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A global multicenter study on reference values: 2. Exploration of sources of variation across the countries

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ABSTRACT

Objectives: The intent of this study, based on a global multicenter study of reference values (RVs) for serum analytes was to explore biological sources of variation (SVs) of the RVs among 12 countries around the world.
Methods: As described in the first part of this paper, RVs of 50 major serum analytes from 13,396 healthy individuals living in 12 countries were obtained. Analyzed in this study were 23 clinical chemistry analytes and 8 analytes measured by immunoturbidimetry. Multiple regression analysis was performed for each gender, country by country, analyte by analyte, by setting four major SVs (age, BMI, and levels of drinking and smoking) as a fixed set of explanatory variables. For analytes with skewed distributions, log-transformation was applied. The association of each source of variation with RVs was expressed as the partial correlation coefficient (r_p).
Results: Obvious gender and age-related changes in the RVs were observed in many analytes, almost consistently between countries. Compilation of age-related variations of RVs after adjusting for between-country differences revealed peculiar patterns specific to each analyte. Judged from the r_p, BMI related changes were observed for many nutritional and inflammatory markers in almost all countries. However, the slope of linear regression of BMI vs. RV differed greatly among countries for some analytes. Alcohol and smoking-related changes were observed less conspicuously in a limited number of analytes.
Conclusion: The features of sex, age, alcohol, and smoking-related changes in RVs of the analytes were largely comparable worldwide. The finding of differences in BMI-related changes among countries in some analytes is quite relevant to understanding ethnic differences in susceptibility to nutritionally related diseases.

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Non-standard abbreviations

Alb	albumin
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AMY	amylase
ARG	Argentina
AST	aspartate aminotransferase
BMI	body mass index
Ca	calcium
CDL	clinical decision limit
CK	creatine kinase
CI	confidence interval
Cl	chloride
CLSI	Clinical and Laboratory Standards Institute
C3	complement component 3
C4	complement component 4
CHN	China
Cre	creatinine
CRM	certified reference materials
CRP	C-reactive protein
CV (b)	CV of the regression slope b
DL	drugs for dyslipidemia; decision limit
DMS	data management system
ETOH	alcohol consumption
Fe	iron
GGT	gamma-glutamyltransferase
Glu	glucose
GBR	Great Britain
GH	growth hormone
HDL-C	HDL-cholesterol
HBV	hepatitis B virus
HCV	hepatitis C virus
HT	drugs for hypertension
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IND	India
IP	inorganic phosphate
JPN	Japan
K	potassium
LAVE	latent abnormal values exclusion
LDH	lactate dehydrogenase
LDL-C	LDL-cholesterol
LL	lower limit
MRA	multiple regression analysis
Me	median
Mg	magnesium
Na	sodium
PAK	Pakistan
PHL	Philippines
RI	reference interval
RMP	reference measurement procedure
RUS	Russia
RV	reference value
r_p	partial correlation coefficient
SAU	Saudi Arabia
SD	standard deviation
SDR	standard deviation ratio
Sk	skewness
Smk	smoking cigarettes
SV	sources of variation
TBil	total bilirubin
TC	total cholesterol
Tf	transferrin
TG	triglycerides
TP	total protein
TUR	Turkey
UA	uric acid
UL	upper limit
ZAF	South Africa
ZAF/Af	African of South Africa
ZAF/NAf	Non-African of South Africa

1. Introduction

The current reference interval (RI) study not only allows determination of RIs but also provides us with an invaluable opportunity to explore and clarify sources of variation (SVs) of each laboratory tests among healthy individuals. In 2004, a multicenter study was conducted in the East and Southeast Asian countries to assess the feasibility of establishing common reference intervals. That study unexpectedly uncovered regional differences in test values for a variety of analytes, especially for inflammatory or nutritional markers, such as IgG, C3, and CRP [1]. To confirm these finding, a large scale study to derive common RIs was conducted in 2009 involving 3500 healthy volunteers recruited by 67 laboratories from 7 countries in East and South-east Asia. By the use of a collective measurement scheme, 72 analytes (25 chemistries and 47 immunoassays) were measured for each specimen [2,3]. The study clearly revealed regional differences in one third of the analytes as determined by use of the criterion of an SD ratio (SDR: between-factor SD divided by between-individual SD) >0.3, which corresponds to the allowable analytical bias based on between-individual SD [4,5]. Notable examples were HDL-C, IgG, C3, CRP, PTH, and folate.

With this background, the current global multicenter study on reference values (RVs) coordinated by the C-RIDL/IFCC was carefully planned with two major objectives: (1) to establish optimal methodologies for derivation of RIs through analysis of real-world datasets gathered from studies around the world, and (2) to explore their SVs including age, gender, BMI, drinking, smoking, geographical regionality and ethnicity. The use of a common protocol with a detailed health-status questionnaire [6], and the scheme of aligning RVs based on the test results of a standard reference serum panel measured in common allowed us to perform the analysis in a well-controlled manner [7]. In the first part of this report on the global study, various methodological issues related to derivation of the RIs were addressed and thoughts on each issue were provided as a consensus among the collaborators [8]. In this second part of the paper, SVs of RVs found in the 12 countries were systematically evaluated, using the same dataset as analyzed in the first part. The main focus of the analyses were as follows:

(1) to evaluate the importance of age, BMI, and levels of alcohol ingestion and smoking as a major SVs of RVs in the various countries (ethnic groups) by use of multiple regression analysis (MRA),
(2) to confirm ethnicity related differences in BMI-related changes in RVs, which were noted for some analytes (ALT, TG, HDL-C, and CRP) in the preliminary analysis [5],
(3) to make a BMI adjusted comparison of RVs among the countries, and
(4) to delineate gender- and age-related profiles of RVs from a large number of datasets compiled from the 12 countries.

2. Methods

2.1. Source data

Included in this interim analysis are the results from 12 countries: China (CHN), Japan (JPN), Philippines (PHL), India (IND), Pakistan (PAK), Saudi Arabia (SAU), Turkey (TUR), Russia (RUS), UK (GBR), South Africa (ZAF), USA, and Argentina (ARG). The demographic profiles of each country's study are as reported in the part 1 of this paper [8]. In brief, the total numbers of subjects were 13,396 (male 6347; female 7049). No ethnicity related distinctions of individuals were made for any countries except South Africa, where RVs were partitioned as Africans and non-Africans. Although the number of individuals above 65 years of age varied greatly, the distributions of ages under 65 were well balanced. Distributions of BMI differed greatly among the countries as shown in Fig. 1. As shown in Table 1 of part 1 [8], the proportions of those who smoked cigarettes were quite comparable among the countries. In contrast, the proportions of individuals who drank alcohol occasionally or regularly differed greatly between countries, partly for religious reasons. In this study of SVs, unlike the study for deriving the