genetic factors may contribute to between-city differences, although we could not distinguish them from environmental factors with the information available in this study.

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Table 4. Components of variations of test results revealed by multiple regression analysis

		Multiple correlation coefficients			*t* Value representing magnitude of associations								
Analytes	n	Sex	Sex + city	Sex + city + other factors	Age	BMI	Cigarette smoking	Alcohol	Regular exercise	Other factor 1		Other factor 2	
AST[e]	570	0.27	0.30	0.41[b]	3.72[b]	3.20	—	—	—	Fruit	2.70	—	—
ALT[e]	570	0.38[a]	0.47[b]	0.55[b]	3.53[b,d]	5.85[a,c]	—	—	—	—	—	—	—
LDH	572	0.07	0.54[a]	0.62[b]	3.81[b,d]	5.37[a,d]	—	—	—	—	—	—	—
ALP	569	0.24[a]	0.27	0.36[b]	4.40[a,d]	—	—	—	—	—	—	—	—
GGT[e]	568	0.45[a]	0.47	0.57[b]	3.57[b,d]	5.93[a]	—	3.90[b]	—	—	—	—	—
CK[e]	572	0.41[a]	0.44	0.49	—	—	—	—	2.95	BW	4.62[a]	—	—
Amylase	568	0.00	0.26[a]	0.33	—	—	—	—	—	BW	−5.01[a]	—	—
TCho	572	0.06	0.16[b]	0.43	8.34[a,d]	—	—	—	—	—	—	—	—
HDL	572	0.37[a]	0.48[b]	0.53	—	−5.43[a]	−2.84	2.83	—	—	—	—	—
TG[e]	570	0.32[a]	0.38[b]	0.50[b]	4.62[a,d]	6.08[a]	—	—	—	—	—	—	—
TP	572	0.05	0.45[a]	0.50	−3.38	—	−3.73[b]	—	—	—	—	—	—
Albumin	572	0.30[a]	0.42[b]	0.47	−6.06[b,c]	—	—	—	—	—	—	—	—
Glb	570	0.16[b]	0.46[a]	0.49	—	—	−3.12	—	—	—	—	—	—
UN	568	0.25[a]	0.50[a]	0.54	3.29	—	—	—	—	Fish	2.83	—	—
CRE	568	0.68[a]	0.69	0.70	—	—	−2.70	—	—	—	—	—	—
UA	571	0.63[a]	0.65	0.69	—	5.43[a]	—	—	—	Fish	2.69	Salty food	−2.97
Na	569	0.18[b]	0.42[a]	0.48	5.62[a,d]	—	—	—	—	Fruit	−3.04	Meat	2.60
K	570	0.09[b]	0.39[a]	0.40	—	—	—	—	—	—	—	—	—
Cl	572	0.13[b]	0.49[a]	0.51	2.61	—	—	—	—	—	—	—	—
aCa	572	0.05	0.27	0.33	—	—	—	—	3.62[b]	—	—	—	—
IP	571	0.15[b]	0.42[a]	0.43	−3.18	—	—	—	—	—	—	—	—
IgG	578	0.18[b]	0.44[a]	0.48	—	—	−4.22[b]	—	—	—	—	—	—
IgA	578	0.05	0.18[b]	0.22	—	—	—	—	—	—	—	—	—
IgM	576	0.36[a]	0.42[b]	0.46	−4.20[b,d]	—	—	—	—	—	—	—	—
C3	575	0.16[b]	0.52[a]	0.63[b]	—	9.99[a]	—	—	—	—	—	—	—
C4	576	0.06	0.45[a]	0.52	3.88[b]	4.64[a]	—	—	—	—	—	—	—
Transferrin	576	0.21[a]	0.25	0.30	—	—	—	—	—	—	—	—	—
TTR	578	0.53[a]	0.57	0.61	—	3.32[b]	—	4.57[b]	—	—	—	—	—
RBP	578	0.48[a]	0.51	0.60[b]	6.38[a]	—	—	5.44[a]	—	—	—	—	—
CysC	576	0.43[a]	0.59	0.65	6.86[a]	—	2.71	—	—	—	—	—	—
CRP[e]	578	0.08[b]	0.38[a]	0.45	—	—	—	—	—	Beans	−3.57[b]	—	—

For R value, [a] increment of >0.2 and [b] increment between 0.08 and 0.20. For *t* value, [a] significance level at $P < 0.00001$ and [b] significance level between 0.00001 and 0.001. (Approximate levels of significance are $t = 2.59$: $P = 0.01$, $t = 3.31$: $P = 0.001$, $t = 3.92$: $P = 0.0001$, and $t = 4.46$: $P = 0.00001$.) [c] Greater tendency in men or [d] greater tendency in women when analyzed separately for each sex, results not shown. [e] Analysis was performed using logarithmically transformed test values. aCa, calcium adjusted for albumin. BW, body weight.

The 7 common analytes measured in the first study (AST, ALT, GGT, TCho, TG, HDL, and CRE), were also included in this study. In the first, we observed moderate between-city differences in test results for all but AST, but we found no apparent differences in this study. This discrepancy may be due to the stricter criteria used for selecting reference individuals. All of these analytes except CRE are related to the metabolic syndrome, and thus between-city differences in its prevalence may have led to differences in test results for those analytes. The SD_{city}/SD_{indiv} for BMI in this study was zero, suggesting there was no difference in the prevalence of obesity in the 6 cities.

For the other analytes measured only in the second study, we observed unexpectedly large between-city differences in test results, especially for LDH, TP, Glb,

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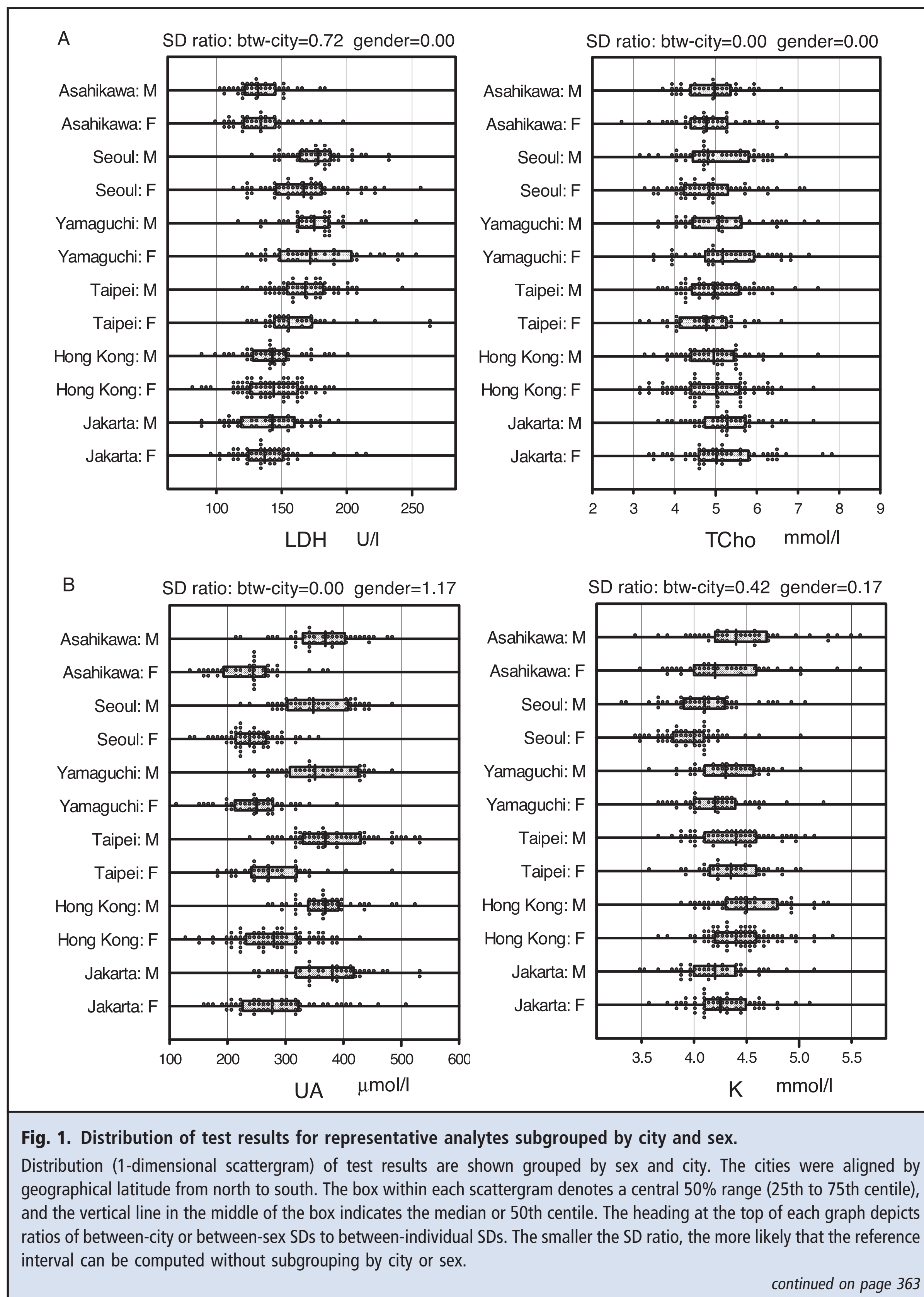

Fig. 1. Distribution of test results for representative analytes subgrouped by city and sex.

Distribution (1-dimensional scattergram) of test results are shown grouped by sex and city. The cities were aligned by geographical latitude from north to south. The box within each scattergram denotes a central 50% range (25th to 75th centile), and the vertical line in the middle of the box indicates the median or 50th centile. The heading at the top of each graph depicts ratios of between-city or between-sex SDs to between-individual SDs. The smaller the SD ratio, the more likely that the reference interval can be computed without subgrouping by city or sex.

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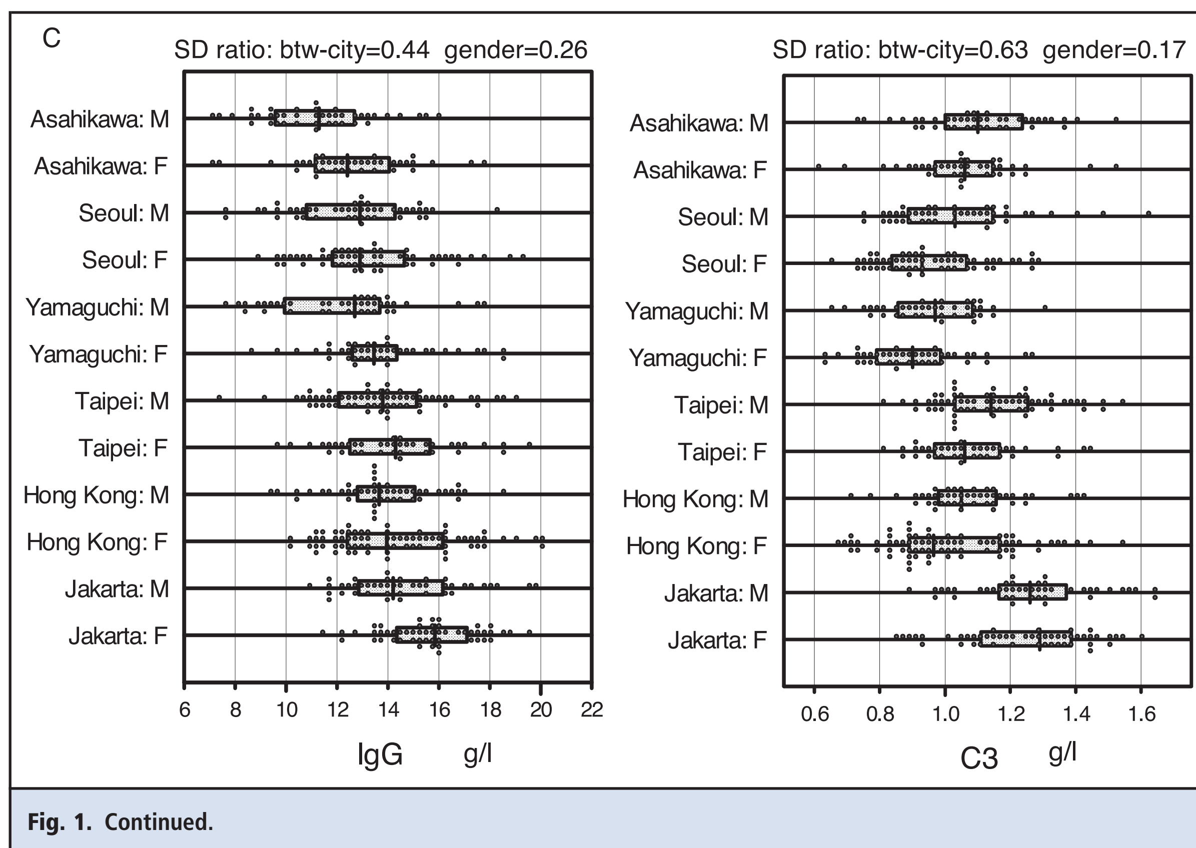

Fig. 1. Continued.

UN, Na, K, Cl, and IP. Lifestyle-related factors may have resulted in spurious between-city differences. However, multiple regression analysis (Table 4) revealed that between-city differences remain highly significant, with only slight changes in the magnitude of R, by inclusion of the lifestyle factors.

As for LDH, no report of regional difference has been published, but plausible factors include (a) hemolysis due to inappropriate sampling procedure in some laboratories, (b) muscular exertion, and (c) genetic factors. Hemolysis-induced LDH increase was unlikely, with no observed correlation between serum concentrations of LDH and K in any city. Furthermore, hemolysis was not reported by the autoanalyzer for any specimen. A different level of exercise could be a factor, because a positive correlation of LDH was observed with both AST and CK (Spearman correlation coefficient rS = 0.446, 0.329, n = 571). However, the questionnaire-based level of exercise showed no association with LDH. Therefore, genetic factors are more likely. Bathum et al. *(14)* reported, after twin-based analysis of test results for LDH, ALT, GGT, and total bilirubin in an elderly group, that the analytes showed high heritability even after adjustment for alcohol consumption and BMI. The large btw-city variation observed for TP and Glb appears to be related to the clear-cut between-city differences in test results for serum proteins, especially for IgG, C3, and C4. Although concentrations of TP and Glb were lower in smokers, multiple regression analysis showed that between-city differences remain highly significant after adjusting for the level of smoking.

Although large regional differences were observed for Na, K, Cl, IP, and UN, the concentrations of these analytes are less dependent on genetic factors. Because their concentrations are under tight physiologic control by homeostatic mechanisms, the observed between-city differences for these analytes are more likely a function of diet and/or the local climate. Geographical differences in potassium concentration appear to be determined by the balance between oral intake and excretion in urine and sweat *(15, 16)*. Because our study population was limited primarily to reference individuals who worked in the hospital laboratory, differences in excretion by perspiration should be negligible. Therefore, variation in dietary intake is likely a more important determinant in our population than differences in potassium excretion. Unfortunately, our questionnaire was not detailed enough to identify dietary

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variation that could account for these differences in potassium or those seen with Na, Cl, and UN.

Our current results indicate there are apparent between-city differences in many analytes. Several criteria have been proposed to aid in deciding whether observed differences in analyte concentrations across groups are large enough to justify using separate RIs rather than common RIs. In the Nordic Project cited above, 3 criteria were considered. The Harris and Boyd *(17)* criterion cited in the CLSI guideline *(6)* uses a formula based on differences in 2 subgroup means adjusted for the sample sizes. The second criterion, proposed by Lahti *(12)*, is based on the percentages of reference values in each subgroup lying outside the upper and lower limits of the common RI derived without partitioning. If the proportion at either end exceeds defined limits, partitioning is permitted. The third criterion, proposed by Fraser et al. *(18)*, is based on the magnitude of biological variation expressed as the standard deviation (s_{bio}), which includes both between- and within-individual components. If the SD accounting for between-subgroup variation is $>0.375s_{bio}$, separate RIs are to be considered.

The first 2 criteria are directly applicable when there are only 2 subgroups. In our case with multiple subgroups, it is difficult to make any judgment by all pairwise comparisons. Recently Gellerstedt *(19)* reported a method for determining the requirement for partitioning multiple subgroups as an extension of Lahti's approach *(12)*. The method focuses on the presence of subgroups with high frequencies ($\geq$4%) of participants outside the common RI. We applied the method to evaluate between-city differences. Acknowledging the limitation of small sample sizes, we found many subgroups exceeding the critical frequencies in all 31 analytes. Therefore we selected Fraser's criterion as more appropriate to aid in deciding whether to use a common RI. Our criterion to justify partition, $SD_{city}/SD_{indiv} \geq 0.4$, is essentially very similar to Fraser's criterion; however, our SD_{indiv} was obtained by removing btw-age, btw-city, and btw-sex components, whereas Fraser's s_{bio} corresponds to gross biological variations without subgrouping by age. Therefore, our SD_{indiv} is generally narrower than s_{bio}. If a large between-city difference exists, the RI without partitioning is always wider than the average RI derived after partitioning. As a result, Fraser's critical SD ($0.375s_{bio}$) also gets wider proportionately, leading to a more conservative judgment not to allow partition. Therefore, compared to Fraser's ratio of 0.375, our critical ratio of 0.4 seems to be either comparable to or more permissive of partitioning. We could set the critical ratio to a higher level in compensation for a narrower measure of between-individual variation; however, SD ratios for between-sex variation or SD_{sex}/SD_{indiv} that exceeded 0.4 were observed for ALT, GGT, CK, HDL, TG, UN, CRE, and UA among the group 1 analytes. Separate RIs for each sex are almost always used for all of the analytes except UN. On the other hand, the analytes with SD_{sex}/SD_{indiv} <0.4 have not traditionally had sex-specific RIs in the 20- to 60-year age interval. This empirical practice began long before discussions of partitioning criteria. In that sense, our critical number of 0.4 seems to be in a practical range.

In summary, this study was not an attempt to define RIs for any population, but rather to demonstrate the presence or absence of differences in analyte concentrations in populations that were as similar as possible except in the city of residence. We observed large between-city differences in some commonly measured analytes among 6 Asian cities, but our sample size and target population was not large enough to generalize the finding to a larger population. Our results suggest that the feasibility of setting common RIs should be confirmed by direct samplings and measurements from the population concerned. Our measure of practical difference, the SD ratio derived from the 3-level nested ANOVA, may be useful in judging the feasibility of adopting a common RI.

Grant/funding Support: In carrying out this study, we did not use any specific grant or fund, but used personal research funds of general use from various sources.
Financial Disclosures: None declared.
Acknowlegments: This study was made possible by the generous support of reagents for the measurements of serum proteins provided by Dade Behring Marburg GmbH, and by the generous support offered by Special Reference Laboratory (SRL), Tokyo, in carrying out the assays for all the analytes. We are very grateful to the eager support and donation of blood by the staffs and associated members of the participating laboratories. We are also grateful to Myron Johnson for his careful editing of this manuscript to improve not only English expressions but also scientific reasoning.

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