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Parametric and non-parametric estimation of reference intervals for routine laboratory tests: an analysis of health check-up data for 260 889 young men in the South Korean military

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ABSTRACT

Objectives Determination of reference intervals (RIs) using big data faces several obstacles due to heterogeneity in analysers, period and ethnicity. The present study aimed to establish the RIs for routine common blood count (CBC) and biochemistry laboratory tests in homogeneous, healthy, male Korean soldiers in their 20s using a large health check-up data set, comparing parametric and non-parametric estimation.

Design A multicentre, cross-sectional study.

Setting Seven armed forces hospitals in South Korea.

Participants A total of 609 649 men underwent health examination when promoted to corporal between January 2015 and September 2021. 260 889 eligible individuals aged 20–25 were included in the analysis.

Main outcomes and measures The RIs were established by parametric and non-parametric methods. In the parametric approach, maximum likelihood estimation was applied to measure the Box-Cox transformation parameter and the values at the 2.5th and 97.5th percentiles were recalculated. The non-parametric approach adopted the Tukey's exclusion test and the values at the 2.5th and 97.5th percentiles were obtained. Classification by body mass index was also performed.

Results The obtained RIs for haematology parameters were comparable between devices. If the values followed a Gaussian distribution, parametric and non-parametric methods were well matched for haematology and biochemical markers. When the values were right-skewed, the upper limits were higher with parametric than with non-parametric methods. Participants with obesity showed higher RIs for CBC, some liver function tests and some lipid profiles than participants without obesity.

Conclusions Using data from healthy, male Korean soldiers in their 20s, we proposed the RIs for CBC and biochemical parameters, comparing parametric and non-parametric estimation. As such approaches based on large data sets become more prevalent, further studies are needed to discriminate eligible individuals and determine RIs in an extrapolated sample.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study was based on 260 889 healthy, male Korean soldiers in their 20s, representing one of the largest samples in the field of reference intervals.
⇒ The reference intervals for haematology and biochemistry markers were estimated and determined using both parametric and non-parametric estimation.
⇒ The reference intervals were compared between obese and non-obese individuals.
⇒ This study was limited to analysis of reference intervals only in young, healthy military soldiers.

is important in supporting decision-making in clinical practice.[1] RIs have been widely calculated by estimating the 0.025 and 0.975 percentiles, 95% CI, from a healthy population.[2] When using non-parametric values, according to the Clinical Laboratory Standards Institute guidelines, it is considered that a minimum of 120 healthy individuals is required to calculate the 90% CI of the upper and lower reference limits. However, since a large volume of data is easily accessible nowadays, analytical approaches based on high-volume data have been conducted in clinical laboratory medicine.[3] In this regard, extension from a few examinees to a real-world population to overcoming the limitation in numbers might be worthy, although appropriate harmonisation of medical records might be challenging.

Direct sampling is a traditional approach that refers to generating RIs from a preselected population with measurement and determination in order. On the contrary, indirect sampling selects results from routine pathology results in a mixed population, which may contain healthy and unhealthy individuals together. Appropriate statistical techniques, such as the truncated maximum

INTRODUCTION

Establishing the reference intervals (RIs) and the reference values for laboratory markers

likelihood method, are then applied to determine RIs.[4] Data mining, a process of extracting and uncovering new information from a large volume of data, is an indirect method of establishing RIs. However, data from diseased individuals may overlap and this heterogeneity is one of the major hurdles of an indirect method.[4]

Several real-world approaches using 'big data' have been performed to build the RIs for blood sampling results. For example, a single-centre study in Korea evaluated the RI for common blood count (CBC) in 804 623 individuals aged 3–99 using a single haematological device.[5] However, whether unhealthy subjects have been excluded is questionable and the study only estimated the CBC values. Another single 'big data' approach in the Netherlands recruited more than seven million test results and rigorously analysed the RIs for 18 commonly used clinical parameters.[6] However, they presumed a healthy population not based on clinical information but the distribution of test results. The heterogeneity originating from the intralaboratory differences and the mixed distribution of healthy and non-healthy populations made calculating RIs from 'big data' challenging.[7]

In this regard, establishing RIs using a large volume of data in a homogeneous healthy adult group might be worthy. Therefore, the present study aims to analyse the health check-up data of about 260 000 healthy Korean soldiers in their 20s, suggest the RIs for CBC and several chemical parameters, partition the samples by body mass index (BMI), and compare the RIs between parametric and non-parametric methods and between analytical instruments.

METHODS
Study participants
As a result of conscription in South Korea, all male citizens perform compulsory military service and undergo physical examination at the Military Manpower Administration before enlistment in the army. Cases of severe physical or mental illnesses are exempted from mandatory military service. Therefore, men without significant illnesses are likely to be serving in the Korean military. After joining the army, about 1 year later, all Korean soldiers undergo additional physical examination when they are promoted to the rank of a corporal from a private first class.

Electronic medical records between January 2015 and September 2021 from the New Defense Medical Information System of the Korean armed forces database were extracted. The medical data of 609 349 men who underwent medical examination before their promotion to the corporal across 18 armed forces hospitals were acquired. Of the hospitals, three (n=41 113) were excluded due to lack of information on haematological and biochemical test devices. Due to heterogeneity in analytical instruments, we then excluded eight hospitals (n=293 799) and selected seven hospitals that use the biochemical devices of the same company and the haematological equipment

from two different companies. We selected soldiers aged 20–25 with a low probability of having a disease and excluded 13 548 aged <20 or >25. Finally, 260 889 healthy Korean soldiers in their 20s were included in the analysis.

Informed consent was waived due to the study's retrospective nature and because anonymous clinical data were used for analysis.

Laboratory tests and analytical instruments
The results for the following haematology test parameters were collected: haemoglobin (Hb, g/L), haematocrit (Hct, %), red blood cell count (RBC, 10^{12}/L), white cell count (WCC, 10^{9}/L) and platelet (PLT, 10^{9}/L). The XN series (Sysmex, Kobe, Japan) were used in five hospitals. Four of them used an XN-1000 analyser and one hospital used an XN-3000. ADVIA 2120i (Siemens Healthcare Diagnostics, Eschborn, Germany) was used in two hospitals.

The results for the following chemistry parameters were collected: creatinine (mg/dL), aspartate aminotransferase (AST, IU/L), alanine aminotransferase (ALT, IU/L), gamma glutamyl transferase (GGT, IU/L), total cholesterol (mg/dL), triglyceride (mg/dL), high-density lipoprotein (HDL) cholesterol (mg/dL), low-density lipoprotein (LDL) cholesterol (mg/dL) and fasting glucose. The Beckman Coulter AU series (Beckman Coulter, Miami, USA) were used to measure these biochemical parameters in seven hospitals. Of the hospitals, four, two and one used AU-480, AU-680 and AU-5800, respectively.

Routine external quality control management procedures for laboratory tests were performed under the Korean Association of External Quality Assessment Service. All hospitals performed internal quality controls and the analytical devices are calibrated in line with the instructions provided by the manufacturers.

Calculation of RIs
Both parametric and non-parametric methods were used to calculate the RIs for haematological and chemical parameters. Considering there is no consensus on which method is appropriate to draw RIs from large-volume data, we adopted both parametric and non-parametric methods. The non-parametric method was applied using steps similar to the process used in the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) study[8]: (1) distributions of the individual parameters were graphically, preliminary inspected using a quantile-quantile graph and a histogram; (2) the Tukey's exclusion test, where values below Q1–1.5×IQR or above Q3+1.5×IQR are excluded, was used to identify outliers; and (3) the values at the 2.5th and 97.5th percentiles were then defined as the lower and upper limits. The parametric method was applied using the sequences adopted in previous studies by Arzideh et al[9] and Zierk et al[10]: (1) a Box-Cox transformation based on kernel density estimation, which may maximise the likelihood function, was used to transform the data into a symmetric distribution; (2) the distribution's 2.5th and 97.5th percentiles were
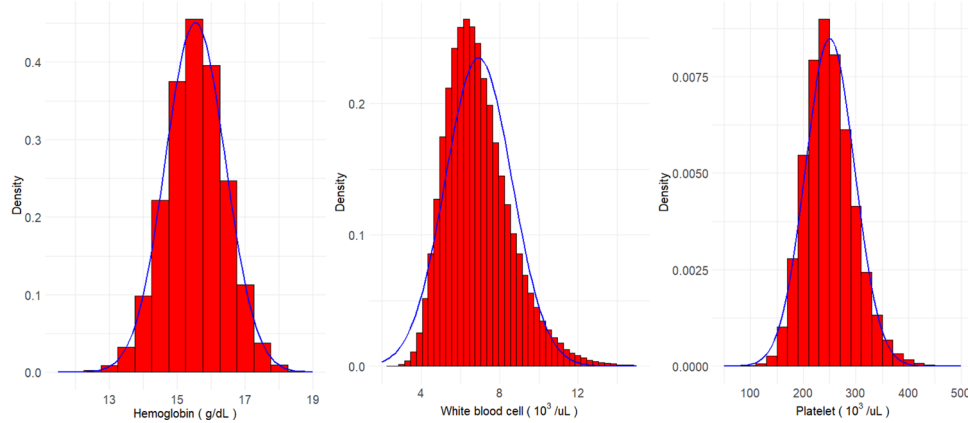
Figure 1 Distribution of haematological markers.

obtained; and (3) the log-transformed value was then recalculated into the real value. In addition, classification according to the presence of obesity, defined by the Korean Society for the Study of Obesity guideline, was performed[11]: non-obese ($<25.0\,\mathrm{kg/m^2}$) and obese (25.0–$30.0\,\mathrm{kg/m^2}$) groups. The Mann-Whitney U test was used to compare differences between obese and non-obese individuals. All statistical analyses were performed using R V.4.1.2 software for Windows (R Development Core Team) and Analyse-it (Analyse-it Software, Leeds, UK).

Patient and public involvement
There is no patient and public involvement.

RESULTS
CBC parameters (eg, Hb, Hct, RBC, WCC and PLT) and biochemical markers (eg, creatinine, AST, ALT, GGT, lipid profiles and fasting glucose) are depicted in figures 1 and 2. WCC and PLT count and most liver

function parameters (eg, AST, ALT and GGT) revealed a non-Gaussian distribution. In terms of lipid profile, triglyceride distribution violated the normality and presented right skewness. These distribution patterns are ascertained in online supplemental tables S1 and S2. The mean value was higher than the median value for WCC, PLT, AST, ALT, GGT and lipid profiles, reflecting a right-skewed distribution.

The RIs for haematology tests determined and established by both parametric and non-parametric methods in healthy Korean soldiers aged 20–25 are tabulated in table 1. Similar RIs between the XN series and ADVIA 2120i were observed in all study subjects. In terms of WCC and PLT, which were distributed with a right tail, the values at the 97.5th percentile were higher in the parametric than in the non-parametric method. In addition, in all examined soldiers, 26.4% were obese and partitioning of RIs according to BMI revealed the values of Hb, Hct, RBC, WCC and PLT to be higher in obese than
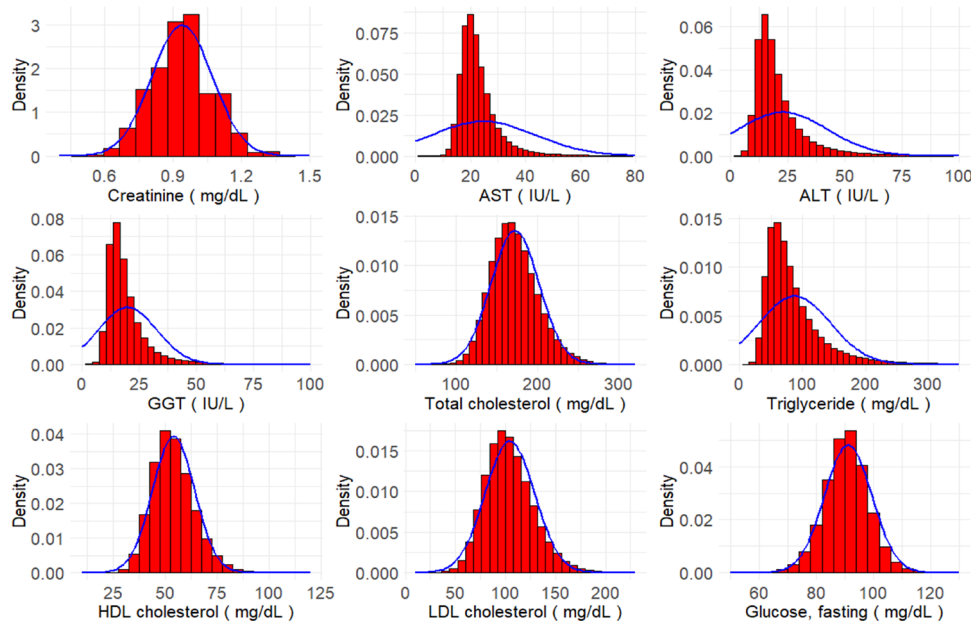
Figure 2 Distribution of creatinine, liver function tests and lipid profiles. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 1 Comparison of RIs for haematological tests in healthy Korean soldiers aged 20–25 years old determined by non-parametric and parametric methods

All

| | XN series (XN-1000 and XN-3000) | | | | | ADVIA 2120i | | | | |
| | | 2.5th percentile | | 97.5th percentile | | | 2.5th percentile | | 97.5th percentile | |
	n	Non-parametric	Parametric	Non-parametric	Parametric	n	Non-parametric	Parametric	Non-parametric	Parametric
Hb (g/L)	180793	139	138	172	172	60466	139	138	172	173
Hct (%)	181524	41.1	40.9	50.5	50.7	60326	41.2	41.0	51.2	51.7
RBC (10¹²/L)	181669	4.6	4.6	5.8	5.8	60900	4.5	4.5	5.7	5.7
WCC (10⁹/L)	179167	4.3	4.3	10.2	10.9	60499	4.3	4.3	9.9	10.5
PLT (10⁹/L)	180909	170	170	339	350	60245	167	167	344	359

Obese (BMI ≥25.0 kg/m²)

| | XN series (XN-1000 and XN-3000) | | | | | ADVIA 2120i | | | | |
| | | 2.5th percentile | | 97.5th percentile | | | 2.5th percentile | | 97.5th percentile | |
	n	Non-parametric	Parametric	Non-parametric	Parametric	n	Non-parametric	Parametric	Non-parametric	Parametric
Hb (g/L)	48537	140	139	173	174	16372	140	139	173	174
Hct (%)	51690	41.4	41.3	50.8	51.0	17396	41.5	41.3	51.6	52.0
RBC (10¹²/L)	51582	4.7	4.6	5.9	5.9	17505	4.6	4.5	5.8	5.8
WCC (10⁹/L)	50971	4.6	4.6	10.6	11.3	17417	4.5	4.5	10.3	10.8
PLT (10⁹/L)	51419	176	175	350	361	17328	172	170	354	370

Non-obese (BMI <25.0 kg/m²)

| | XN series (XN-1000 and XN-3000) | | | | | ADVIA 2120i | | | | |
| | | 2.5th percentile | | 97.5th percentile | | | 2.5th percentile | | 97.5th percentile | |
	n	Non-parametric	Parametric	Non-parametric	Parametric	n	Non-parametric	Parametric	Non-parametric	Parametric
Hb (g/L)	131096	138	137	171	172	44328	138	137	172	172
Hct (%)	130931	41.0	40.8	50.4	50.5	44035	41.0	40.9	51.1	51.5
RBC (10¹²/L)	130648	4.6	4.6	5.8	5.8	44255	4.5	4.5	5.7	5.7
WCC (10⁹/L)	128919	4.2	4.3	10.0	10.8	43970	4.2	4.2	9.7	10.3
PLT (10⁹/L)	130129	169	168	334	345	43742	166	166	338	355

The non-parametric method determined the RIs by excluding the outliers using Tukey's test and adopting the values at the 2.5th and 97.5th percentiles.
The parametric method determined the RIs using the recalculated values at the 2.5th and 97.5th percentiles followed by Box-Cox transformation.
BMI, body mass index; Hb, haemoglobin; Hct, haematocrit; PLT, platelet; RBC, red blood cell; RI, reference interval; WCC, white cell count.

Table 2 Comparison of RIs for biochemistry tests in healthy Korean soldiers aged 20–25 years old determined by non-parametric and parametric methods

		2.5th percentile		97.5th percentile	
All	**n**	**Non-parametric**	**Parametric**	**Non-parametric**	**Parametric**
Creatinine (mg/dL)	245 869	0.7	0.7	1.2	1.2
AST (IU/L)	245 879	14.0	14.6	33.0	51.0
ALT (IU/L)	245 860	9.0	8.9	37.0	69.0
GGT (IU/L)	245 873	9.0	9.0	32.0	49.7
Total cholesterol (mg/dL)	245 699	122.0	122.0	228.0	237.0
Triglyceride (mg/dL)	223 134	34.0	34.5	154.0	232.0
HDL cholesterol (mg/dL)	238 172	37.0	37.0	73.3	76.3
LDL cholesterol (mg/dL)	238 171	63.0	63.0	152.0	159.0
Glucose, fasting (mg/dL)	245 880	76.0	75.0	105.0	107.0
		2.5th percentile		**97.5th percentile**	
Obese (BMI ≥25.0 kg/m^2)	**n**	**Non-parametric**	**Parametric**	**Non-parametric**	**Parametric**
Creatinine (mg/dL)	64 905	0.7	0.7	1.2	1.2
AST (IU/L)	60 668	15.0	15.0	37.0	63.0
ALT (IU/L)	60 096	10.8	11.0	53.0	106.0
GGT (IU/L)	61 029	10.0	10.0	42.7	72.0
Total cholesterol (mg/dL)	64 845	125.0	125.0	241.0	249.0
Triglyceride (mg/dL)	60 449	37.0	37.8	205.0	301.0
HDL cholesterol (mg/dL)	62 943	35.0	35.0	69.0	72.0
LDL cholesterol (mg/dL)	63 170	67.0	67.0	163.0	170.1
Glucose, fasting (mg/dL)	64 496	78.0	77.0	107.0	108.0
		2.5th percentile		**97.5th percentile**	
Non-obese (BMI <25.0 kg/m^2)	**n**	**Non-parametric**	**Parametric**	**Non-parametric**	**Parametric**
Creatinine (mg/dL)	174 499	0.7	0.7	1.2	1.2
AST (IU/L)	166 055	14.0	14.0	32.0	46.0
ALT (IU/L)	165 833	9.0	9.0	32.0	49.0
GGT (IU/L)	167 123	9.0	9.0	28.0	38.0
Total cholesterol (mg/dL)	173 968	121.0	121.0	223.0	230.7
Triglyceride (mg/dL)	161 294	33.0	34.0	137.0	194.0
HDL cholesterol (mg/dL)	168 015	38.0	38.0	74.5	77.6
LDL cholesterol (mg/dL)	168 061	62.0	62.0	145.0	152.0
Glucose, fasting (mg/dL)	173 468	76.0	75.0	105.0	106.0

The non-parametric method determined the RIs by excluding the outliers using Tukey's test and adopting the values at the 2.5th and 97.5th percentiles.

The parametric method determined the RIs using the recalculated values at the 2.5th and 97.5th percentiles followed by Box-Cox transformation.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RI, reference interval.

in non-obese individuals (p<0.001). This level of difference was irrespective of analytical devices and methods.

The established RIs for common chemistry examinations in healthy Korean soldiers in their 20s according to the parametric and non-parametric methods are summarised in table 2. The parametric method presented higher RIs than the non-parametric approach for AST, ALT, GGT and triglyceride levels. Some lipid profiles (eg, total cholesterol,

triglyceride and LDL cholesterol) were higher in obese than in non-obese soldiers (p<0.001), while HDL cholesterol level was lower in obese soldiers (p<0.001).

DISCUSSION

The present study inspected a large volume of data from haematological and biochemical laboratory tests

in 260 889 healthy Korean soldiers aged 20–25, with the individuals classified by obesity status. Both parametric and non-parametric methods were used to calculate the RIs. Within the study period, no significant differences by year were observed. The analytical devices and the population were stable over the study period. However, the distribution of data among the seven hospitals was not equal (online supplemental figure 1). In CBC tests, the estimated RIs were comparable between parametric and non-parametric methods. The estimated RIs showed concordance between analytical instruments, and obesity was associated with the difference in the RIs for haematological tests. The upper limit values of some chemistry markers were higher in the parametric than in the non-parametric method. This might have originated from the right-skewed distribution of raw data, where the Tukey's test excludes a bunch of data in the right tail. In addition, we showed that the RIs for biochemical parameters differed between obese and non-obese individuals.

This study is the largest to suggest RIs, with the strength of evaluating a group of non-diseased adults of a single ethnicity in their 20s. Of all enlisted men within the study period, about 2% were multicultural.[12] Considering more than 3000 individuals are required to derive the RIs for each decade of life in their 20s, 30s and 40s,[13] there is a paucity of data establishing the RIs for the age group in their 20s from a large population. The current results from a large number of samples could be advantageous, considering that a small sample size may produce selection bias[14] and that variation in estimating the RIs for a population is largely affected by the sample size. The established RIs align with the previous results from big data approaches.

An analysis using the data set of the Canadian Health Measures Survey used the non-parametric rank method to establish the RIs for common haematological parameters and showed comparable values for Hb, Hct, RBC, WCC and PLT with the current study.[15] In terms of partitioning, we observed that the RIs for haematological values were higher in obese than in non-obese individuals. Several studies support this observation, linking obesity, proinflammatory cytokines and increased erythropoiesis. Waist circumference was positively correlated with Hb, Hct, RBC, WCC and PLT.[16] A nationwide population-based cohort study revealed that high Hb level was associated with increased OR for metabolic syndrome.[17] We also obtained positive correlation coefficients between BMI and all examined haematological indices with p<0.001 (data not shown). Given that a prediction model proposed an increasing trend in obesity in Korean adults, estimating 62% of men would have obesity by 2030,[18] a careful interpretation of laboratory tests by BMI might be necessary.

The established RIs for AST, ALT, GGT, total cholesterol, triglyceride, LDL cholesterol and fasting glucose levels were higher in obese than in non-obese, while HDL cholesterol was lower in obese participants. Elevated liver enzyme levels are associated with obesity in the adult population.[19] However, due to interlaboratory variability in the upper limit of normal liver enzyme levels, an RI based on strictly defined reference population by different analysers should be established.[20] This study agrees with the previous report regarding lipid profiles, where total cholesterol, LDL cholesterol and triglyceride levels were higher in obese than in non-obese individuals.[21] Therefore, it might be plausible to determine the RIs for liver function tests and lipid profiles based on an individual's clinical condition.

A big data-driven approach to determining RIs has been introduced to clinical laboratory medicine for several years. The application of big medical data has also facilitated advances in bioinformatics, precision medicine, public health informatics and artificial intelligence in imaging.[22] Using stored laboratory results has an advantage in terms of data availability. This merit makes collecting data from specific subpopulations more accessible. For example, Tyler et al[23] showed that the RIs in critically ill patients were significantly different from those in healthy outpatients, indicating cautious decision-making in the intensive care unit based on the RIs from a healthy control. Another merit lies in the representativeness of the RIs from big data. A truly large number of study participants may permit generation of population-representative reference limits. A Canadian nationally representative health survey enrolled 11 999 samples of citizens aged 3–79, and robust RIs spanning from children to the elderly were extracted.[15]

However, several hurdles exist when estimating RIs using big data. This approach does not guarantee high-quality RIs due to mixed, incomplete clinical data. Several statistical methods have been introduced to improve the reliability of RIs. For example, Ammer et al[24] developed the *refineR* algorithm, which can be freely accessible by downloading open-source R package. Other approaches, such as *kosmic* algorithm[25] or the truncated minimum χ^2 method,[26] might improve the generalisability of indirect methods in calculating RIs from real-world data. In addition, data used in the indirect approach may involve noise.[27] It might be very challenging to separate healthy from non-healthy individuals. The intersection point between those individuals could be determined using Box-Cox transformation following kernel density estimation,[10] which was adopted in the current study. Although the range of age covered in this study was only 6 years, the study participants were likely healthy individuals. We used health check-up data from the outpatient setting, which is recommended because this sample selection could minimise the enrolment of patients in acute pathophysiological conditions.[14]

In terms of methods for RI determination, both direct and indirect approaches have been used. The direct method calculates RIs from predefined healthy individuals, whether selecting them before collecting blood samples, a priori, or after, a posteriori. True randomisation would be important in this method, but requires extensive cost, time and effort to represent the target

population. Generally, the direct method recruits small numbers of the population, which could cause selection bias.[4] The indirect method selects a large number of individuals from a mixed population, which may contain not only non-diseased but also diseased individuals, and measures RIs based on robust statistical methods.[4] Because the method could not select healthy individuals using predetermined specific criteria, individuals who are diseased or in a subclinical state could influence the RIs. In this regard, individuals in the current study are large in number, are unlikely to have a disease and are of a single race. This may be advantageous in combining the merits of direct and indirect methods.

This study has several limitations. First, because only healthy military soldiers in their 20s were included in the analysis, data classification according to parameters such as age, sex or ethnicity was not feasible. Second, although individuals in the current study were considered healthy, noise due to unrecognised or subclinical health status could exist. For example, a soldier was healthy at enlistment, but a disease might newly occur before the physical examination. Third, an examination of CBC differential profiles was not performed. Despite these limitations, with sufficiently large sample size, the study showed the RIs for CBC and common clinical chemistry parameters in healthy Korean men in their 20s. Considering sample size planning has been crude in many studies[28] and several obstacles in recruiting appropriate participants may preclude a sufficiently large sample size, this study has merit in providing RIs within homogeneous healthy young adults using an adequately large sample and in showing differences in RIs for haematological markers and common chemical tests between obese and non-obese individuals.

CONCLUSIONS

By analysing a large sample size of healthy soldiers, we suggest the RIs for CBC and common clinical chemistry parameters in homogeneous Korean men in their 20s using parametric but non-parametric methods. We observed differences in the RIs for haematology markers and common biochemical tests between obese and non-obese individuals, and clinicians should interpret the test results cautiously according to their anthropometric values.

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