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Nationwide Multicenter Reference Interval Study for 28 Common Biochemical Analytes in China

Liangyu Xia, MD, Ming Chen, MD, Min Liu, MD, Zhihua Tao, MD, Shijun Li, PhD, Liang Wang, PhD, Xinqi Cheng, MD, Xuzhen Qin, MD, Jianhua Han, MD, Pengchang Li, MD, Li'an Hou, MD, Songlin Yu, MD, Kiyoshi Ichihara, MD, and Ling Qiu, MD

Abstract: A nationwide multicenter study was conducted in the China to explore sources of variation of reference values and establish reference intervals for 28 common biochemical analytes, as a part of the International Federation of Clinical Chemistry and Laboratory Medicine, Committee on Reference Intervals and Decision Limits (IFCC/C-RIDL) global study on reference values.

A total of 3148 apparently healthy volunteers were recruited in 6 cities covering a wide area in China. Blood samples were tested in 2 central laboratories using Beckman Coulter AU5800 chemistry analyzers. Certified reference materials and value-assigned serum panel were used for standardization of test results. Multiple regression analysis was performed to explore sources of variation. Need for partition of reference intervals was evaluated based on 3-level nested ANOVA. After secondary exclusion using the latent abnormal values exclusion method, reference intervals were derived by a parametric method using the modified Box–Cox formula.

Test results of 20 analytes were made traceable to reference measurement procedures. By the ANOVA, significant sex-related and age-related differences were observed in 12 and 12 analytes, respectively. A small regional difference was observed in the results for albumin, glucose, and sodium. Multiple regression analysis revealed BMI-related changes in results of 9 analytes for man and 6 for woman. Reference intervals of 28 analytes were computed with 17 analytes partitioned by sex and/or age.

In conclusion, reference intervals of 28 common chemistry analytes applicable to Chinese Han population were established by use of the latest methodology. Reference intervals of 20 analytes traceable to reference measurement procedures can be used as common reference intervals, whereas others can be used as the assay system-specific reference intervals in China.

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Abbreviations: Alb = albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AMY = amylase, AST = aspartate aminotransferase, BMI = body mass index, Ca = calcium, CDL = clinical decision limit, CI = confidence intervals, CK = creatine kinase, Cl = chloride, CLSI = Clinical and Laboratory Standards Institute, CRE = creatinine, C-RIDL = Committee on Reference Intervals and Decision Limits, CV(b) = CV of the regression slope b, CV = coefficient of variation, Fe = ion, GGT = γ-glutamyltransferase, Glu = glucose, HDL-C = high-density lipoprotein-cholesterol, IFCC = International Federation of Clinical Chemistry, IP = inorganic phosphate, K = potassium, LAVE = latent abnormal values exclusion, LDH = lactate dehydrogenase, LDL-C = low-density lipoprotein-cholesterol, LIP = lipase, LL = lower limit, Me = median, Mg = magnesium, MRA = multiple regression analysis, Na = sodium, NP = nonparametric, P = parametric, P5P = pyridoxal-5-phosphate, RI = reference interval, RMP = reference measurement procedure, r_p = partial correlation coefficient, SD = standard deviation, SDR = standard deviations ratio, SOP = standard operation procedure, SV = sources of variation, TBil = total bilirubin, TC = total cholesterol, Tf = transferrin, TG = triglycerides, TP = total protein, TTR = transthyretin, UA = uric acid, UL = upper limit.

INTRODUCTION

Reference intervals (RIs) are widely used in the process of making a medical diagnosis, therapeutic management decision, or other physiological assessment. RIs are supposed to be established or verified for each analyte and specimen source in every clinical laboratory. In reality, however, very few laboratories or manufacturers carry out their own reference interval studies. Other laboratories and manufacturers refer to RIs from studies done a few decades ago, when both the analytical methods and life style of the population were very different. So it is a very important task to carefully establish RIs by the laboratory based on standard protocols. The Clinical and Laboratory Standards Institute (CLSI) published and updated a guideline[1] for laboratories and manufactures to perform their own reference interval studies. IFCC/C-RIDL recently published 2 papers including a protocol and standard operation procedures (SOPs) for multicenter RI studies.[2,3] A serum panel with assigned values traceable to the reference measurement procedures (RMP) was introduced in the multicenter study. It is meant for the alignment of test results among laboratories and for promoting common use of RIs through standardization.[4]

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From the Department of Clinical Laboratory, Peking Union Medical College Hospital, Chinese Academic Medical Science and Peking Union Medical College (LX, XC, XQ, JH, PL, LH, SY, LQ), Beijing, China; and Department of Clinical Laboratory Medicine, Institute of Surgery Research, Daping Hospital, the Third Military Medical University (MC), Chongqing, China; Department of Clinical Laboratory, the first Affiliated Hospital, Sun Yat-sen University (ML), Guangzhou, China; Department of Clinical Laboratory, the Second Affiliated Hospital of Zhejiang University School of Medicine (ZT), Hangzhou, China; Department of Clinical Laboratory, the First Affiliated Hospital of Dalian Medical University (SL), Dalian, China; Department of Clinical Laboratory, Xinjiang Medical University (LW), Urumuqi, China; and Faculty of Health Sciences, Yamaguchi University Graduate School of Medicine (KI), Ube, Japan.
Correspondence: Ling Qiu, Department of Clinical Laboratory, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Dongcheng District, Beijing, China (E-mail: lingqiubj@163.com).
LX, MC, ML, ZT, SL, and LW contributed equally to this study.
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In China, only 12.2% of all the laboratories use self-established RIs or verified RIs, others adopt RIs from reagent inserts or reference articles, according to the nationwide survey conducted in 2010.[5] There are a few reports on RIs for Chinese population,[6–9] but there have been few large population-based nationwide studies of RIs which adopt up-to-date research design and employ advanced statistical methods. Therefore, in 2013, we voluntarily chose to participate from China in the global RIs study coordinated by IFCC/C-RIDL. In this study, we organized and conducted a multicenter nationwide RI study for Chinese Han population using the protocol and SOPs[2] provided by IFCC/C-RIDL. The study was aimed to: (1) explore possible regional differences in reference values of major analytes among cities in 6 provinces; (2) define RIs to be used nationwide for as many analytes as possible. In this manuscript, we describe the analytical results obtained from this first nationwide multicenter RI study in China for 28 commonly tested biochemical analytes, which adopted the scheme of centralized measurements, up-to-date statistical methods, and strict procedure for standardization.

METHOD

Ethics

This study was approved by the Ethics Committee of Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science. Written informed consents were obtained from all participants prior to the study.

Selection of Reference Population

Source of Reference Population

A total of 3148 apparently healthy individuals were recruited from 6 cities covering wide areas of China, which represented regions of Northeast (Dalian), Northwest (Urumqi), North (Beijing), East (Shanghai), Southwest (Chongqing), and South (Guangdong). All the participants should be living in the city for at least 1 year. Sixty individuals were required for each sex and for every age group as 19 to 29, 30 to 39, 40 to 49, 50 to 64, except for the group of >65 years, for which group 20 individuals were required. Therefore, the target number of subjects per region was 520 [60 × 2 (sexes) × 4 (4 age groups)] + 40 [20 × 2 (sexes)]. Please note that test results from those >65 years of age were meant for analyses after worldwide accumulation of reference values and thus were not analyzed in this Chinese study.

Exclusion Criteria

The exclusion criteria were determined based on the protocol provided by IFCC/C-RIDL.[2] Every participant was required to complete the questionnaire form to judge the eligibility. The height, weight, and blood pressure were measured on site. The exclusion criteria were as follows:

1. Presence of acute or chronic diseases which require medical intervention including: respiratory diseases, circulation system diseases, liver or kidney diseases, acute and chronic infections, metabolic and nutritional diseases, autoimmune diseases, endocrine diseases, hematological diseases, and malignant tumor.
2. Febrile conditions or use of antibiotics in the past 2 weeks.
3. History of being a hospital in-patient or seriously ill during the previous 4 weeks.
4. Surgery in the past 6 months.
5. Blood donation in the previous 3 months.
6. Female participants who are pregnant, breastfeeding, or within 1 year after childbirth
7. Known carrier state for hepatitis B virus, hepatitis C virus, or human immunodeficiency virus.
8. Blood pressure: systolic pressure ≥160 mm Hg or diastolic pressure ≥100 mm Hg.
9. Body mass index (BMI) ≥ 28 or ≤18.5 kg/m^2.
10. Excessive alcohol consumption (average alcohol consumption ≥75 g ethanol/day).

Laboratory Analysis

Sample Collection and Processing

Preparation for sampling, sampling and sample processing procedures were performed according to the IFCC/C-RIDL protocol.[2] The participants were required to avoid excessive eating or drinking, unusual strenuous exercise within 3 days before the sampling, and sampling on the day after working a night shift.

After overnight fasting for 10 to 14 hours, with no water drinking 1 hour before and no smoking half an hour before, 9 mL blood was collected into tubes with serum separator gel (SST II, Becton-Dickinson, or Vacuette, Greiner Bio-One GmbH, Austria). At 15 to 30 min after sampling, the samples were centrifuged at 1200 g for 10 min at room temperature. The serum from each tube was promptly divided into 4 aliquots of 1 mL each, using well-sealed freezing containers, and was immediately stored at −80°C. The samples, packed in dry ice, were sent to the central laboratory in Beijing within 2 months except for those taken in Chongqing, which were tested by the another central laboratory located in Chongqing.

Tests and Analyzers

Beckman Coulter reagents for AU series were used on AU5800 analyzers in clinical laboratories of Peking Union Medical College Hospital, Beijing, and Daping Hospital, Chongqing, which acted as the second central laboratory for this study. Twenty-eight items including total protein (TP), albumin (Alb), Urea, uric acid (UA), creatinine (CRE), total bilirubin (TBil), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), inorganic phosphate (IP), glucose (GLU), total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL)-cholesterol (HDL-C), low-density lipoprotein (LDL)-cholesterol (LDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ-glutamyltransferase (GGT), creatine kinase (CK), amylase (AMY), lipase (LIP), ion (Fe), magnesium (Mg), transthyretin (TTR), and transferrin (Tf) were measured for each serum sample. The assay methods for the analytes and the manufacture stated traceability are listed in Supplemental Table 1, http://links.lww.com/MD/A723.

Quality Control and Traceability

Commercial controls with assigned values were used to monitor the quality of the test results. The results must be within acceptable range shown in the inserts. A mini panel of 5 sera from healthy individuals was prepared and measured in singleton on each day of measurement to monitor the stability of the assays.[3]

To evaluate the traceability and make comparison of the results from the 2 central laboratories, a panel of 40 sera

prepared from healthy individuals provided by the IFCC/C-RIDL was measured in both centers.[4] The panel was value assigned based on certified reference materials and equivalent value assigned sera[3] for 21 analytes including TP, Urea, UA, CRE, TBil, Glu, TC, TG, HDL-C, LDL-C, Na, K, Cl, Ca, IP, ALT, AST, CK, GGT, Tf, and TTR.

Data Analyses

Multiple regression analysis (MRA) was performed to identify sources of variation (SVs) possibly influencing the test results,[10] including region, sex, age, BMI, levels of cigarette smoking, daily alcohol consumption, and regular physical exercise with respective levels categorized into 4, 5, and 8 grades as follows: none, $\leq$250, 250 to 500, 500 $<$ pack $\times$ year; none, $\leq$12.5, 12.5 to 25, 25 to 50, 50$<$ g ethanol/day; none, 1 to 7 days/week. In the analysis of regional differences by use of dummy variables, Beijing was set as the reference region. A given explanatory variable was considered to be of practical importance when its standardized partial regression coefficient, which corresponds to the partial correlation coefficient (r_p), was $>$0.15, which corresponds to $P \approx 0.0$ with the large data size around 1500.

Standard deviations (SD) attributable to 3 major SVs[10] of test results: between-region (SD-reg), between-sex (SD-sex), between-age (SD-age), together with net between-individual (SD-btw-ind) were computed by 3-level nested ANOVA. The relative magnitude of each SD was expressed as the ratio of SD (SDR) over SD-btw-ind: SDR_{sex}, SDR_{age}, and SDR_{reg} for SD due to sex, age, and region, respectively. An SDR $>$ 0.3 was regarded as a guide to consider partitioning reference values by the SV.[3,10]

The latent abnormal values exclusion (LAVE)[10] method was applied as a method for secondary exclusion with Alb, UA, Glu, TG, LDL-C, HDL-C, AST, ALT, GGT, LDH, CK, and CRP set as the reference test items for judging the need for exclusion. Modified Box–Cox power transformation formula was used to transform reference values into the Gaussian distribution.[10] The RI was first computed as the mean $\pm$ 1.96 $\times$ SD of the transformed reference values selected by the LAVE method, and then reverse transformed to the original scale. The 90% confidence intervals (CI) of the lower and upper limits of the RIs (LLs and ULs) were calculated by use of the bootstrap method through random resampling of the same dataset for 100 times. Making use of the procedure, both LLs and ULs were optimized by taking their averages of the repeatedly derived values.

RESULTS

Profile of the Population

Age and sex distributions of the participants in the 6 cities are shown in Table 1. The average age and SD of participants were 41.8 $\pm$ 14.1 (n = 1509) years for man and 42.2 $\pm$ 13.8 (n = 1639) years for woman. As a whole, male female ratio was almost equal (52.1% vs 47.9%). The BMI was 23.6 $\pm$ 2.9 (n = 1507) for man and 22.2 $\pm$ 2.8 (1639) for woman. 31.8% of the male participants were smokers whereas females seldom smoke. About 35% of participants had different levels of exercise and the ratios of those who do regular exercise were almost equal for both sexes. A total of 26.2% of the male participants had different levels of drinking whereas females seldom drink (Figure 1).

Traceability

The serum panel results of Beijing were compared to the assigned values and the results of Chongqing. The results of the comparison is shown in Supplemental Figure 1, http://links.lww.com/MD/A722; as correlation matrix graphs made from assigned values, and test results from Beijing and Chongqing for each analyte. Urea, UA, TBil, CRE, Glu, TG, Na, K, Cl, Ca, IP, CK, Tf, and TTR were regarded as traceable to the RMP based on the comparison of Beijing's results with the assigned values.

TABLE 1. Age and Sex Composition of the Participants From the 6 Cities of China

Sex	Region	19–29 Years	30–39 Years	40–49 Years	50–64 Years	65 Years	Total
Male	Beijing	54	64	53	53	20	244
	Hangzhou	58	62	60	63	14	257
	Chongqing	63	55	63	59	20	260
	Guangzhou	60	59	59	66	19	263
	Dalian	68	54	60	57	22	261
	Urumqi	59	59	42	53	11	224
	Total	362	353	337	351	106	1509
Female	Beijing	64	68	62	80	19	293
	Hangzhou	61	66	63	63	13	266
	Chongqing	60	66	63	62	16	267
	Guangzhou	59	59	60	64	22	264
	Dalian	62	55	65	60	18	260
	Urumqi	66	66	61	75	21	289
	Total	372	380	374	404	109	1639
Total	Beijing	118	132	115	133	39	537
	Hangzhou	119	128	123	126	27	523
	Chongqing	123	121	126	121	36	527
	Guangzhou	119	118	119	130	41	527
	Dalian	130	109	125	117	40	521
	Urumqi	125	125	103	128	32	513
	Total	734	733	711	755	215	3148

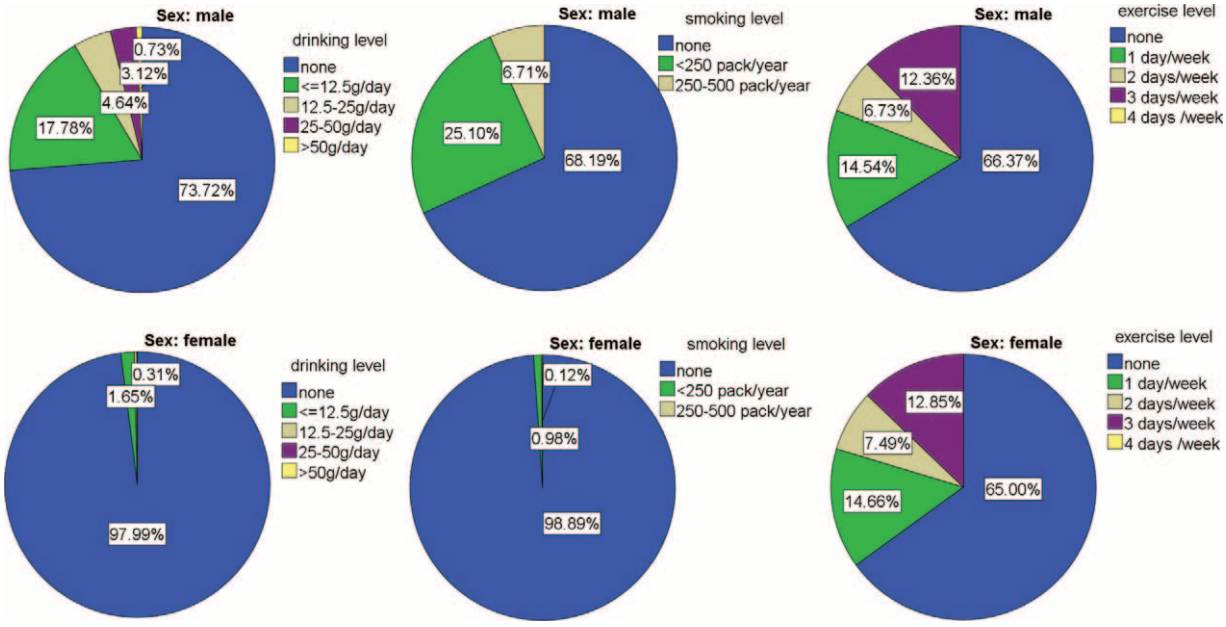

FIGURE 1. Distribution of drinking, smoking, and exercise levels of the reference population. Pie charts were made for each sex to show proportions of levels for 3 life-style habits.

The results of Chongqing were converted to those of Beijing by using the major axis regression line when the coefficient of variation (CV) of the regression line slope, CV(b), was below the critical value of 5.5%.[4] The results for the analyte were judged convertible and merged except for those of Na, Cl, Mg, and TBil. The failure of the latter 4 analytes was attributed to the narrow coverage of the test ranges by the serum panel. However, the SD ratio (SDR) representing between-laboratory differences computed by use of the 1-way analysis of variance[3] were 0.24, 0.09, 0.44, 0.14, respectively for the analytes, and thus their results except Mg were merged without conversion (Supplemental Table 2, http://links.lww.com/MD/A724; Mg test results from Chongqing were not included in derivation of the RI.

The major axis linear regression between the assigned values and merged values of Chongqing to Beijing was used to assess the need for recalibration of test results.[4] When difference in the lower or upper limit of the RI before and after recalibration was >1/4 of SD of the original RI, recalibration of test results was performed by using regression coefficients (intercepts and slopes). This criterion corresponds to Frasers' allowable limit of bias. By this criterion, the recalibration based on the assigned values was performed for TP, GGT, TC, HDL-C, and LDL-C.

Regional Variations of Reference Values

The MRA results in Table 2 showed that test results for Glu from Beijing were higher than those from other cities except Chongqing with $r_p < -0.15$. Dalian showed a largest negative shift. We could not identify the reason, but the time between sampling and serum separation was carefully controlled to be identical. Both males and females of Hangzhou had a significantly high level of Na, and Guangzhou had a high level of UA, compared to Beijing. Males of Urumqi had a significantly high level of ALT than other 5 cities. Males of Chongqing and Urumqi both had much higher GGT than other cities. Though MRA results (r_p) of LIP, Mg, K, and Cl were above 0.15, we considered the actual difference are very small and not of practical importance (Table 3).

Age- and Sex-Related Changes of Results

Age-related changes were analyzed by combination of MRA and 3-level nested ANOVA (Table 4), and 1-dimensional scattergrams (Figure 3). We observed that 5 analytes changed significantly with age in both sexes: Alb, LDL-C, TC, TG, and Urea. Alb steadily decreased with age, especially in males, and others increased. ALP, AMY, CK, Fe, GGT, Glu, LDH, and TG increased with age only in females. In males, the level of lipids increased mainly until the age of 40, whereas in female, the increase occurred more prominently after 40 years of age. The level of SDRs for sex (SDR_{sex}) was significantly high ($\geq$0.30) for 13 analytes: ALT, AST, CK, CRE, Fe, GGT, HDL-C, IP, TBil, TG, TTR, UA, and Urea.

Other Sources of Variations of the Results

MRA results (Table 2) revealed that test results for 7 analytes (UA, TG, HDL, ALT, GGT, CK, and AMY) in females and 10 analytes (UA, TC, TG, HDL-C, LDL-C, ALT, GGT, CK, AMY, TTR) in males were related to BMI. Of all the related analytes, only HDL-C and AMY decreased with the increase of BMI. Alcohol-related changes for GGT and TTR, and smoking-related change in TP were observed in males. No appreciable changes in test results in association with exercise level were noted in any analyte in both sexes.

Reference Intervals

RIs were derived both parametrically (P) and nonparametrically (NP) with LAVE (−), LAVE (allowing 1 abnormal value), and LAVE (allowing no abnormal value). Most analytes had generally identical RIs determined by both P and NP methods, except for TBil, Glu, TG, AST, ALT, GGT, CK, and LIP, the ULs of which were higher derived by NP than by

TABLE 2. MRA Results for Sources of Variation of Reference Intervals in Males and Females

		Regions									
Analytes	**n**	**Urumqi**	**Dalian**	**Hangzhou**	**Guangzhou**	**Chongqing**	**Age**	**BMI**	**Ethanol Level**	**Smoking Level**	**Exercise Level**
Male											
TP	1449	−0.04	−0.11	−0.07	−0.12	0.08	−0.19	0.04	0.02	−0.17	0.00
Alb	1451	0.00	0.11	−0.05	−0.11	0.07	−0.50	0.01	0.05	−0.04	−0.01
Glu	1444	−0.18	−0.33	−0.16	−0.19	0.04	0.22	−0.01	0.07	0.00	−0.01
TBil	1450	0.07	−0.02	−0.02	−0.07	0.07	−0.03	−0.07	0.04	−0.12	0.03
Urea	1449	0.01	0.02	0.03	−0.08	−0.05	0.29	0.01	0.05	−0.02	0.02
CRE	1450	−0.07	−0.14	0.04	0.12	0.03	0.03	0.06	−0.03	−0.05	0.02
UA	1450	0.04	−0.02	0.03	0.15	0.06	−0.15	0.21	0.08	0.00	−0.01
TC	1451	−0.01	0.07	−0.03	0.14	0.02	0.15	0.17	0.10	0.12	0.01
TG	1450	0.10	−0.08	0.02	0.07	0.09	0.08	0.31	0.06	0.09	−0.01
HDL-C	1450	−0.05	−0.04	−0.04	−0.01	0.02	0.02	−0.31	0.12	−0.06	0.02
LDL-C	1450	−0.07	−0.06	−0.05	0.13	−0.09	0.13	0.21	0.05	0.12	0.00
ALT	1445	0.16	0.02	0.06	0.07	0.07	−0.09	0.34	−0.01	−0.01	−0.02
AST	1449	0.07	−0.06	0.02	0.00	−0.01	0.08	0.13	0.07	−0.04	−0.03
LDH	1450	−0.03	0.10	0.08	−0.04	−0.25	0.09	0.10	0.06	−0.05	−0.02
ALP	1447	0.09	0.02	0.06	0.05	0.13	−0.02	0.02	−0.06	0.06	−0.03
GGT	1428	0.12	0.16	0.09	0.12	0.12	0.02	0.30	0.17	0.12	−0.01
CK	1441	−0.11	−0.12	−0.06	0.03	−0.12	−0.09	0.11	0.02	−0.04	−0.02
AMY	1451	0.03	−0.12	0.01	0.12	0.03	0.17	−0.15	−0.05	−0.13	0.01
LIP	1192	0.03	−0.01	0.05	0.17		0.15	0.09	0.01	−0.01	0.01
Na	1193	−0.15	−0.10	0.21	0.08		0.00	0.04	0.01	−0.02	−0.01
K	1450	−0.15	−0.12	−0.25	−0.22	−0.23	0.07	−0.03	0.02	0.04	0.03
Ca	1451	−0.02	−0.09	−0.06	−0.08	0.10	−0.35	−0.01	0.08	−0.04	0.03
Cl	1192	−0.06	0.11	0.11	−0.05		0.04	0.00	0.05	0.03	−0.03
IP	1449	−0.04	−0.08	0.03	−0.01	0.05	−0.25	−0.01	−0.04	0.13	0.00
Mg	1449	0.05	0.17	0.10	−0.10	−0.03	0.08	0.04	0.07	0.05	0.02
Fe	1450	0.03	0.06	0.02	−0.08	0.02	0.01	−0.01	0.07	0.07	−0.01
Tf	1449	0.02	−0.06	−0.12	−0.20	0.05	−0.12	0.15	0.07	0.07	−0.01
TTR	1450	0.03	0.14	−0.06	0.02	−0.05	−0.21	0.15	0.20	0.09	0.04
Female											
TP	1591	−0.07	−0.14	−0.02	0.01	0.09	−0.07	−0.04	−0.01	−0.03	0.02
Alb	1590	−0.05	0.12	0.05	−0.04	0.12	−0.21	−0.09	0.00	0.00	−0.01
Glu	1589	−0.18	−0.27	−0.06	−0.02	0.10	0.25	−0.13	0.01	0.02	0.01
TBil	1591	0.11	0.07	0.07	0.00	0.07	0.05	−0.05	−0.02	0.01	0.03
Urea	1589	−0.04	0.01	0.03	−0.06	0.09	0.40	0.03	0.03	0.00	0.00
CRE	1591	−0.13	−0.12	0.02	−0.02	−0.03	0.19	−0.03	0.00	0.01	−0.04
UA	1591	0.01	0.03	0.07	0.25	0.08	0.00	0.17	0.05	0.02	0.02
TC	1590	−0.09	−0.01	−0.09	0.10	−0.02	0.41	0.04	0.03	−0.02	−0.03
TG	1591	0.07	−0.01	0.10	0.03	0.10	0.32	0.24	0.04	0.00	0.01
HDL-C	1591	−0.13	−0.15	−0.13	0.05	−0.01	0.03	−0.30	0.01	−0.03	−0.02
LDL-C	1590	−0.10	−0.09	−0.09	0.07	−0.09	0.40	0.09	0.03	−0.02	−0.03
ALT	1588	0.10	0.08	0.09	0.08	0.05	0.26	0.21	−0.03	−0.03	−0.01
AST	1589	0.00	−0.02	0.06	0.04	−0.05	0.38	0.05	−0.02	−0.05	0.00
LDH	1590	−0.01	0.03	0.10	0.00	−0.20	0.34	0.11	−0.04	−0.02	−0.02
ALP	1588	0.05	−0.02	0.08	−0.01	0.10	0.41	0.12	−0.06	−0.01	0.00
GGT	1587	0.06	0.04	0.04	0.04	0.03	0.22	0.20	0.02	−0.01	0.01
CK	1588	−0.05	−0.09	0.02	0.13	−0.06	0.16	0.16	−0.03	−0.03	0.01
AMY	1589	−0.05	−0.11	−0.05	0.02	−0.05	0.03	−0.17	−0.01	−0.04	0.02

TABLE 2. (*Continued*)

Analytes	n	Regions					Age	BMI	Ethanol Level	Smoking Level	Exercise Level
		Urumqi	Dalian	Hangzhou	Guangzhou	Chongqing					
LIP	1324	−0.01	0.07	0.08	0.07		0.14	0.00	0.05	0.01	0.03
Na	1324	−0.06	0.02	0.21	0.21		0.11	0.02	−0.01	−0.11	−0.03
K	1591	−0.24	−0.12	−0.26	−0.16	−0.24	0.11	0.04	0.00	−0.02	0.02
Ca	1591	−0.11	−0.05	−0.01	−0.06	0.15	0.01	−0.06	−0.03	−0.01	0.00
Cl	1323	−0.19	0.02	0.15	0.00		0.01	0.05	−0.02	−0.06	−0.01
IP	1588	−0.10	−0.07	0.01	0.03	0.10	−0.04	−0.11	−0.01	−0.03	0.02
Mg	1590	0.05	0.22	0.09	−0.03	−0.02	0.27	−0.09	−0.03	0.03	0.01
Fe	1591	0.05	0.12	0.04	−0.06	0.02	0.07	−0.05	0.02	0.00	−0.02
Tf	1546	−0.02	−0.11	−0.06	−0.08	−0.02	−0.26	0.14	0.02	0.02	−0.03
TTR	1591	−0.05	0.10	−0.03	0.04	−0.13	0.02	0.08	0.10	0.04	0.02

Alb = albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AMY = amylase, AST = aspartate aminotransferase, BMI = body mass index, Ca = calcium; CK = creatine kinase, Cl = chloride, CRE = creatinine, Fe = iron, GGT = γ-glutamyltransferase, Glu = glucose, HDL-C = high-density lipoprotein-cholesterol, IP = inorganic phosphate, K = potassium, LDL-C = low-density lipoprotein-cholesterol, LIP = lipase, Mg = magnesium, Na = sodium, TBil = total bilirubin, Tf = transferrin, TG = triglycerides, TP = total protein, TTR = transthyretin, UA = uric acid.

the P method. We observed that the ULs of some analytes decreased significantly when stricter secondary exclusion criteria were applied. It was seen for Glu, TG, TC, HDL-C, LDL-C, AST, ALT, LDH, ALP, GGT, CK, and LIP in female. And in males, those analytes were UA, TG, TC, AST, ALT, LDH, CK, GGT, and LIP. Examples of the comparison of RIs for both males and females by P and NP methods with or without LAVE method are shown in Figure 2. Those for other analytes, see Supplemental Figure 2 and Supplemental Figure 3, http://links.lww.com/MD/A722. As a general rule, we derived the RIs by use of the P method without applying the LAVE procedure for analytes whose RIs did not change much by the procedure, such as TP, TBil, Na, K, and Ca. On the other hand, for those analytes which are sensitive to the LAVE procedure, such as TG, AST, ALT, GGT, and CK, we adopted the RIs by use of P method with LAVE (abn = 1). We did not adopt RIs by LAVE (abn = 0), because it led to narrow RIs by too strict exclusion of individuals with latent diseases. The final RIs of this study and comparison with other studies are listed in Table 5.

DISCUSSION

Traceability

Judging from test results of the serum panel, Urea, TBil, UA, CRE, Glu, TG, Na, K, Cl, Ca, IP, CK, Tf, and TTR were regarded as traceable to the reference materials. The results of TP, GGT, TC, HDL-C, and LDL-C were recalibrated by use of the major axis linear regression[4] based on test results for the serum panel which has assigned values for those analytes.[3] Therefore, the RIs derived for them in this study are applicable to any Chinese laboratory as long as the assays are standardized and regional differences in test results do not exist. The serum panel results for ALT and AST were lower than the assigned values, it is considered to be lack of Pyridoxal-5-phosphate (P5P) in the reagent for the 2 items. As the values of Alb, TBil, Mg, Fe, LDH, ALP, AMY, and LIP of the serum panel were not assigned by IFCC/C-RIDL in this study, the results of these analytes should be regarded as Beckman AU-specific RIs.

Exclusion of Latent Diseases

When determining the RI, the first thing is to define reference individual as appropriately as possible in consideration of many properties which constitute the status of healthiness by consensus among the investigators. We asked a series of questions and measured the anthropometric parameters including BMI and blood pressure to exclude those who possibly possessed pathological conditions. However, we may still include a lot of individuals who have latent diseases, especially those of high prevalence among apparently healthy individuals in China such as metabolic syndrome and diabetes mellitus.[11–13] The LAVE method, which depends on association of abnormal test results among test items affected by those diseases, is considered a good way to deal with such situation.[10,14,15] By application of the LAVE method with the stricter criteria,[3] ULs derived were lowered in both P and NP methods. At the same time, the gaps of the ULs between P and NP methods became narrower. This is because more and more abnormal results from individuals with latent diseases were removed by the stricter LAVE criteria.

Sources of Variations

In conducting the study on RIs, another important aspect is to explore SVs of each test item. Age, sex, BMI, and region are

TABLE 3. Regional Difference of Reference Intervals

Analytes	City	Male and Female				Male				Female			
		n	LL	Me	UL	n	LL	Me	UL	n	LL	Me	UL
UA	Beijing	364	184	308	524	155	270	384	531	208	183	262	413
	Dalian	384	194	317	494	190	243	367	495	191	181	273	406
	Gungzhou	363	211	346	571	183	233	394	599	177	207	304	474
	Hangzhou	361	195	319	543	185	242	376	547	175	176	271	398
	Urumqi	338	194	307	523	135	267	380	549	196	181	269	394
	Chongqing	387	212	325	528	180	288	381	563	201	197	283	395
Glu	Beijing	361	4.26	5.01	5.93	151	4.30	5.11	6.02	210	4.23	4.94	5.86
	Dalian	386	3.74	4.51	5.37	195	3.71	4.52	5.43	192	3.78	4.48	5.41
	Gungzhou	353	3.95	4.94	5.89	175	3.72	4.88	5.84	179	4.23	4.98	6.00
	Hangzhou	356	3.71	4.84	5.71	186	3.57	4.87	5.72	168	3.98	4.79	5.73
	Urumqi	339	4.06	4.70	5.68	138	4.13	4.85	5.79	197	4.03	4.61	5.45
	Chongqing	383	4.48	5.17	6.04	184	4.52	5.25	6.11	199	4.44	5.13	5.98
Na	Beijing	355	136	140	143	149	137	140	144	206	136	139	143
	Dalian	376	136	139	142	185	137	140	142	190	136	139	142
	Gungzhou	356	136	140	143	178	137	140	143	178	136	139	143
	Hangzhou	355	137	141	145	183	138	141	145	172	137	141	145
	Urumqi	333	136	140	144	135	136	140	144	194	135	140	144
	Chongqing	378	137	141	145	182	138	141	145	199	136	141	146
ALT	Beijing	367	7	15	39	155	10	19	55	213	7	13	25
	Dalian	383	8	16	41	192	10	19	52	195	7	14	32
	Gungzhou	372	8	17	38	186	10	20	45	183	8	14	28
	Hangzhou	358	8	17	43	185	10	20	54	174	7	14	28
	Urumqi	337	8	18	57	138	12	26	74	196	8	14	32
	Chongqing	389	8	16	47	185	9	21	50	201	7	13	32
GGT	Beijing	362	8	15	51	151	11	20	65	207	8	13	36
	Dalian	382	9	17	56	188	11	22	69	191	9	14	30
	Gungzhou	364	9	17	53	181	11	21	63	185	9	14	32
	Hangzhou	361	8	17	62	185	10	22	65	175	8	13	41
	Urumqi	334	8	17	66	133	13	26	87	198	8	13	33
	Chongqing	388	8	17	80	184	10	24	109	201	8	13	38
LIP	Beijing	351	6.4	17.8	45.0	148	5.9	17.0	46.8	201	7.2	18.1	45.2
	Dalian	373	7.0	17.8	43.2	183	5.6	15.6	40.2	189	8.8	20.0	43.7
	Gungzhou	352	8.0	19.7	46.4	176	7.3	19.8	45.7	176	9.3	19.7	47.6
	Hangzhou	351	8.1	19.0	39.7	179	8.2	17.8	35.8	171	8.5	20.3	41.7
	Urumqi	326	6.0	17.8	39.3	133	6.0	17.7	44.7	194	6.1	18.0	40.7

ALT = alanine aminotransferase, GGT = γ-glutamyltransferase, Glu = glucose, LIP = lipase, Na = sodium, UA = uric acid.

considered the most important SVs. With the use of MRA and nested-ANOVA, we reconfirmed age- and sex-related variations in many of the common analytes. Though our multicenter study covered a wide area of China, no significant regional differences were discovered in most analytes, expect Na, UA, ALT, GGT, and Glu. Test results of Na were higher in Hangzhou, which may reflect higher consumption of salt in foods. The ULs of UA for both male and female from Guangzhou were much higher than other cities. It is consistent with the report that Guangzhou had a higher prevalence of hyperuricemia than other cities.[16] Higher UL of ALT for Urumqi may be due to higher BMI compared to other areas. ULs of GGT for males in both Chongqing and Urumqi are high. In interpreting the deviation of their ULs, we have to note that GGT has a very skewed distribution and thus its 90% CI of the UL is much wider than other analytes when RIs were determined province by province using a small sample. Anyway, a higher daily consumption of alcohol may partially explain the high GGT in Chongqing. However, after adjustment of alcohol consumption by use of MRA, GGT is not so high (compared with Beijing) in Chongqing and Urumqi (Table 2).

The life habit such as smoking may be another important SV to affect the RIs. The relation of smoking, drinking, and exercise with RIs were analyzed. We confirmed alcohol-related changes for GGT, TTR, and smoking-related changes for TP in males.[17,18] No such relations were seen in females, which is obviously due to very low proportion of females with habits of smoking and drinking.

Once notable SVs were observed, it is important to determine its relative magnitude of variation associated with the SV in order to evaluate the necessity of developing separate RIs according to the source. Several methods, such as Harris–Boyd method,[19] Lahti method,[20] and Ichihara method[18] have been proposed for use. We adopted the Ichihara method in this study, which depends on 1-way ANOVA or 2 or multilevel nested ANOVA. It computes the SD ratio (SDR), which is the SD due

TABLE 4. Sex-, Region-, and Age-Related Changes Evaluated by 3-Level Nested ANOVA

Item	SDR_Reg (M, F)	SDR_Sex (<50, ≥50)	SDR_Age (M, F)
Alb	0.00 (0.20, 0.22)	0.23 (0.47, 0.00)	0.55 (0.62, 0.32)
ALP	0.00 (0.17, 0.07)	0.26 (0.63, 0.21)	0.46 (0.15, 0.42)
ALT	0.00 (0.09, 0.16)	0.59 (0.76, 0.17)	0.31 (0.00, 0.09)
AMY	0.12 (0.06, 0.00)	0.10 (0.19, 0.00)	0.16 (0.22, 0.37)
AST	0.00 (0.24, 0.25)	0.32 (0.47, 0.00)	0.34 (0.13, 0.28)
Ca	0.17 (0.26, 0.28)	0.09 (0.34, 0.21)	0.37 (0.07, 0.14)
CK	0.00 (0.10, 0.10)	0.59 (0.71, 0.35)	0.22 (0.00, 0.65)
Cl	0.21 (0.14, 0.19)	0.18 (0.15, 0.00)	0.17 (0.31, 0.19)
CRE	0.00 (0.24, 0.15)	1.37 (1.60, 1.16)	0.18 (0.08, 0.20)
Fe	0.00 (0.11, 0.18)	0.41 (0.43, 0.36)	0.03 (0.29, 0.31)
GGT	0.00 (0.34, 0.28)	0.67 (0.80, 0.40)	0.30 (0.15, 0.47)
Glu	0.38 (0.41, 0.37)	0.00 (0.00, 0.00)	0.41 (0.29, 0.35)
HDL-C	0.00 (0.10, 0.20)	0.51 (0.56, 0.37)	0.11 (0.10, 0.08)
IP	0.00 (0.20, 0.30)	0.43 (0.30, 0.76)	0.33 (0.08, 0.07)
K	0.26 (0.39, 0.34)	0.00 (0.00, 0.00)	0.14 (0.05, 0.13)
LDH	0.30 (0.11, 0.09)	0.00 (0.20, 0.07)	0.00 (0.11, 0.51)
LDL-C	0.15 (0.21, 0.19)	0.00 (0.30, 0.23)	0.48 (0.30, 0.60)
LIP	0.01 (0.14, 0.08)	0.11 (0.09, 0.06)	0.19 (0.23, 0.15)
Mg	0.22 (0.19, 0.23)	0.13 (0.24, 0.00)	0.25 (0.40, 0.23)
Na	0.35 (0.20, 0.09)	0.03 (0.00, 0.00)	0.25 (0.21, 0.00)
TBil	0.00 (0.11, 0.10)	0.37 (0.39, 0.29)	0.10 (0.09, 0.01)
TC	0.07 (0.12, 0.18)	0.00 (0.21, 0.30)	0.48 (0.31, 0.58)
Tf	0.00 (0.27, 0.00)	0.39 (0.48, 0.18)	0.27 (0.13, 0.32)
TG	0.00 (0.22, 0.10)	0.31 (0.49, 0.00)	0.43 (0.30, 0.51)
TP	0.15 (0.17, 0.20)	0.00 (0.00, 0.22)	0.29 (0.24, 0.13)
TTR	0.00 (0.16, 0.21)	0.91 (1.08, 0.51)	0.29 (0.10, 0.24)
UA	0.00 (0.14, 0.25)	1.06 (1.18, 0.76)	0.23 (0.16, 0.21)
Urea	0.00 (0.09, 0.13)	0.36 (0.47, 0.27)	0.44 (0.35, 0.48)

Alb = albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AMY = amylase, AST = aspartate aminotransferase, BMI = body mass index, Ca = calcium; CK = creatine kinase, Cl = chloride, CRE = creatinine, Fe = iron, GGT = γ-glutamyltransferase, Glu = glucose, HDL-C = high-density lipoprotein-cholesterol, IP = inorganic phosphate, K = potassium, LDL-C = low-density lipoprotein-cholesterol, LIP = lipase, Mg = magnesium, Na = sodium, SDR = standard deviations ratio, TBil = total bilirubin, TC = total cholesterol, Tf = transferrin, TG = triglycerides, TP = total protein, TTR = transthyretin, UA = uric acid.

to a given factor divided by the SD_{BI}, or SD due to between-individual variation. The guide value for considering the partition is SDR = 0.3, which corresponds to the allowable limit of bias (B_A) in analytical system:[3] that is, the ratio of allowable bias is $B_A/SD_{TB} \leq 0.25$, where SD_{TB} represents total biological SD (= SD_{BI} unadjusted for SD due to sex and age) and is generally larger than our SD_{BI}. Thus, the ratio was raised to 0.30 in consideration of the general difference between SD_{TB} and SD_{BI}. The ratio was regarded appropriate for most cases. However, actual scatter plots for comparing test results across provinces or for sex-specific age profiling of test results are of relevance in interpreting SDR and for deciding whether or not to partition reference values. For example, we observed a steady decline of Alb by increasing age with $SDR_{age} = 0.55$. However, actual difference of the RI between age-subgroups is slight. Similarly, the SDR_{reg} for K is 0.39, but actual between province differences are rather small and of no clinical significance. The same thing is true to TP, Na, and Cl. The common features of these special analytes are the narrow range of the RI for these test results, and thus SDR tends to be exaggerated by placing relatively smaller SD_{BI} (≈1/4th of the RI) in the denominator. Another example of need for caution in using SDR is the case of LDH. Although SDR_{sex} is 0.0, when SDR_{age} was computed for each sex separately, SDR_{age} is 0.51 for males and 0.11 for females. This implies that between-sex difference was masked due to the interaction of age on the analysis of sex-related change. Therefore, SDR_{sex} was computed after subgrouped by age at 50 years. Without partition at age of 50, 14 analytes (50%) have $SDR_{sex} > 0.3$, whereas 18 analytes (64%) have $SDR_{sex} > 0.3$ with age <50 and only 8 analytes (29%) have $SDR_{sex} > 0.3$ with age ≥50 (Table 4).

In fact, there are multiple test items which showed differential effect of sex on age-related change, as shown in Figure 3. In males, nutritional markers (TG, TC, LDL-C, ALT, AST, and GGT) increased from 20 to 40 years of age and unchanged or decreased thereafter. This mode of change reflects life style change with age, whereas in females, they increased from 30 to 60 with steeper changes between 45 and 55. The steeper part is interpreted as reflecting hormonal changes pecular to females. These observations point to the importance of computing SDR_{sex} and SDR_{age} in multiple ways and interpreting the values in reference to the sex- and age-related profiles.

In this study, the ULs of the RIs for TG, TC LDL-C, and UA are all higher than the clinical decision limits (CDLs),[21,22] although they became appreciably lowered by application of the LAVE method, which effectively reduced the influence of metabolic conditions. Further attempt of applying stricter exclusion criteria in the recruitment might match the RIs a little closer to the CDLs, but it makes *a prior* study of the RI impossible with difficulty of recruitment. Therefore, it is important to reduce the influence of latent disease, but we cannot make the ULs identical with the CDLs (Figure 3).

In fact, CDLs were determined by consensus among experts of the diseases targeted by the CDLs for early detection and intervention (based on clinical experiences and epidemiological study), whereas the RIs are determined from individuals with as many healthy attributes as possible so that they serve as references for the healthy status, but they are not meant for distinguishing the healthy from the disease. Therefore, the RIs of these items with known CDLs should not be confused with the RIs. The CDLs should be primarily used for judging the need for preventing or diagnosing the diseases, but the RIs are still necessary for use in other clinical situations which are unrelated to the CDL-associated diseases.

Comparison of RIs With Other Studies

The RIs of CRE, TBil, K, AST, LDH, ALP, GGT, CK, and AMY showed great difference compared with those from the reagent inserts (Table 5). So, laboratories should evaluate the clinical applicability of the RIs from reagent inserts before use.

The RIs of TP, Alb, Urea, CRE, TBil, Na, K, Cl, Ca, IP, MG, Fe, AST, ALT, LDH, ALP, and GGT are close to those from Health occupation standards of China,[23–29] which were approved by the Ministry of Health P. R. China, whereas the RI of AMY is much lower than that from the standard, which may because that the results of AMY were not standardized in our study.

Compared to another multicenter study by Mu et al[9] in China, which aimed at liver function tests, RIs of ALT for females, TBil, AST, and LDH are very close. ULs of ALT and GGT for males are a little higher in our study. This may be

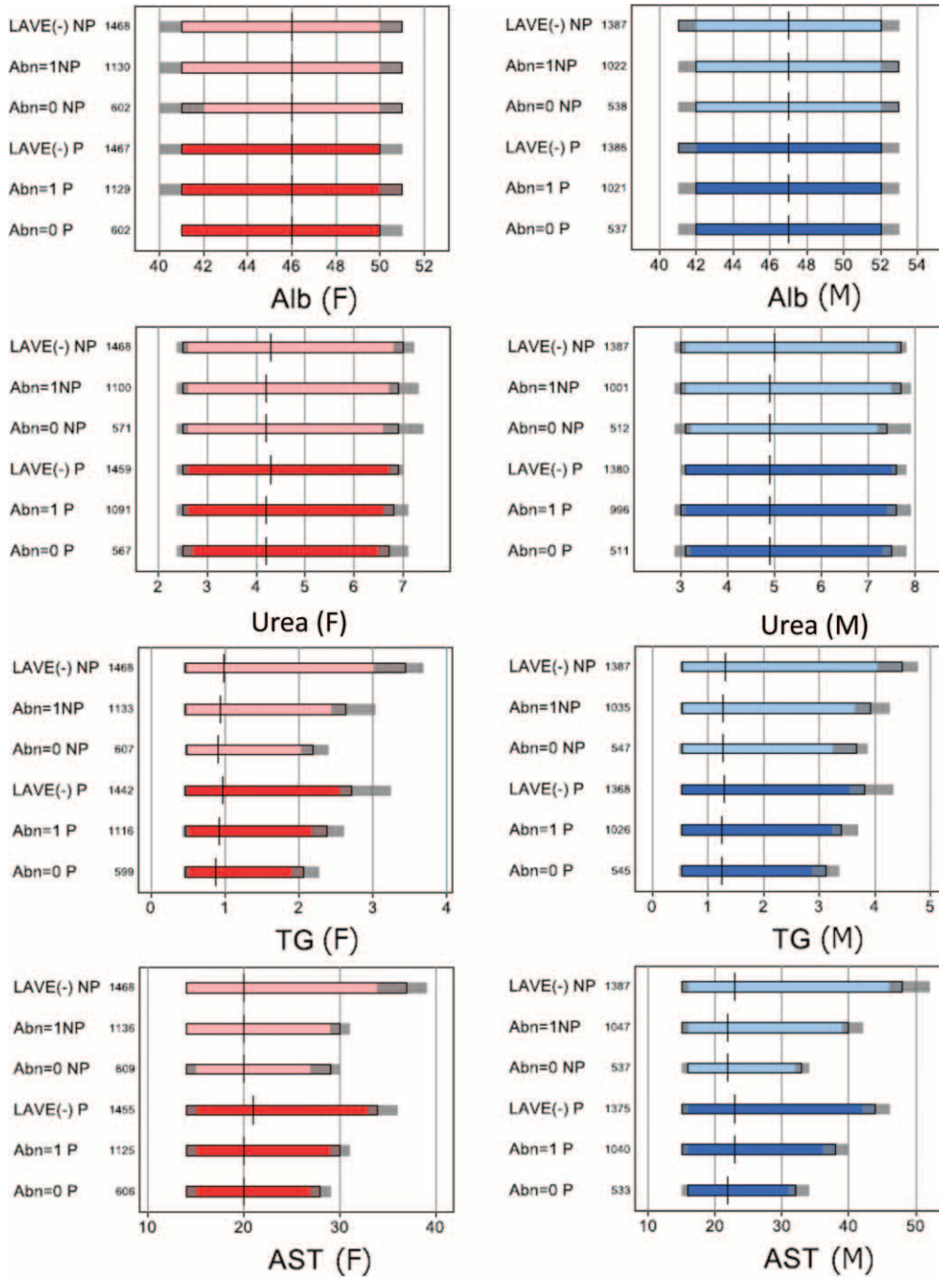

FIGURE 2. Comparison of RIs derived by parametric (P) and nonparametric (NP) methods with or without latent abnormal values exclusion (LAVE) method. The RIs were derived in 6 ways: by P or NP method with LAVE (abn = 1) or LAVE (abn = 0) or without application of LAVE method, where abn = 1 or abn = 0 implies allowance of 1 or no abnormal result among reference test items in accepting each record for use in deriving the RI. Each horizontal bar represents the RI, and the vertical line in the center corresponds to the midpoint. The shades on both ends of the bar represent 90%CI for the limits of the RI predicted by the bootstrap method of 100 repetitive computations. The results for 4 representative analytes for males (M) and females (F) are shown in this figure. The same figures for all other analytes are available in Suppl Figure 2 for M and in Suppl Figure 3 for F. F = females, LAVE = latent abnormal values exclusion, M = males, NP = nonparametric, P = parametric, RI = reference intervals.

attributable to the different exclusion criteria. In Mu's study, those who consumed alcohol >30 g/day and/or with fatty liver disease had been excluded, whereas in our study, alcohol consumption and fatty liver disease were not considered in the recruitment because of planned use of the LAVE method. There were ~2.3% of the participants who consumed >25 g/day in our study. But the UL of GGT for females in Mu's study (43U/L) is about 39% higher than that in our study, which is

TABLE 5. RIs Derived by a Parametric Method With or Without LAVE and Comparison With Those From Reagent Inserts and Other Studies

		RIs Derived by a Parametric Method in Our Study (Beckman, AU)					Reagent Insert (Beckman, AU)			Health Occupational Standard of China (23-29)				Turkey's Study (Abbott,Architect)(30)				Saudi Arabia's Study (Abbott,Architect)(31)		
Analytes	Unit	LAVE	Age	M+F	M	F	M+F	M	F	Age	M+F	M	F	Age	M+F	M	F	M+F	M	F
TP		LAVE (−)		65–79			66–83				65–85				66–82			62–77		
Alb		LAVE (−)		41–52			35–52				40–55				41–49			39–50		
Urea	μmol/L	LAVE (−)	20–49		3.1–7.6	2.4–6.4	2.8–7.2			20–59		3.1–8.0	2.6–7.5	<50		2 95–7 20	2.21–6.12		2.8–7.3	2.1–6.4
			50–64			3.0–7.7				60–79		3.6–9.5	3.1–8.8	>=50			2.85–7.96			
CRE	μmol/L	LAVE (−)			57–102	42–73		64–104	49–90	20–59		57–97	41–73			59–92	50–71		66–111	50–74
										60–79		57–111	41–81							
UA	μmol/L	LAVE (abn = 1)			247–540	178–406		208.3–428.4	154.7–357.0							226–458	166–345		223–444	148–321
TBil	μmol/L	LAVE (−)			7.1–28.8	6.0–22.0	5–21					5.9–30.4	5.0–23.8			3.8–24.1	2.7–15.9		3.6–22.4	2.2–15.5
Glu	mmol/L	LAVE (abn = 1)		4.0–5.9			4.1–5.9								3.96–5.88			4.0–5.9		
TG	mmol/L	LAVE (abn = 1)			0.53–3.43	0.46–2.28	<1.7 (NCEP)							<50		0.53–3.39	0.46–2.52		0.50–3.58	0.39–1.60
														>=50			0.64–3.55			
TC	mmol/L	LAVE (abn = 1)	20–49		3.19–6.16	3.12–5.68	<5.2							<50	3.22–6.45	3.20–6.42	3.20–6.38	3.5–6.36		
			50–64			3.65–6.87								>=50			3.93–7.92			
HDL−C	mmol/L	LAVE (−)			0.80–1.95	0.91–2.18	1.03–1.55 (NCEP)									0.85–1.52	0.95–1.56		0.74–1.76	0.98–2.19
LDL−C	mmol/L	LAVE (abn = 1)	20–49		1.33–3.83	1.28–3.41	<2.6 NCEP							<50	1.47–3.92	1.60–4.01	1.32–3.92	1.80–4.34		
			50–64			1.75–4.43								>=50			1.78–4.91			
Na	mmol/L	LAVE (−)		136–144			136–146				137–147				137–144			135–144		
K	mmol/L	LAVE (−)		3.7–4.7			3.5–5.1				3.5–5.3				3.7–4.9			3.7–4.9		
Cl	mmol/L	LAVE (−)		101–109			101–109				99–110				99–107			101–111		
Ca	mmol/L	LAVE (−)		2.16–2.48			2.20–2.65				2.11–2.52				2.15–2.47			2.11–2.56		
IP	mmol/L	LAVE (−)		0.84–1.48			0.84–1.45				0.85–1.51				0.80–1.40			0.81–1.44		
MG	mmol/L	LAVE (−)		0.78–1.00				0.73–1.06	0.77–1.03		0.75–1.02				0.77–1.06			0.71–0.96		
Fe	μmol/L	LAVE (−)			10–34	5–30		12.5–32.2	10.7–32.2			10.6–36.7	7.8–32.2						7.9–29.6	3.7–26.0
AST	U/L	LAVE (abn = 1)			16–39	14–34		<50	<35			15–40	13–35			13–30	11–25		11–28	10–24
ALT	U/L	LAVE (abn = 1)			10–59	6–34		<50	<35			9–50	7–40			9–57	7–28		7–39	5–18
LDH	U/L	LAVE (abn = 1)	20–49		120–224	112–202		<248	<247		120–250				126–220			10–238		
			50–64			133–233														
ALP	U/L	LAVE (abn = 1)	20–49		50–124	39–96		30–120		20–49		45–125	35–100	<50	38–112	43–116	34–97	39–114		
			50–64			52–127				50–79			50–135	>=50			47–133			
GGT	U/L	LAVE (abn = 1)			10–62	8–31	9–64					10–60	7–45			11–69	7–33		11–65	7–21
CK	U/L	LAVE (abn=1)			65–277	44–180		<171	<145			50–310	40–200			48–227	34–131		54–266	27–138
AMY	U/L	LAVE (−)		29–92			22–80				35–135				34–119			31–117		
Tf	gL	LAVE (−)			1.83–3.02	1.95–3.52	2.0–3.6												2.0–3.2	2.1–3.9
TTR	gL	LAVE (−)			0.21–0.39	0.18–0.32	0.2–0.4													
LIP	U/L	LAVE (−)		7–46			<67													

Alb = albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AMY = amylase, AST = aspartate aminotransferase, BMI = body mass index, Ca = calcium; CK = creatine kinase, Cl = chloride, CRE = creatinine, Fe = iron, GGT = γ-glutamyltransferase, Glu = glucose, HDL-C = high-density lipoprotein-cholesterol, IP = inorganic phosphate, K = potassium, LAVE = latent abnormal values exclusion, LDH = lactate dehydrogenase, LDL-C = low-density lipoprotein-cholesterol, LIP = lipase, Mg = magnesium, Na = sodium, SDR = standard deviations ratio, TBil = total bilirubin, TC = total cholesterol, Tf = transferrin, TG = triglycerides, TP = total protein, TTR = transthyretin, UA = uric acid.

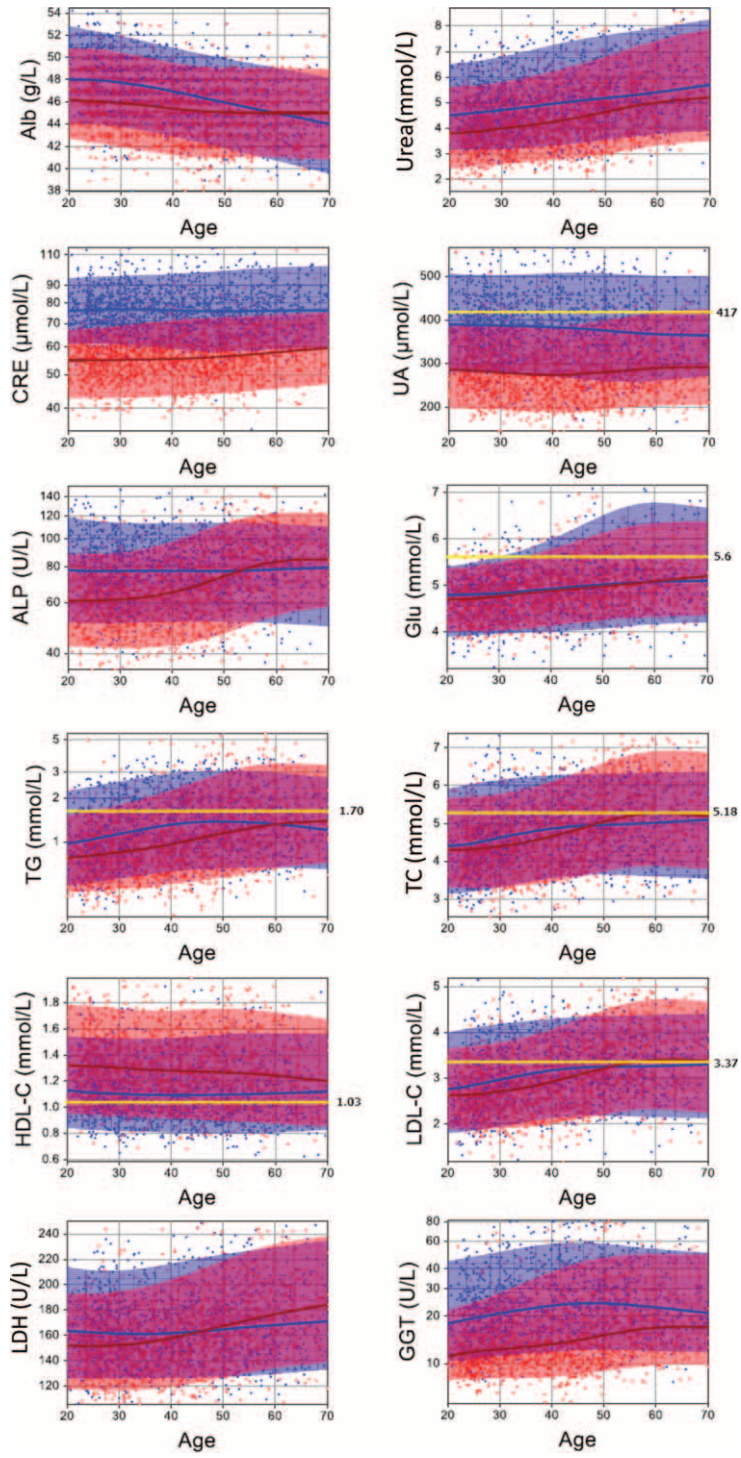

FIGURE 3. Sex- and age-related profiles of reference values. Two-dimensional scattergrams are shown for 12 typical test items with higher SDR_{sex} and/or SDR_{age}. Blue and red dots denote males and females, respectivey, all from individuals without any abnormal results in the reference test items. The central curves of both sexes were drawn by smoothly connecting median values, which were computed at each year-by-year moving age segment of 5-year spans. The outer curves on both sides of the centers represent those of the 2.5 and 97.5 percentiles. Horizontal yellow lines shown in the graphs for UA, Glu, TG, TC, HDL-C, and LDL-C represent clinical decision limits (CDLs) specified in clinical guidelines. CDLs = clinical decision limits, Glu = glucose, HDL-C = high-density lipoprotein-cholesterol, LDH = lactate dehydrogenase, LDL-C = low-density lipoprotein-cholesterol, SDR = standard deviations ratio, TC = total cholesterol, TG = triglycerides, UA = uric acid.

31 U/L with LAVE (abn = 1). We get 43U/L by LAVE(−) method, which is the same as the one by Mu's study. From this finding, it can be said that the LAVE method is more appropriate than Mu's method when applied to those apparently healthy individuals.

The RI of TP differed much between Mu's and our studies. We found that the RIs from Turkey (66 − 82 g/L with Abbott Architect 8000),[30] Saudi (62−77 g/L with Abbott Architect 16000), Hongkong (66−80 g/L with Roche Modular)[8] are close to ours (65−79 g/L) whereas that from Shuo Yang's study(68−86 g/L with Roche Modular)[7] is close to Mu's(67.9−85.1 g/L for 4 cities and 70.1−90.1 g/L for another 2 cities).

The RI of Alb in our study is lower than Mu's study. They used 3 different assay systems and the RIs differed with each other. The final RI was adjusted according to the results from the 3 assay systems. Among the 3 systems, RI from AU system (described as Olympus in the article) is ∼42−52 g/L, which is very close to ours (41−52 g/L). So it is important to have specific RI for each assay system.

As compared to Turkey's and Saudi Arabia's[31] multicenter study which used the same protocol with us, the RIs of TP, Alb, CRE, Glu, Na, K, Cl, Ca, IP, and Mg, Fe, LDH, ALP, GGT are close with each other. Among the 3 countries, Turkey has a highest lipids level, and Saudi Arabia has the lowest ALT level, which appear to be mainly due to difference in ethnicity and life style, and partly due to diffences in assay systems.

In conclusion, this is the largest multicenter RI study in China involving 28 commonly measured tests aimed at adult population, which also is a part of global multicenter collaborative project for derivation of RIs. Age and sex differences were observed in many items and it was important to decide the need for partitioning RIs based on combined analysis of nested-ANOVA and sex and age profile of reference values. No regional differences of practical importance were observed in any of the test items. The LAVE method was indispensable to derive the RIs more appropriately by minimizing the influence of metabolic disorders.

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