A nationwide multicentre study in Turkey for establishing reference intervals of haematological parameters with novel use of a panel of whole blood

Yesim Ozarda*[1], Kiyoshi Ichihara[2], Ebubekir Bakan[3], Harun Polat[3], Nurinnisa Ozturk[3], Nurcan K. Baygutalp[3], Fatma Taneli[4], Yesim Guvenc[4], Murat Ormen[5], Zubeyde Erbayraktar[5], Nurten Aksoy[6], Hatice Sezen[6], Meltem Demir[7], Gulcin Eskandari[8], Gurbuz Polat[8], Nuriye Mete[9], Hatice Yuksel[9], Husamettin Vatansev[10], Fatma Gun[10], Okhan Akin[11], Ozlem Ceylan[11], Tevfik Noyan[12], Ozgul Gozlukaya[12], Yuksel Aliyazicioglu[13], Sevim Kahraman[13], Melahat Dirican[1], Gul Ozlem Tuncer[1], Shogo Kimura[2], Pinar Eker[14]

[1]Department of Medical Biochemistry, Uludag University School of Medicine, Bursa, Turkey
[2]Department of Laboratory Sciences, Faculty of Health Sciences, Yamaguchi University Graduate School of Medicine, Ube, Japan
[3]Department of Medical Biochemistry, Ataturk University School of Medicine, Erzurum, Turkey
[4]Department of Medical Biochemistry, Celal Bayar University School of Medicine, Manisa, Turkey
[5]Department of Medical Biochemistry, Dokuz Eylul University School of Medicine, Izmir, Turkey
[6]Department of Medical Biochemistry, Harran University School of Medicine, Urfa, Turkey
[7]Department of Medical Biochemistry, Medicalpark Hospital, Antalya, Turkey
[8]Department of Medical Biochemistry, Mersin University School of Medicine, Mersin, Turkey
[9]Department of Medical Biochemistry, Dicle University School of Medicine, Diyarbakir, Turkey
[10]Department of Medical Biochemistry, Selçuk University School of Medicine, Konya, Turkey
[11]Department of Medical Biochemistry, Keçiören Teaching and Research Hospital, Ankara, Turkey
[12]Department of Medical Biochemistry, Ordu University School of Medicine, Ordu, Turkey
[13]Department of Medical Biochemistry, Karadeniz Teknik University School of Medicine, Trabzon, Turkey
[14]Department of Medical Biochemistry, Kuzey Laboratories, Fatih Sultan Mehmet Hospital, Istanbul

*Corresponding author: yesim@uludag.edu.tr

Abstract

Introduction: A nationwide multicentre study was conducted to establish well-defined reference intervals (RIs) of haematological parameters for the Turkish population in consideration of sources of variation in reference values (RVs).

Materials and methods: K2-EDTA whole blood samples (total of 3363) were collected from 12 laboratories. Sera were also collected for measurements of iron, UIBC, TIBC, and ferritin for use in the latent abnormal values exclusion (LAVE) method. The blood samples were analysed within 2 hours in each laboratory using Cell Dyn and Ruby (Abbott), LH780 (Beckman Coulter), or XT-2000i (Sysmex). A panel of freshly prepared blood from 40 healthy volunteers was measured in common to assess any analyser-dependent bias in the measurements. The SD ratio (SDR) based on ANOVA was used to judge the need for partitioning RVs. RIs were computed by the parametric method with/without applying the LAVE method.

Results: Analyser-dependent bias was found for basophils (Bas), MCHC, RDW and MPV from the panel test results and thus those RIs were derived for each manufacturer. RIs were determined from all volunteers' results for WBC, neutrophils, lymphocytes, monocytes, eosinophils, MCV, MCH and platelets. Gender-specific RIs were required for RBC, haemoglobin, haematocrit, iron, UIBC and ferritin. Region-specific RIs were required for RBC, haemoglobin, haematocrit, UIBC, and TIBC.

Conclusions: With the novel use of a freshly prepared blood panel, manufacturer-specific RIs' were derived for Bas, Bas%, MCHC, RDW and MPV. Regional differences in RIs were observed among the 7 regions of Turkey, which may be attributed to nutritional or environmental factors, including altitude.

Key words: multicentre study; reference intervals; complete blood count; haematology; Turkey

Received: February 09, 2017 *Accepted: May 08, 2017*

Introduction

In recent years, the Committee on Reference Intervals and Decision Limits (C-RIDL) of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) proposed a country-wide multicentre study for the derivation of reference intervals (RIs) in a harmonized way by recruiting a sufficient number of reference individuals together with the use of an issued protocol and standard operating procedures (SOPs) (1,2). The protocol recommends centralized measurements to avoid assay platform dependent differences in test results. For international comparison, the use of a panel of sera is set as the key strategy for aligning test results among laboratories (3). The global RIs project initiated by C-RIDL involving many countries, including Turkey, aimed to promote harmonized derivation of reliable country-specific RIs through multicentre studies and to compare reference values (RVs) among the countries using these strategies (4). We joined the global project and conducted a nationwide multicentre study to establish RIs of the Turkish population for biochemical parameters and to explore sources of variation in RVs, including regionality (5).

After establishing the RIs for biochemical analytes, another multicentre study was initiated to establish RIs for haematological parameters. Haematological parameters, especially the complete blood count (CBC), are the most commonly measured tests in clinical laboratories and it is well known that the RIs of haematological parameters vary with age and gender and require population-specific RIs (6). According to the European Directive 98/79 on *in vitro* diagnostic medical devices, diagnostic kit manufacturers are obliged to supply their clients with appropriate reference RIs for use with their assay platforms and reagents. Furthermore, the International Organization for Standardization Standard 15189 for clinical laboratory accreditation states that each laboratory should periodically re-evaluate its own RIs (7,8). However, despite these facts and requirements, attempts to establish specific RIs for haematology parameters are still uncommon and are applied to insufficient sample sizes. There have been a limited number of attempts (6,9,10) to conduct appropriate multicentre studies to achieve this goal, because with the exception of the concentration of haemoglobin, there are no standard reference materials; native samples must be measured fresh and cannot be measured or re-analysed after storage (9).

Turkey consists of 7 geographical regions, which extend more than 1600 km from the Aegean Sea in the west to the Iranian border in the east. Turkey encompasses an area of 780,580 km^2 with a population of approximately 80 million (11). There are large differences in altitude among the regions, and altitude is well known to have a significant effect on CBC parameters (12). These facts aroused our interest in investigating the RIs of haematological parameters nationwide among the 7 regions of Turkey. The study aimed to 1) establish well-defined RIs of haematological parameters for nationwide use with high precision from a large number of healthy volunteers, 2) evaluate the utility of latent abnormal values exclusion (LAVE) methods for reducing the influence of latent anaemia, 3) explore possible regional differences in the RVs among the 7 regions, and 4) investigate analyser dependent bias in test results by a novel scheme of preparation and common measurement of a panel of fresh blood.

Materials and methods

Subjects

The study was conducted from January 2015 to December 2015. With a recruitment quota of ≥ 400 volunteers per geographical region, a total of 3363 healthy individuals participated in the study; assays were performed by 12 laboratories from the 7 geographical regions of Turkey. Healthy individuals were selected in accordance with the EP28-A3C guideline (13). The target age range was 18 to 79 years. A questionnaire regarding general health and lifestyle was used for the selection of reference individuals. The essential items required for the comparison of the centres are body mass index (BMI), special diet, records of medicines and/or

supplements regularly taken, habits of smoking, alcohol consumption per week (roughly expressed grams of ethanol), and frequency and strength of physical exercise. Exclusion criteria were applied at the time of recruitment according to the IFCC/C-RIDL protocol (2). The volunteers gave written informed consent to participate in the study, and they were informed of the results on request. The study protocol, the contents of the informed consent form, and the general health and lifestyle questionnaire were approved by the Ethics Committee of Uludag University School of Medicine.

Methods

The procedures for blood collection were performed according to the IFCC/C-RIDL protocol (2). The time of the sampling was set at 7–10 am after overnight fasting. For harmonization, the same blood collection tubes made by Becton Dickinson (BD Diagnostics, Oxford, England) were used in all laboratories. For CBC, 2 mL of venous blood was drawn into a vacuum tube containing potassium 2 ethylene-diamine-tetraacetic acid (K_2 EDTA). For iron (Fe), total and unsaturated iron binding capacity (TIBC and UIBC), and ferritin, 5 mL of blood was drawn into a vacuum tube with gel serum separator (SST II) tubes. The sera samples were left thirty to sixty minutes to clot formation prior centrifugation at 1200g for 10 minutes at room temperature and the sera were stored at – 80 ± 2 ºC for up to 6 months until analysis.

Haematological analyses were performed for 20 CBC parameters: white blood cell count (WBC), neutrophil absolute count (Neu), neutrophil percentage (Neu%), lymphocyte absolute count (Lym), lymphocyte percentage (Lym%), monocyte absolute count (Mon), monocyte percentage (Mon%), basophil absolute count (Bas), basophil percentage (Bas%), eosinophil absolute count (Eos), eosinophil percentage (Eos%), red blood cell count (RBC), haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count (PLT) and mean platelet volume (MPV). The EDTA blood samples were analysed within 2 hours in each of the 12 participating laboratories using 4 different analysers from 3 manufacturers: Cell Dyn 3700 and Ruby "A" (Abbott Diagnostics, IL, USA); LH780 "BC" (Beckman Coulter Diagnostics, CA, USA), and Sysmex XT-2000i "S" (Sysmex Corporation, Kobe, Japan). Fe, UIBC and TIBC were analysed in each serum sample using 10 different analysers made by 4 manufacturers as shown in Table 1.

Panels of whole blood and sera

As a key scheme of confirming comparability of test results among the collaborating laboratories, two panels of specimens were produced in a laboratory in Istanbul. One was a panel of whole bloods, and the other was a panel of sera. For the first panel, 21 mL of venous blood was taken into 3 K_2EDTA tubes (7.0 mL draw volume) and for the second panel, 24 mL of blood was collected into gel 3 SST II tubes (8.5 mL draw volume) from each volunteer. The blood collection tubes made by BD (BD Diagnostics, Oxford, England) were used for the preparation of the both panels. Both included specimens freshly prepared from 40 healthy volunteers, but from different individuals for each panel. A total of 12 sets of the blood panels were produced by aliquoting 1.5 mL of blood from each individual into Eppendorf tubes immediately after drawing blood. Similarly, a total of 12 sets of the serum panels were produced by aliquoting 1 mL of serum from each individual into Eppendorf tubes after serum separation. Both blood and serum panels were placed into polystyrene boxes packed with ice bars to keep the temperature between 10–20 °C and were then distributed to each laboratory by airplane or by car within 12 hours after production then measured after the delivery on the same day and at the same time of day in each participating laboratory.

Quality control

Internal and external quality controls (QC) were performed in the participating laboratories to monitor the stability of the assay. The two levels of internal QC materials (low and high control) used for analytical coefficients of variation determina-

Table 1. Analytical systems used for the measurements together with CV_A data

		CV_A, %										Analytical system		
Centre	**Control**	**WBC**	**RBC**	**Hb**	**Hct**	**MCV**	**MCH**	**MCHC**	**RDW**	**PLT**	**MPV**	**Fe**	**Fer**	**CBC**	**Fe**	**Fer**
Bursa	C1	1.98	0.62	0.88	0.84	0.65	0.65	0.47	1.40	2.84	1.19	3.69	3.95	Cell Dyne (A)	Arcitect 16000 (A)	Architect i2000 (A)
	C2	1.67	0.54	0.69	1.01	0.51	0.69	0.45	1.53	2.38	0.79	2.41	3.21			
İzmir	C1	1.95	0.83	0.86	0.80	0.67	0.60	0.42	1.71	2.76	1.22	3.55	3.37	LH780 (BC)	AU 5800 (BC)	Unicell DXI 800 (BC)
	C2	1.80	0.52	0.62	0.92	0.55	0.68	0.44	1.62	2.69	0.96	2.72	3.64			
Manisa	C1	2.44	0.86	1.04	1.25	0.70	0.64	0.50	1.62	3.42	1.75	3.81	4.78	LH780 (BC)	ADVIA 1800 (S)	ADVIA Centaur (S)
	C2	2.16	0.94	0.74	1.00	0.63	0.70	0.52	1.63	2.78	1.14	2.86	4.02			
Antalya	C1	1.93	0.76	1.11	0.93	0.59	0.61	0.48	1.69	3.12	1.32	3.05	3.24	2000i (S)	Cobas Integra800(S)	Cobas E601(S)
	C2	2.01	0.60	0.78	0.98	0.65	0.67	0.49	1.64	2.95	0.99	2.93	3.55			
Mersin	C1	1.79	0.74	0.94	1.22	0.69	0.62	0.65	1.45	3.15	0.98	3.44	4.04	2000i (S)	Arcitect 8000 (A)	ADVIA Centaur (S)
	C2	1.90	0.88	0.70	1.18	0.64	0.64	0.59	1.68	3.40	1.14	3.79	3.77			
Diyarbakır	C1	1.84	0.92	0.85	0.97	0.61	0.54	0.42	1.36	2.87	1.01	2.57	3.29	Rubi (A)	Arcitect 16000 (A)	Cobas E601(R)
	C2	1.61	0.90	0.87	1.11	0.58	0.59	0.54	1.67	2.65	1.17	2.89	3.76			
Urfa	C1	1.82	0.83	1.15	0,95	0.60	0.59	0.39	1.55	3.26	1.73	3.05	3.91	Cell Dyne (A)	Arcitect 16000 (A)	Architect i2000 (A)
	C2	1.76	0.74	0.81	1.26	0.50	0.64	0.48	1.71	2.93	1.60	3.39	3.88			
Ankara	C1	2.42	0.81	0.73	0.82	0,62	0.67	0.51	1.64	2.77	1.45	4.13	3.76	LH 780 (BC)	AU 680 (BC)	Unicell DXI 800 (BC)
	C2	1.95	0.53	0.69	1.10	0.57	0.69	0.47	1.55	2.90	1.22	3.92	3.50			
Konya	C1	1.99	0.93	1.20	0.84	0.69	0.61	0.44	1.28	2.65	1.14	3.60	4.19	Cell Dyne (A)	Arcitect 16000 (A)	Cobas E601(S)
	C2	1.78	0.77	0.92	0,98	0.61	0.68	0.49	1.49	2.47	0.85	3.01	3.90			
Erzurum	C1	1.79	0.71	1.14	0.88	0.59	0.67	0.40	1.54	2.82	1.27	3.76	3.79	LH 780 (BC)	AU 5800 (BC)	Unicell DXI 800 (BC)
	C2	2.15	0.50	0.77	1.00	0.51	0.63	0.41	1.40	2.65	0.78	3.11	3.35			
Ordu	C1	2.03	0.89	1.09	0.99	0.68	0.59	0.49	1.19	3.33	0.96	2.95	4.41	Cell Dyne (A)	Arcitect 8000 (A)	Architect i2000 (A)
	C2	1.96	0.75	0.82	1.00	0.64	0.62	0.45	1.49	3.27	1.09	2.87	3.63			
Trabzon	C1	1.64	0.80	0.93	1.29	0.63	0.60	0.53	1.30	2.93	1.02	3.16	3.84	LH 780 (BC)	AU 5800 (BC)	Unicell DXI 800 (BC)
	C2	2.06	0.54	0.70	1.34	0.51	0.61	0.52	1.74	2.42	0.94	2.88	3.52			

The desirable limits for CV_As were set as half of within-individual biologic variation (CV_I) as reported on the Westgard website; https://www.westgard.com/biodatabase1.ht (WBC = 11.4, RBC = 3.2, Hb = 2.85, Hct = 2.7, MCV = 1.4, MCH = 1.4, MCHC = 1.06, RDW = 3.5, PLT = 9.1, MPV = 4.3, Fe = 26.5, Fer = 14.2). CV_A - analytical variation. C1 - control 1 (low). C2 - control 2 (normal). A - Abbott, BC - Beckman Coulter, S - Sysmex, R – Roche.

tion were supplied by A (Abbott Diagnostics, IL, USA) for A users, BC (Beckman Coulter Diagnostics, CA, USA) for BC users, and S (Sysmex Corporation, Kobe, Japan) for S users. Randox International Quality Assessment Scheme (RIQAS) Haematology External Quality Assessment (EQA) Programme was used in all the participating laboratories. The analytical coefficient of variation (CV_A) was computed for each analyte from the results of repeated measurements of the internal quality control material measured in each laboratory. The desirable limits for between-day and within-day CV_As were set as a half of the within-individual CV (CV_I) reported on the Westgard website (14). The within- and between-day CV_As for all analytes, listed in Table 1, did not exceed the desirable limits.

Statistical analysis

In order to evaluate the magnitude of between-laboratory bias in test results of the blood/serum panel or those of volunteers' samples, the standard deviation (SD) representing between-laboratory variation (SD_{BL}) was computed based on one-way ANOVA. The relative magnitude of SD_{BL} to that of residual SD (or net between-individual SD: SD_{BI}) was computed as the SD ratio (SDR): $SDR_{BL} = SD_{BL} / SD_{BI}$. For detailed analysis of sources of variation of RVs, SDRs for between-gender (SDR_{gender}), between-age subgroup differences (SDR_{age}) and between-region (SDR_{BR}), were computed based on 3-level nested ANOVA (15). In the analysis of Eos, Eos%, Bas, Bas%, and ferritin, test results were transformed logarithmically because of their skewed distribution patterns. For those parameters, any subset of SD derived in the logarithmic scale (SD^T) was back-transformed (16).

Multiple regression analysis (MRA) was performed to identify factors possibly associated with the test results, including age, BMI, altitude of the regions above sea level, and level of cigarette smoking, alcohol drinking and physical exercise. In the analysis, dummy variables representing the Turkish regions, with Marmara set as the reference region, were also introduced to adjust for any possible influence of place of residence on RVs.

Judgment of analytical bias among the laboratories from the panel test results

Between-laboratory SDR computed from the panel test results (SDR_{BL1}) was used to assess the analyser dependent bias in test results among the laboratories. We adopted SDR > 0.30 as a guide value for judging the analytical bias among the laboratories. If there was only one laboratory showing an obvious bias, we excluded the panel test results from that laboratory and recomputed the SDR_{BL1}. If SDR_{BL1} remained > 0.30, we then checked for the consistency of the findings in volunteers' test results (SDR_{BL2}) as described below before deciding on the need for haematology analyser specific analysis of RVs.

The criterion for partitioning reference values and derivation of reference intervals

In the absence of bias in the panel test results ($SDR_{BL1} \leq 0.3$), SDR_{BL2} of > 0.3 was regarded as a regional difference requiring partition for the derivation of RIs. For the parameters found to have large between-manufacturer differences ($SDR_{BM} > 0.3$) in the panel test results, we partitioned the RVs by manufacturer.

The lower and upper limits (LL and UL) of the RIs were derived by the parametric method after normalizing the data distribution using the modified Box-Cox power transformation method (15). The 90% confidence intervals (CIs) for LL and UL were estimated by use of the bootstrap method through iterative resampling 100 times. Using this procedure, the final LL and UL were set as the average after 100 iterations.

As a method for secondary exclusion of RVs to cope with a high prevalence of latent anaemia, the LAVE method was applied by allowing one abnormal result in 7 reference test items (Hb, Hct, MCV, Fe, UIBC, TIBC, and ferritin) which reflect anaemic disorders (15-17). Thus, the RIs were derived in two ways, either with or without the LAVE method. The choice between the two RIs was made by the ratio of the difference in the two LLs (or ULs) to the SD comprising the RI, which corresponds to between-individual SD (SD_{BI}), as follows (17):

$$\Delta LL \text{ ratio} = | LL_{-} - LL_{+} | / (UL_{+} - LL_{+}) / 3.92$$

$$\Delta UL \text{ ratio} = | UL_{-} - UL_{+} | / (UL_{+} - LL_{+}) / 3.92$$

where LL_{+}, LL_{-} (or UL_{+}, UL_{-}) represent LL (or UL) determined with/without the LAVE method, respectively. We set the critical value for ΔLL (or ΔUL) ratio as 0.25 in analogy to the theory of acceptable analytical bias in laboratory tests since the numerator of ΔLL (or ΔUL) ratio is a bias by the choice of derivation method and the denominator corresponds to SD_{BI} (14).

Results

Analytical bias in test results among the laboratories

The age and gender distributions of the participants from the 7 regions of Turkey are shown in Table 2. The male to female ratio was close to 1.0. The majority of participants (2914; 86.6% of the total) were between 20 and 59 years old (Table 2).

To see any analyser dependent bias in the measurements among the 12 laboratories, the between-laboratory SDR for the panel test results (SDR_{BL1}) was computed as shown in Column 2 of Table 3. $SDR_{BL1} > 0.3$ was noted for 10 parameters (Neu, Neu%, Mon, Mon%, Bas, Bas%, MCV, MCHC, RDW, and MPV). The implication of the bias was then evaluated in reference to the actual distributions of the panel test results among the 12 laboratories as shown in Figure 1.

For Neu and Neu%, an obvious bias in measurements from Urfa was identified in Figure 1- {2,3} due to an unknown technical problem. However, removal of the results led to a reduction in SDR_{BL1} from 0.48 to 0.26 for Neu, and from 0.60 to 0.00 for Neu%. On the other hand, the between-laboratory SDRs for Neu and Neu% based on volunteers' test results (SDR_{BL2}) shown in Column 6 of Table 3 were 0.20 and 0.15, respectively. Therefore, we judged that neither analyser dependent bias nor regional difference existed for Neu and Neu%, and thus all the results from the 12 laboratories could be combined to derive the RIs.

For MCV, we observed in Figure 1 - {15} that there was a similar problem of bias in the measurements from Mersin and again removal of the results led

Table 2. Age and gender of the volunteers from the 7 regions of Turkey

Gender, N	Region	18-29 y	30-39 y	40-49 y	50-59 y	60-69 y	70-79 y	Total (N)
	Aegean	46	36	37	49	15	6	189
	Black Sea	63	75	73	56	31	1	299
	Central Anatolia	63	89	55	38	38	7	290
Male, 1614	Eastern Anatolia	63	61	47	25	20	7	223
	Marmara	35	52	48	21	6	11	173
	Mediterranean	40	36	32	37	20	21	186
	Southeastern Anatolia	58	57	61	43	26	9	254
	Aegean	42	64	41	48	35	3	233
	Black Sea	61	77	65	44	26	1	274
	Central Anatolia	76	73	68	59	34	11	321
Female, 1746	Eastern Anatolia	72	60	55	32	14	9	242
	Marmara	55	74	67	25	11	1	233
	Mediterranean	42	45	41	44	20	28	220
	Southeastern Anatolia	48	37	48	53	38	2	226
Total (N)		764	836	738	574	334	117	3363

y – years old.

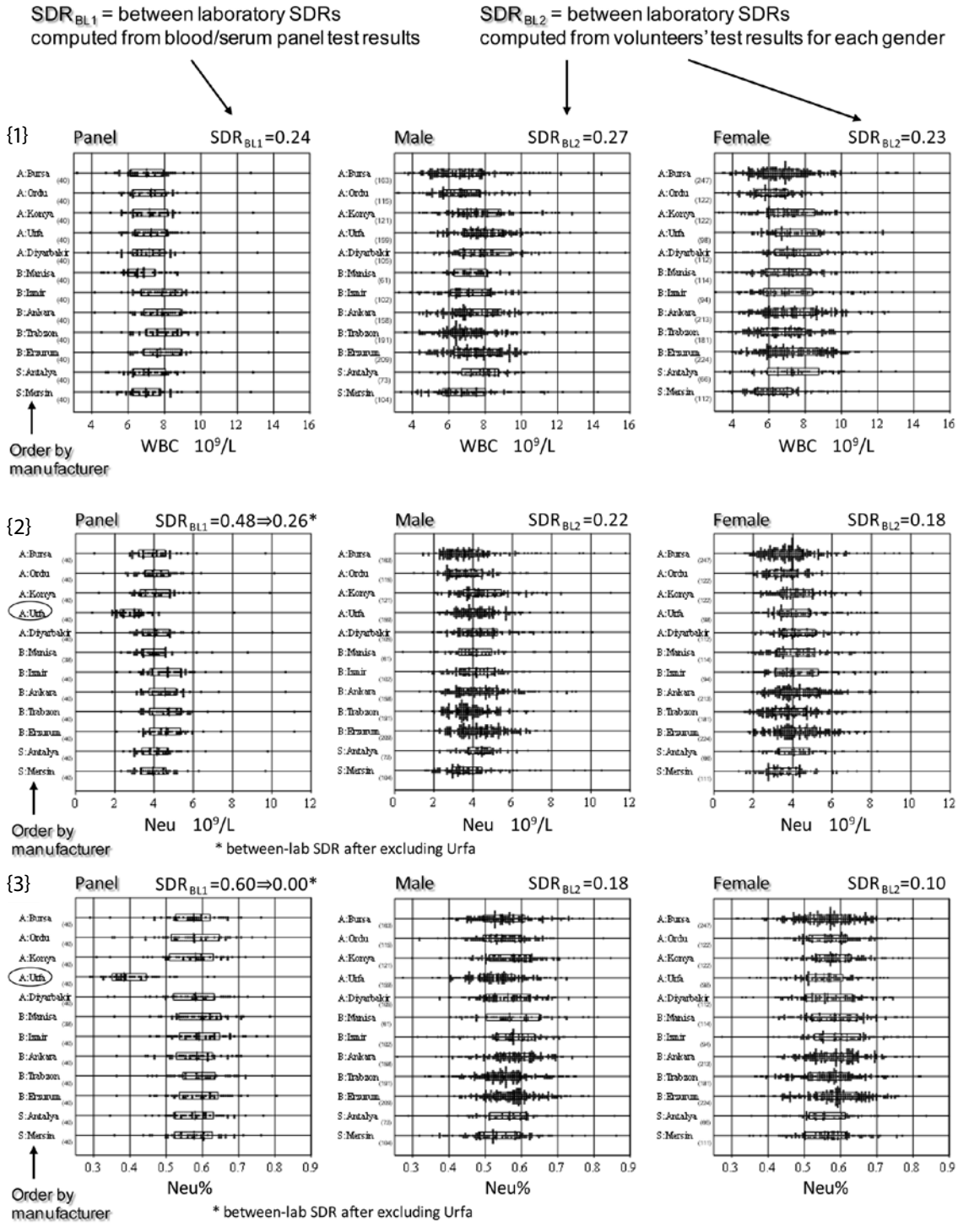
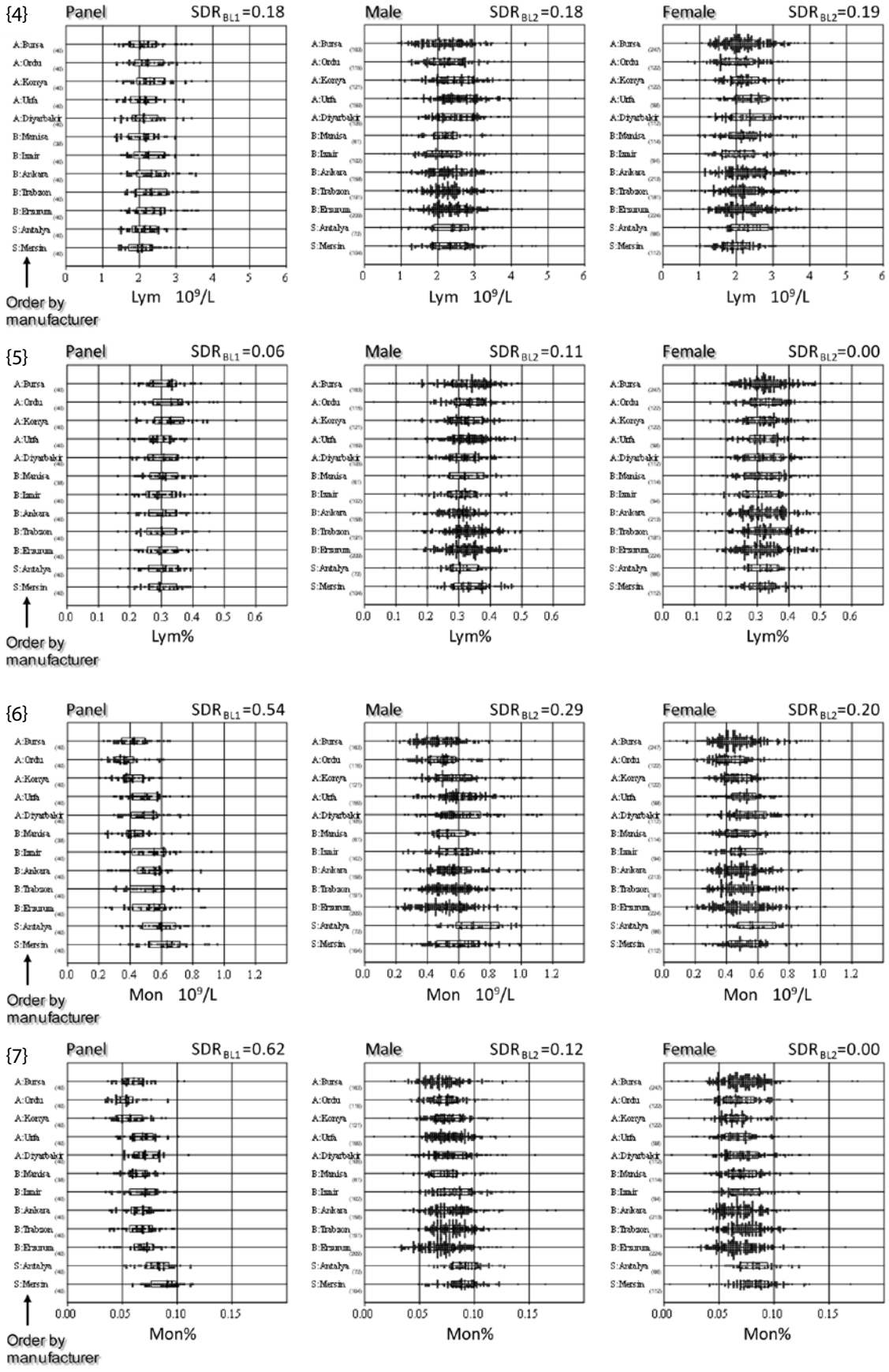
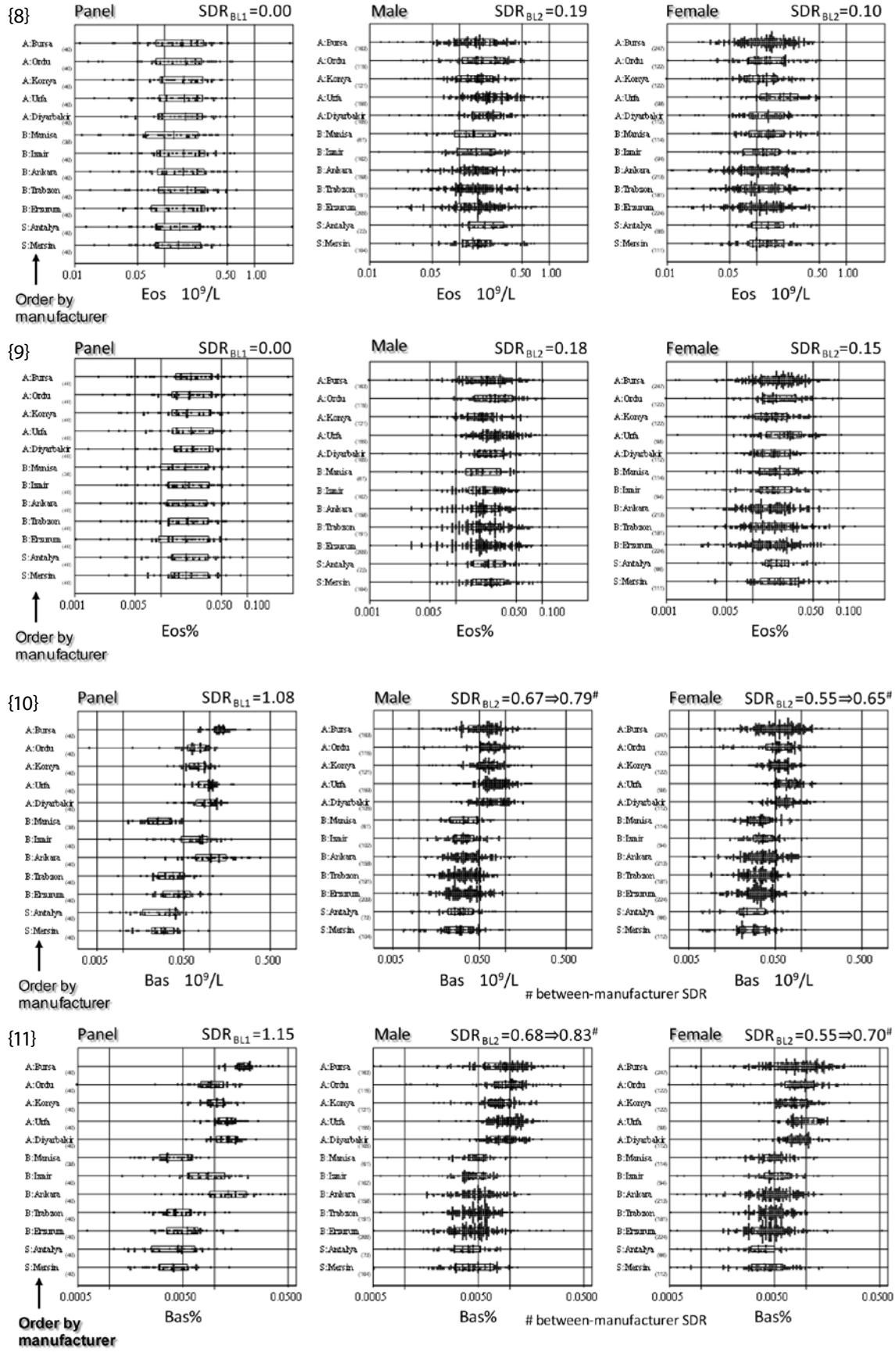
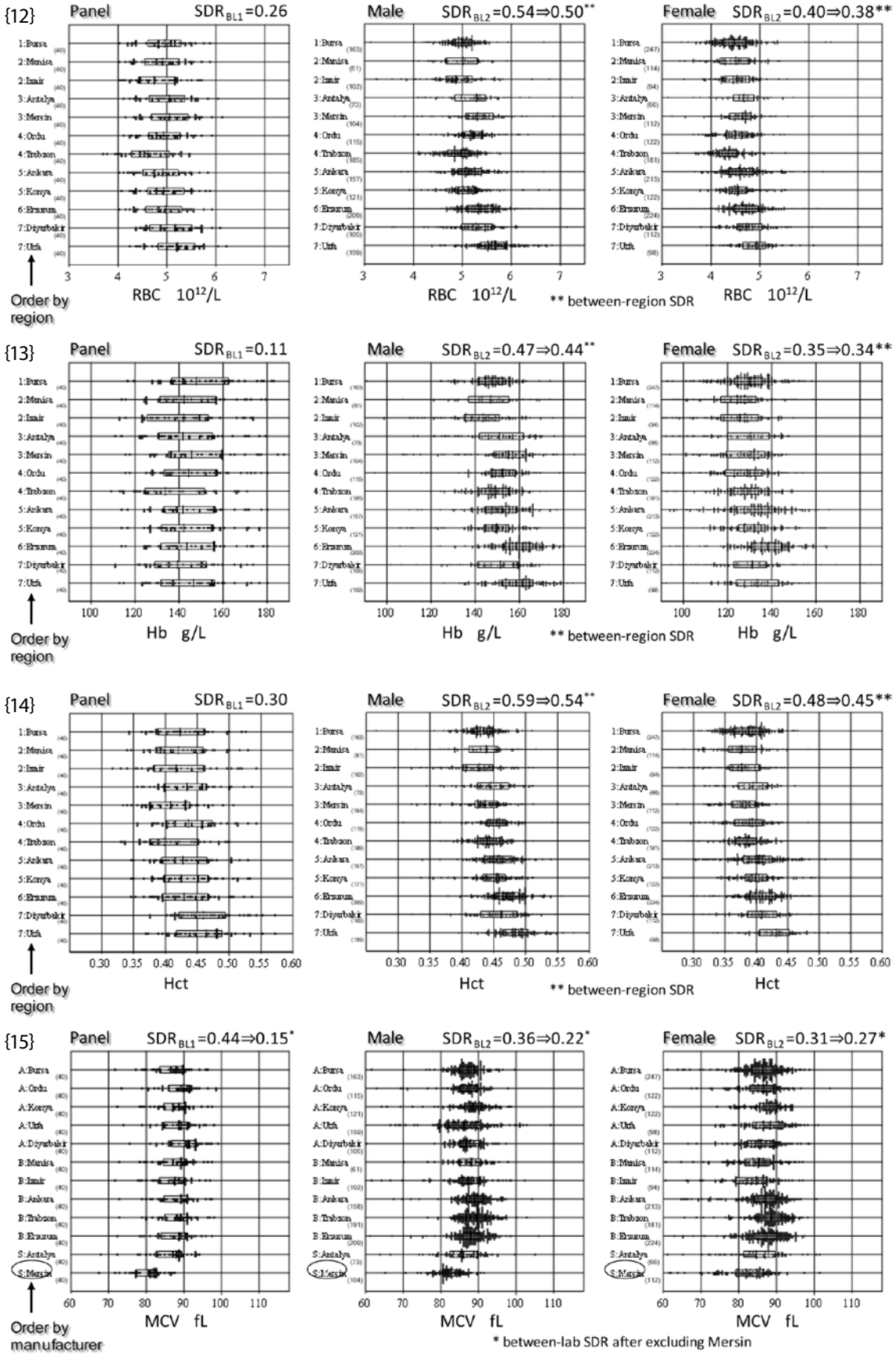
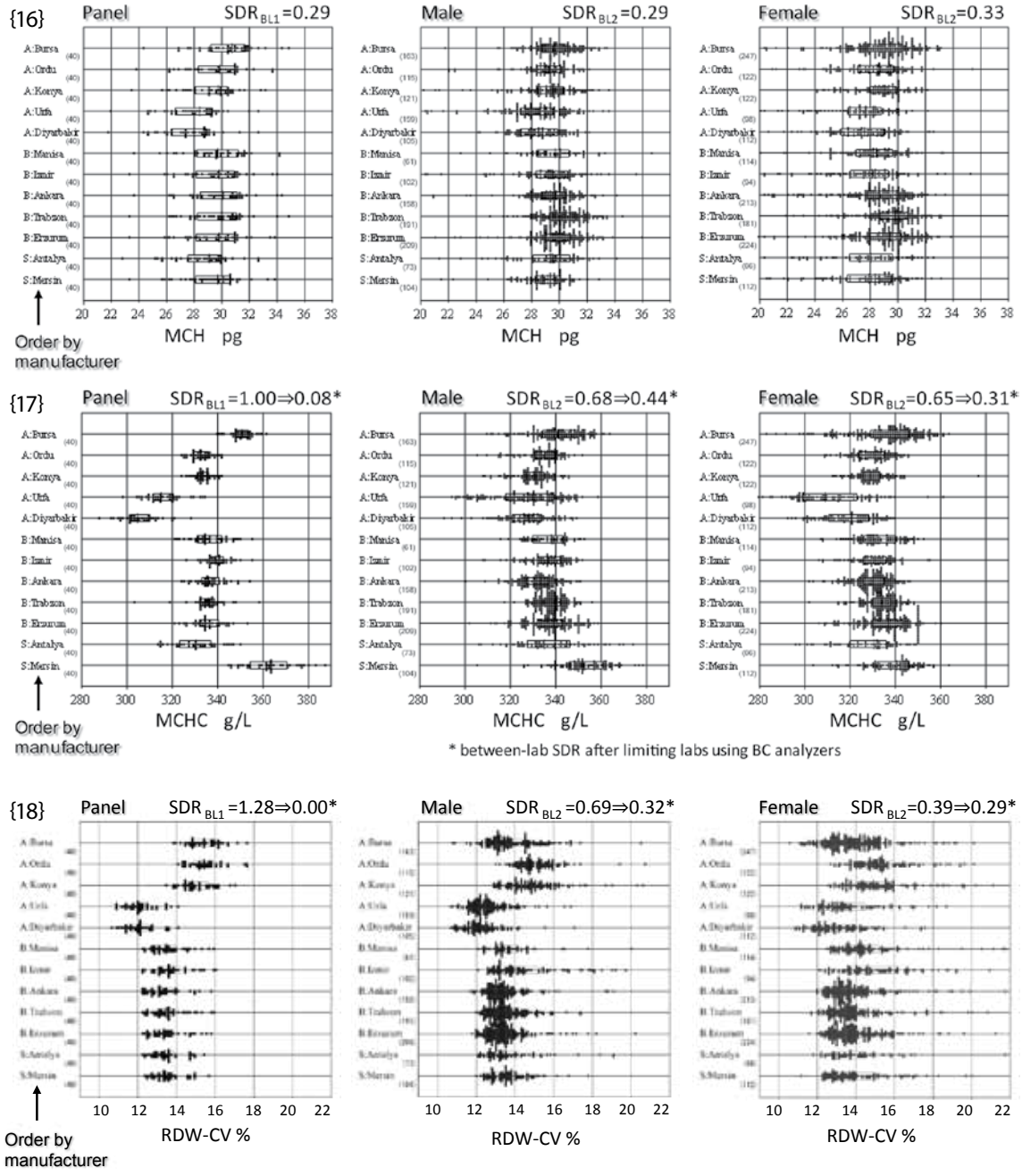


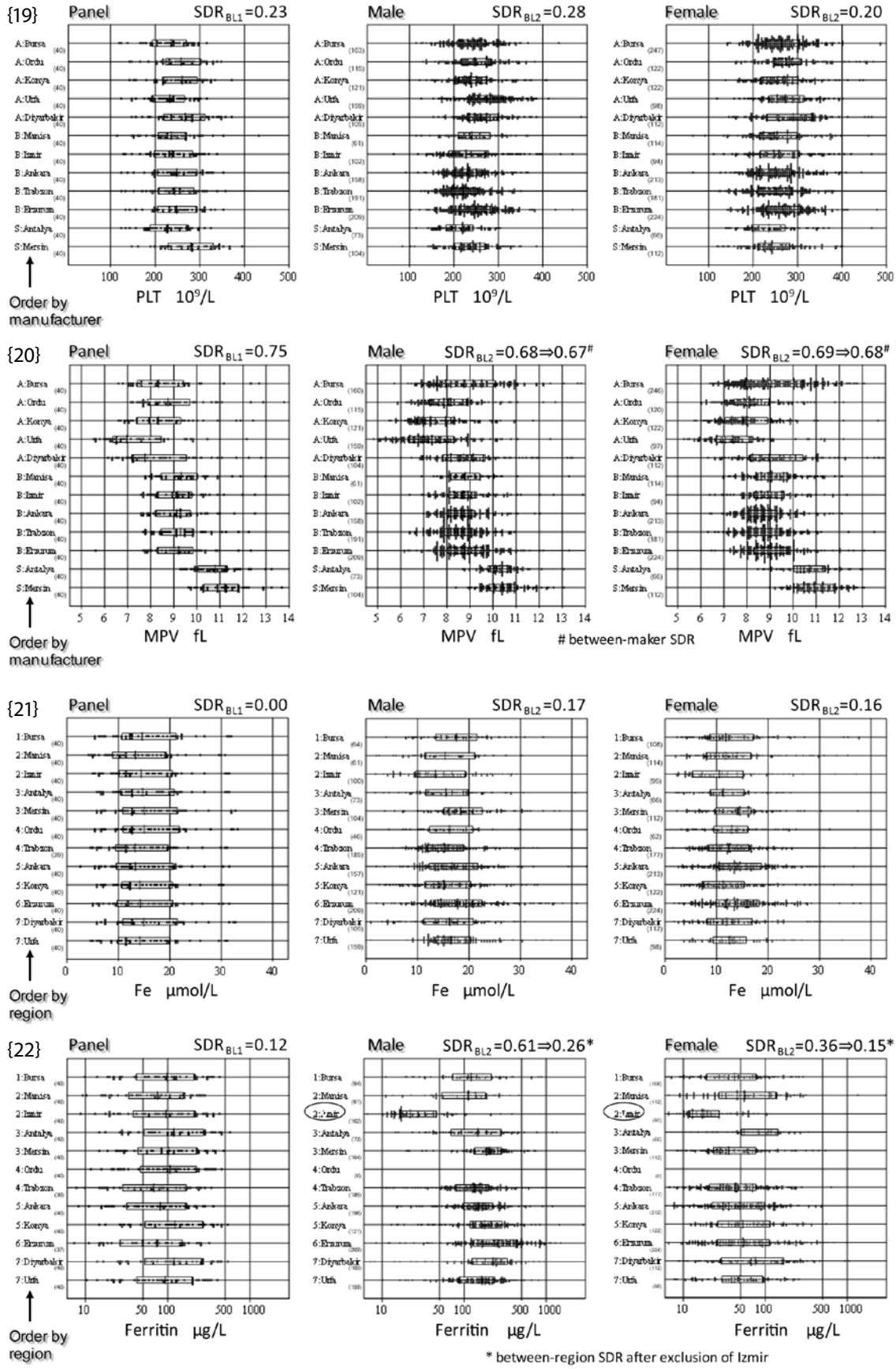
Figure 1. Between-laboratory comparison of test results for the blood/serum panel and volunteers' samples
For all 12 laboratories, the distributions of test results for all haematological parameters were drawn for the blood/serum panels (left graphs) and for volunteers' test results of males and females (middle and right graphs). The 12 laboratories are placed in the order of the manufacturers (A: Abbott; BC: Beckman Coulter; S: Sysmex), for WBC, Neu, Neu%, Lym, Lym%, Mon, Mon%, Eos, Eos%, Bas, Bas%, MCV, MCH, MCHC, RDW, PLT and MPW but, for RBC, Hb, Hct, Fe, Ferritin, UIBC and TIBC due to our judgement of regional differences, the laboratories are aligned in the order of geographical regions (1: Marmara, 2: Aegean, 3: Mediterranean, 4: Black Sea, 5: Central Anatolia, 6: East Anatolia, 7: South East Anatolia).
The horizontal box in each scattergram represents the central 50% range, and the vertical line in the centre denotes the median point.


{4}
Panel
SDR BL1 =0.18
Male
SDR BL2 =0.18
Female
SDR BL2 =0.19
A:Bursa
A:Ordu
A:Konya
A:Urfa
A:Diyarbakir
B:Manisa
B:Izmir
B:Ankara
B:Trabzon
B:Erzurum
S:Antalya
S:Mersin
0 1 2 3 4 5 6
Lym 10^9/L
Order by manufacturer
{5}
Panel
SDR BL1 =0.06
Male
SDR BL2 =0.11
Female
SDR BL2 =0.00
0.0 0.1 0.2 0.3 0.4 0.5 0.6
Lym%
Order by manufacturer
{6}
Panel
SDR BL1 =0.54
Male
SDR BL2 =0.29
Female
SDR BL2 =0.20
0.0 0.2 0.4 0.6 0.8 1.0 1.2
Mon 10^9/L
Order by manufacturer
{7}
Panel
SDR BL1 =0.62
Male
SDR BL2 =0.12
Female
SDR BL2 =0.00
0.00 0.05 0.10 0.15
Mon%
Order by manufacturer


{8}
Panel
SDR BL1 =0.00
Male
SDR BL2 =0.19
Female
SDR BL2 =0.10
A:Bursa
A:Ordu
A:Konya
A:Urfa
A:Diyarbakir
B:Manisa
B:Izmir
B:Ankara
B:Trabzon
B:Erzurum
S:Antalya
S:Mersin
0.01
0.05
0.50
1.00
Eos 10^9/L
Order by manufacturer
{9}
Panel
SDR BL1 =0.00
Male
SDR BL2 =0.18
Female
SDR BL2 =0.15
0.001
0.005
0.050
0.100
Eos%
Order by manufacturer
{10}
Panel
SDR BL1 =1.08
Male
SDR BL2 =0.67⇒0.79#
Female
SDR BL2 =0.55⇒0.65#
0.005
0.050
0.500
Bas 10^9/L
Order by manufacturer
between-manufacturer SDR
{11}
Panel
SDR BL1 =1.15
Male
SDR BL2 =0.68⇒0.83#
Female
SDR BL2 =0.55⇒0.70#
0.0005
0.0050
0.0500
Bas%
Order by manufacturer
between-manufacturer SDR


{12}
Panel
SDR BL1 =0.26
Male
SDR BL2 =0.54⇒0.50**
Female
SDR BL2 =0.40⇒0.38**
RBC 10^12/L
** between-region SDR
Order by region
{13}
Panel
SDR BL1 =0.11
Male
SDR BL2 =0.47⇒0.44**
Female
SDR BL2 =0.35⇒0.34**
Hb g/L
** between-region SDR
Order by region
{14}
Panel
SDR BL1 =0.30
Male
SDR BL2 =0.59⇒0.54**
Female
SDR BL2 =0.48⇒0.45**
Hct
** between-region SDR
Order by region
{15}
Panel
SDR BL1 =0.44⇒0.15*
Male
SDR BL2 =0.36⇒0.22*
Female
SDR BL2 =0.31⇒0.27*
MCV fL
* between-lab SDR after excluding Mersin
Order by manufacturer


{16}
Panel
SDR BL1 =0.29
Male
SDR BL2 =0.29
Female
SDR BL2 =0.33
A:Bursa
A:Ordu
A:Konya
A:Urfa
A:Diyarbakir
B:Manisa
B:Izmir
B:Ankara
B:Trabzon
B:Erzurum
S:Antalya
S:Mersin
20 22 24 26 28 30 32 34 36 38
MCH pg
Order by manufacturer
{17}
Panel
SDR BL1 =1.00⇒0.08*
Male
SDR BL2 =0.68⇒0.44*
Female
SDR BL2 =0.65⇒0.31*
280 300 320 340 360 380
MCHC g/L
Order by manufacturer
* between-lab SDR after limiting labs using BC analyzers
{18}
Panel
SDR BL1 =1.28⇒0.00*
Male
SDR BL2 =0.69⇒0.32*
Female
SDR BL2 =0.39⇒0.29*
10 12 14 16 18 20 22
RDW-CV %
Order by manufacturer
* between-lab SDR after limiting labs using BC and Sy analyzer


{19}
Panel
SDR$_{BL1}$=0.23
Male
SDR$_{BL2}$=0.28
Female
SDR$_{BL2}$=0.20
PLT 10^9/L
Order by manufacturer
{20}
Panel
SDR$_{BL1}$=0.75
Male
SDR$_{BL2}$=0.68⇒0.67#
Female
SDR$_{BL2}$=0.69⇒0.68#
MPV fL
between-maker SDR
Order by manufacturer
{21}
Panel
SDR$_{BL1}$=0.00
Male
SDR$_{BL2}$=0.17
Female
SDR$_{BL2}$=0.16
Fe µmol/L
Order by region
{22}
Panel
SDR$_{BL1}$=0.12
Male
SDR$_{BL2}$=0.61⇒0.26*
Female
SDR$_{BL2}$=0.36⇒0.15*
Ferritin µg/L
Order by region
* between-region SDR after exclusion of Izmir

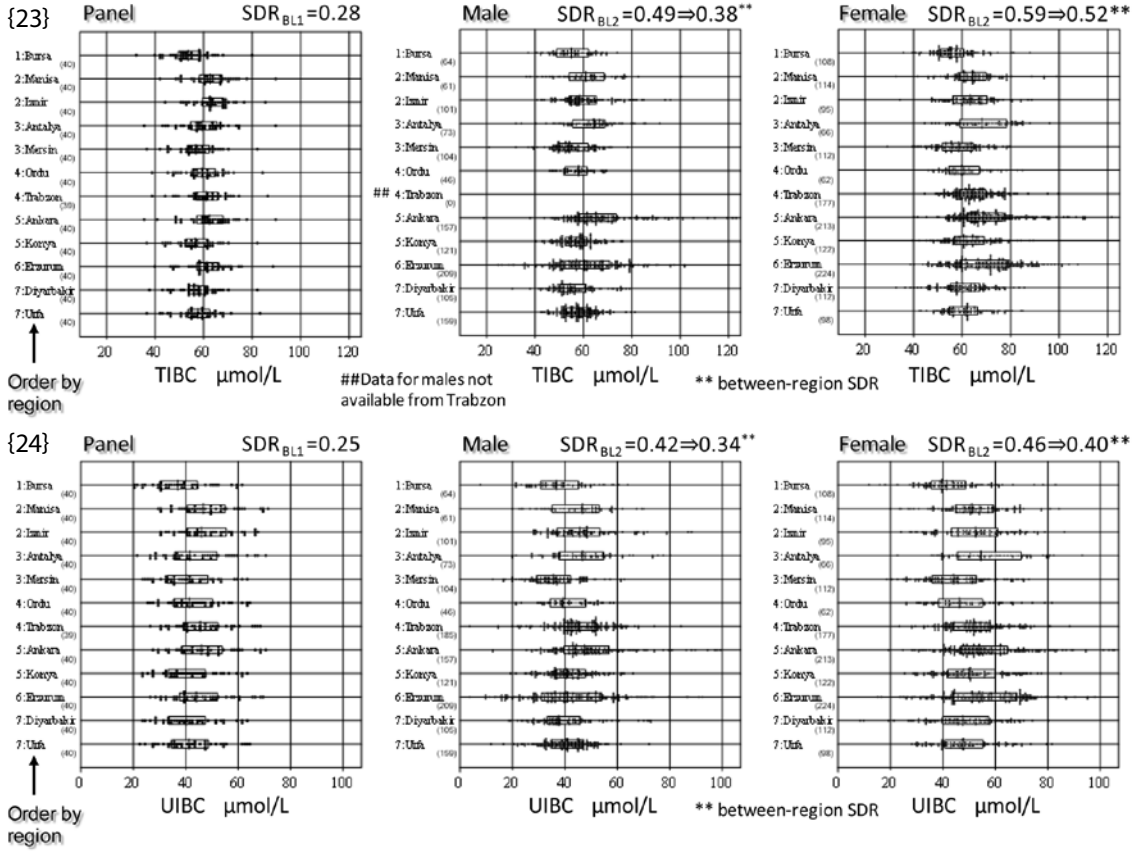


Figure 1 (continued). Between-laboratory differences computed as SD ratio (SDR) were denoted as SDR_{BL1} for the panel test results and as SDR_{BL2} for volunteers' test results. SDR > 0.30 was used as a guide value for judging the presence of analytical bias or regional difference among the laboratories. The laboratories which showed a prominent bias were indicated with a circle around the name. In special situations where a laboratory showed obvious bias, analyser dependency, or regionality of test results, the SDR was recomputed after excluding Urfa for Neu and Neu%, after excluding Mersin for MCV, after regrouping test results by manufacturers for Bas, Bas%, and MPV, after regrouping laboratories by region for RBC, Hb, Hct, UIBC and TIBC, after limiting results to laboratories using BC and S analysers for RDW, after limiting test results to laboratories using BC analysers for MCHC, and after excluding results from Izmir for ferritin.

to a reduction in SDR_{BL1} from 0.44 to 0.15. After removal of the biased test results, SDR_{BL2} reduced below 0.3 as shown in Column 6 of Table 3, and thus we chose to combine all the results for derivation of the RI for MCV.

For Mon and Mon%, we observed apparent between-laboratory differences in the panel test results (SDR_{BL1} of 0.54 and 0.62, respectively) with a tendency of analyser dependent bias. However, SDR_{BL2} based on volunteers' results were < 0.3 for males and females as shown in Figure 1 - {6,7} and Column 6 of Table 3. Thus, we assumed that monocytes in the blood panel, which were measured 13 hours after preparation, were not stable during transportation at 10 – 20 °C. Therefore, we ignored the panel test results and decided to combine the results for Mon and Mon% from all the laboratories to derive the RIs.

For Bas and Bas%, a large between-laboratory difference was observed in the panel test results (SDR_{BL1} of 1.08 and 1.15, respectively) and in the volunteers' test results (SDR_{BL2} of 0.61 and 0.62, respectively). This indicated the analyser dependency of Bas and %Bas measurements as shown in Figure 1 - {10,11}. By grouping the haematology analysers used in the 12 laboratories under the headings of the 3 manufacturers, the between-manufacturer SDR (SDR_{BM}) of Bas and Bas% were com-

Table 3. Analyses of between-laboratory differences in test results of the blood/serum panel and volunteers' specimens to assess the need for partitioning reference values

Test item	Panel test results					Volunteers' test results		
	SDR_{BL1}		SDR-gender	SDR-age (M, F)	SDR_{BL2} (M, F)	SDR_{BR}	SDR_{BM}	Scheme for deriving RIs
	All centres	Aft excl						
WBC	0.24	-	0.11	0.00 (0.00, 0.00)	0.25 (0.27, 0.23)	-	-	RI from all labs' results
Neu	**0.48**	0.26*	0.00	0.05 (0.03, 0.07)	0.20 (0.22, 0.18)	-	-	RI from all labs' results
%Neu	**0.60**	0.00*	0.10	0.10 (0.10, 0.10)	0.15 (0.18, 0.10)	-	-	RI from all labs' results
Lym	0.18	-	0.10	0.07 (0.10, 0.00)	0.18 (0.18, 0.19)	-	-	RI from all labs' results
%Lym	0.06	-	0.00	0.14 (0.15, 0.12)	0.07 (0.11, 0.00)	-	-	RI from all labs' results
Mon	**0.54**	-	0.31	0.14 (0.16, 0.02)	0.12 (0.29, 0.20)	-	-	RI from all labs' results
%Mon	**0.62**	-	0.23	0.07 (0.12, 0.00)	0.07 (0.12, 0.00)	-	-	RI from all labs' results
Eos	0.00	-	0.25	0.03 (0.02, 0.04)	0.15 (0.19, 0.10)	-	-	RI from all labs' results
%Eos	0.00	-	0.23	0.05 (0.00, 0.07)	0.16 (0.18, 0.15)	-	-	RI from all labs' results
Bas	**1.08**	-	0.04	0.17 (0.13, 0.18)	**0.61** (**0.67**, **0.55**)	-	**0.71** (**0.79**, **0.65**)	RIs for 3 manufacturers
%Bas	**1.15**	-	0.00	0.00 (0.10, 0.23)	**0.62** (**0.68**, **0.57**)	-	**0.76** (**0.83**, **0.70**)	RIs for 3 manufacturers
RBC	0.26	-	**1.00**	0.16 (0.24, 0.00)	**0.49** (**0.54**, **0.40**)	**0.45** (**0.50**, **0.38**)	-	RIs for 7 regions for each sex
Hb	0.11	-	**1.26**	0.19 (0.28, 0.00)	**0.41** (**0.47**, **0.35**)	**0.39** (**0.44**, **0.34**)	-	RIs for 7 regions for each sex
Hct	0.30	-	**1.20**	0.11 (0.17, 0.00)	**0.53** (**0.59**, **0.48**)	**0.50** (**0.54**, **0.45**)	-	RIs for 7 regions for each sex
MCV	**0.44**	0.15†	0.17	0.07 (0.11, 0.03)	**0.33** (**0.36**, **0.31**) 0.25 (0.22, 0.27)†	-	-	RI from all labs' results
MCH	0.29	-	0.27	0.00 (0.00, 0.00)	**0.30** (0.29, **0.33**)	-	-	RI from all labs' results
MCHC	**1.00**	0.08§	0.27	0.00 (0.00, 0.00)	**0.66** (**0.68**, **0.65**) 0.36 (**0.44**, 0.31)§	-	-	RI for BC
RDW	**1.28**	0.00‖	0.21	0.13 (0.30, 0.00)	**0.50** (**0.69**, **0.39**) 0.30 (0.32, 0.29)‖	-	-	RI for BC + Sy
PLT	0.23	-	0.23	0.10 (0.08, 0.11)	0.24 (0.28, 0.20)	-	-	RI from all labs' results

MPV	**0.75**	-	0.00	0.00 (0.00, 0.00)	**0.68** (**0.68**, **0.69**)	-	**0.67** (**0.60**, **0.68**)	RIs for 3 manufacturers
Fe	0.00	-	**0.40**	0.11 (0.16, 0.00)	0.17 (0.17, 0.16)	-	-	RIs from all labs' results for each sex
UIBC	0.25	-	**0.43**	0.00 (0.09, 0.00)	**0.44** (**0.42**, **0.46**)	**0.37** (**0.34**, **0.40**)	-	RIs for 7 regions for each sex
TIBC	0.28	-	0.29	0.00 (0.00, 0.00)	**0.55** (**0.49**, **0.59**)	**0.46** (**0.38**, **0.52**)	-	RIs for 7 regions
Ferritin	0.12	**-**	**0.84**	**0.35** (0.00, **0.49**)	**0.49** (**0.61**, **0.36**) 0.20 (0.26, 0.15)‡	-	-	RIs from all labs' results for each sex

SDR - standard deviation ratio, the ratio of the standard deviation for a given factor to that for a net between-individual variation. By use of 3-level nested ANOVA, the magnitudes (SD) of between-sex, -age, -region variation were computed relative to the net between-individual SD as SDR. SDR-sex, SDR-age, and SDR-region denote SDR for between-sex, between-age, and between-region differences, respectively. The SDRs in parentheses represent those computed after partitioning data to males (M) and females (F) by use of 2-level nested ANOVA, setting age and birth place (or region) as the target factors. The bold characters indicate SDR > 0.3. SDR_{BL1} - between laboratory SDR based on panel test results. Aft excl - after exclusion. SDR_{BL2} - between laboratory SDR based on volunteers' test results. SDR_{BR} - between region SDR. SDR_{BM} - between manufacturer SDR. RIs - reference Intervals. BC – Beckmann Coulter. Sy – Sysmex.

*after excluding results from Urfa. †after excluding results from Mersin. ‡after excluding results from Izmir. §after limiting to laboratories using BC analysers. ‖after limiting to labs using BC and Sy analysers.

puted as 0.71 and 0.76, respectively (Column 7 of Table 3). This indicated a need to derive RIs for Bas and Bas% after partition into the three manufacturers. In this finding of manufacturer dependency of test results for Bas and Bas%, it is notable that the between-laboratory difference was more prominent for the panel test results (SDR_{BL1}) than the volunteers' results (SDR_{BL2}). We presumed a time and temperature dependent instability of basophils in the blood panel as noted for monocytes.

For MCHC, RDW, and MPV, we noted apparent bias among the 12 laboratories with SDR_{BL1} of 1.00, 1.28 and 0.75, respectively. Similar between-laboratory differences were also observed in volunteers' test results as indicated by SDR_{BL2} of 0.66, 0.50, and 0.68. For MCHC, as shown in Figure 1 - {17}, in the laboratories using A and S analyser, the volunteers' results were not consistent despite the use of the same analyser. Therefore, we were obliged to derive RIs only for laboratories using BC analysers. For RDW, as shown in Figure 2 - {18}, the distribution of volunteers' results showed a wide fluctuation among the laboratories using A analysers. Therefore, we decided to derive the RI for RDW only from the results measured with BC and S analysers. For MPV, as shown in Figure 1 - {20}, the volunteers' results were dependent on the analyser. This observation was confirmed by the high SDR_{BM} (0.67) shown in Column 7 of Table 3. Therefore, we decided to derive RIs separately for each manufacturer.

Regional differences in reference values

For the remaining parameters which showed no analyser dependent bias with $SDR_{BL1} \leq 0.3$, we examined between-laboratory differences in volunteers' results by computing SDR_{BL2} as shown in Column 6 of Table 3. The following findings were obtained.

No obvious between-laboratory difference was observed with $SDR_{BL2} \leq 0.3$ for WBC, Neu and Neu%, Mon and Mon%, Eos and Eos%, Lym and Lym%, MCH, PLT, and Fe. Therefore, RIs were derived after merging the volunteers' results from all 12 laboratories.

Obvious between-laboratory difference with $SDR_{BL2} > 0.3$ were observed for RBC, Hb, Hct, UIBC, TIBC, and ferritin. For ferritin, the high SDR_{BL2} was attributable to an obvious bias in the Izmir results (Figure 1 - {22}) despite the fact that the panel test results did not show any bias. After exclusion of

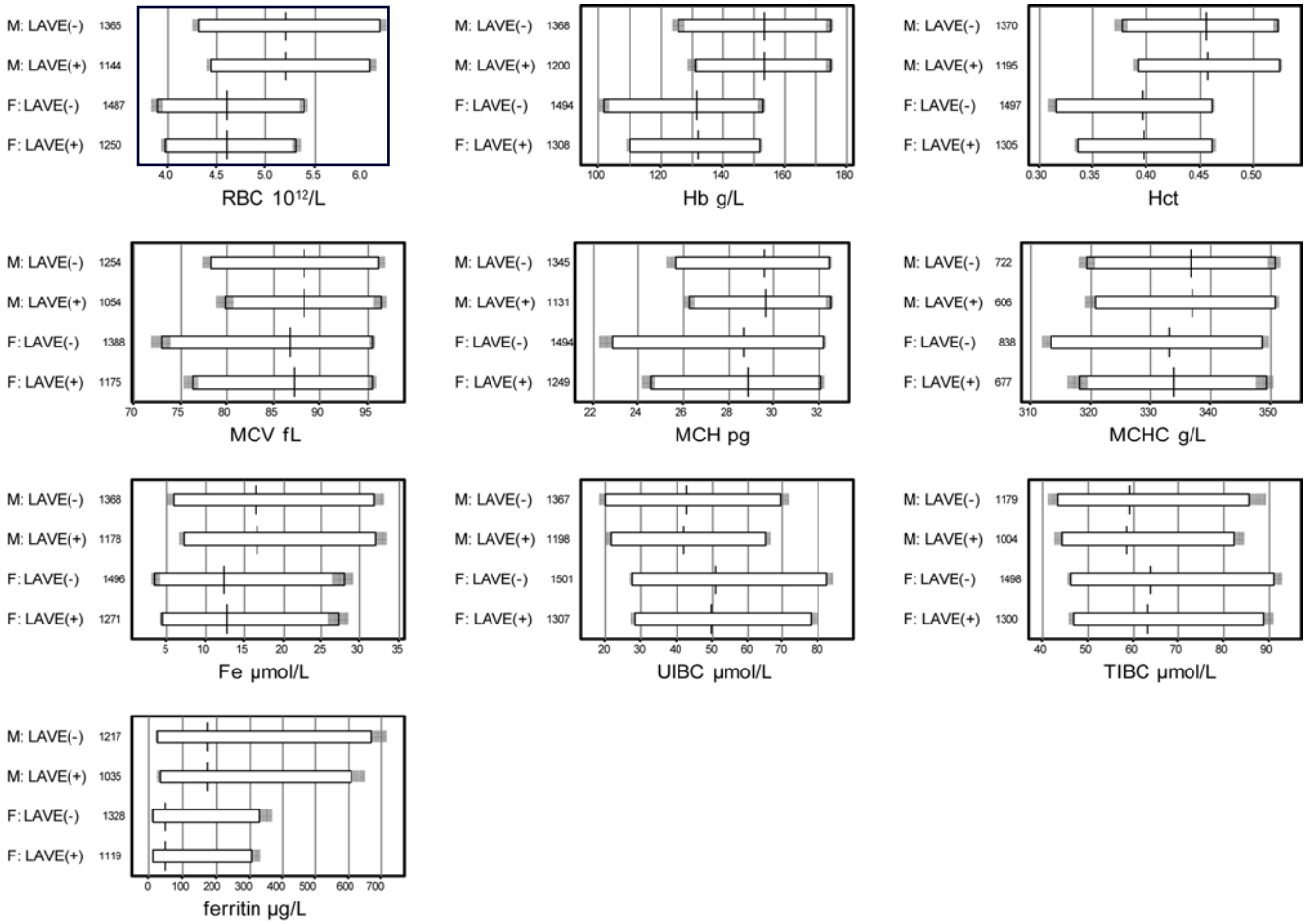


Figure 2. Comparison of reference intervals derived with or without applying LAVE method
RIs were derived parametrically in two ways with/without LAVE method. Each RI was depicted by the horizontal bar with shades on both ends corresponding to the 90% CI derived by the bootstrap method (using iteration of 100 times). The lower and upper limits of each RI were determined as the average of the 100 iterations. The LAVE method was applied in order to reduce the influence of latent anaemia with the use of the following test items as reference for exclusion: Hb, Hct, MCV, Fe, UIBC, TIBC, and ferritin. One abnormal value among them was allowed in the selection process. The data used for derivation of the RIs for MCV and ferritin were those which remained after removing biased results from Mersin and Izmir, respectively. For MCHC, derivation of the RIs was applied with the results from the laboratories using the BC analysers.

the results, SDR_{BL2} decreased from 0.49 to 0.20 (Column 6 of Table 3), so we decided to derive the RI from all the other laboratory results. For RBC, Hb, Hct, UIBC, and TIBC, we regrouped the 12 laboratories into 7 geographical regions, and recomputed between-region SDR (SDR_{BR}) as shown in Column 7 of Table 3. The SDR_{BR} for RBC, Hb, Hct, UIBC, and TIBC were found to be 0.45, 0.39, 0.50, 0.37 and 0.46. Therefore, the RIs for these parameters were derived for each region as shown in Column 8 of Table 3. As described below, we presumed that this regional difference was partly attributable to the altitude of the city where each collaborating laboratory was located.

Multiple regression analysis to assess sources-of-variation of test results

MRA was performed for each gender as shown in Table 4. By setting standardized partial regression coefficients (rp) ≥ 0.20 as a practically significant level, an age-related decrease of RVs was noted for RBC, Hb, and Hct only in males and an age-related increase was noted for RDW in males, and for ferri-

Table 4. Multiple regression analyses of results (rp) for sources of variation of reference values in males and females

Test Item	Male								Female							
	N	R	Altitude	Age	BMI	DrkLvl	SmkLvl	ExerLvl	N	R	Altitude	Age	BMI	DrkLvl	SmkLvl	ExerLvl
WBC	1526	0.262	0.103	0.053	0.077	-0.015	**0.227**	- 0.019	1670	0.234	0.105	- 0.144	0.179	0.005	0.107	0.021
Neu	1526	0.240	0.119	0.098	0.020	0.001	0.189	- 0.029	1669	0.202	0.109	- 0.130	0.129	0.009	0.088	0.038
%Neu	1526	0.179	0.100	0.149	- 0.064	0.018	0.051	- 0.002	1669	0.119	0.078	- 0.082	0.013	0.009	0.024	0.040
Lym	1526	0.218	0.052	- 0.112	0.150	- 0.029	0.138	- 0.002	1670	0.203	0.063	- 0.098	0.192	-0.017	0.078	- 0.012
%Lym	1526	0.186	- 0.041	- 0.177	0.086	- 0.009	-0.064	0.005	1670	0.082	- 0.035	0.046	0.023	- 0.029	- 0.021	- 0.024
Mon	1526	0.161	- 0.037	0.065	0.000	0.064	0.118	- 0.046	1670	0.102	- 0.008	- 0.076	0.057	0,064	0.030	- 0.015
%Mon	1526	0.142	-	0.038	-0.068	0.113	-0.071	- 0.026	1670	0.161	-	0.072	-0.125	0.097	- 0.060	- 0.021
Eos	1526	0.187	- 0.023	0.046	0.080	- 0.058	0.158	0.033	1669	0.153	- 0.064	0.071	0.067	0.005	0.075	- 0.017
%Eos	1526	0.138	- 0.062	0.034	0.055	- 0.057	0.091	0.041	1669	0.173	- 0.103	0.127	0.006	0.003	0.043	- 0.024
Bas*	876	0.130	0.040	- 0.001	0.048	- 0.064	0.085	- 0.027	976	0.098	0.064	- 0.002	0.038	- 0.001	0.063	- 0.003
%Bas*	876	0.066	0.006	0.003	0.010	- 0.056	0.016	- 0.018	976	0.088	0.021	0.093	-0.046	0.001	0.029	-0.021
RBC	1526	0.337	0.172	**- 0.284**	0.103	- 0.048	0.050	- 0.008	1670	0.232	**0.204**	0.005	0.102	0.009	0.030	0.013
Hb	1526	0.379	**0.228**	**- 0.273**	0.101	- 0.014	0.129	- 0.006	1670	0.277	**0.270**	0.066	0.004	0.045	0.040	0.003
Hct	1526	0.390	**0.279**	**- 0.222**	0.090	- 0.062	0.135	0.013	1670	0.301	**0.278**	0.092	0.038	0.004	0.055	0.011
MCV†	1428	0.181	0.059	0.150	-0.040	0.021	0.106	0.015	1563	0.127	0.059	0.126	- 0.084	-0.001	0.028	- 0.012
MCH	1526	0.107	0.038	0.058	-0.027	0.046	0.075	- 0.002	1670	0.126	0.077	0.079	- 0.102	0.039	0.013	- 0.016
MCHC‡	705	0.155	- 0.019	-0.152	0.075	0.004	- 0.007	- 0.048	803	0.132	0.113	- 0.030	- 0.002	0.035	- 0.022	- 0.032
RDW*	876	0.297	-	0.111	- 0.079	0.036	0.011	0.023	976	0.062	-	- 0.011	- 0.018	0.058	0.023	0.012
PLT	1526	0.162	0.067	- 0.115	0.073	- 0.081	0.018	0.002	1670	0.174	0.040	- 0.174	0.143	- 0.008	- 0.003	0.028
MPV‡	705	0.151	0.099	- 0.026	0.014	0.090	0.070	0.037	803	0.077	0.025	0.052	0.009	0.039	- 0.027	- 0.012
Fe	1365	0.188	0.086	- 0.114	- 0.056	0.067	0.045	- 0.015	1477	0.149	0.100	0.081	- 0.091	0.049	0.066	0.020
UIBC	1366	0.150	0.001	0.035	0.109	- 0.045	- 0.059	0.009	1477	0.209	0.147	- 0.111	0.118	- 0.055	0.008	0.005
TIBC	1181	0.154	0.079	- 0.007	0.121	- 0.019	- 0.043	- 0.006	1477	0.268	**0.232**	- 0.090	0.092	- 0.039	0.046	0.017
Ferritin§	1224	0.231	**0.224**	0.039	0.082	0.033	0.015	0.008	1322	0.427	0.098	**0.394**	0.042	0.019	- 0.060	- 0.040

Standardized partial regression coefficients (rp) is listed in the table with rp ≥ 0.20 marked by bold letter. For the analysis of RDW and %Mon, altitude was not included because of multicollinearity related to a bias in locations of laboratories. For the analysis of Eos, %Eos, Bas, %Bas, and Ferritin, test results were logarithmically transformed to adjust for their skewed distributions.

R - multiple correlation coefficient. BMI - Body Mass Index. DrkLvl - Drinking Level. SmkLvl - Smoking Level. ExerLvl - Exercise Level.

*Data limited to laboratories using Beckmann Coulter and Sysmex analysers. †Data from Mersin were not included. ‡Data limited to laboratories using Beckmann Coulter analysers. §Data from Izmir were not included.

tin in females. Each volunteer was assigned an altitude corresponding to the city of residence. The value of the altitude was set to that of the location of the municipal government. An altitude-related increase was found for Hb, Hct and ferritin in males, and for RBC, Hb, Hct, and TIBC in females. A smoking-related increase with $r_p \geq 0.2$ was observed only for WBC in males. A strong age-related increase with rp ≥ 0.394 was observed for ferritin in females (Table 4). BMI and alcohol-related changes were all well below the critical level of $r_p \geq 0.2$.

Derivation of reference intervals

The basic scheme for deriving the RI in consideration of analyser dependent bias and regional differences in RVs has been described in the previous sections. Additional considerations required were the need for partition of RVs by gender and age subgroups as well as the need for secondary exclusion with the use of the LAVE method to cope with latent anomia.

The calculated RIs and 90% CIs for haematological parameters in males and females (M+F), males (M), and females (F) are shown in Table 5. For partition by gender, we found it necessary for RBC, Hb, Hct, Fe, UIBC, and ferritin based on the criteria of $SDR_{gender} > 0.3$ as shown in Table 6. The RIs for these parameters were given for M and F separately (Table 6). For partition by age subgroup, $SDR_{age} > 0.3$ was only noted for ferritin in females as shown in Column 5 of Table 3. Therefore, RVs of ferritin were partitioned at 45 years of age (Table 6).

To judge the need for the LAVE method, we computed the RIs in two ways with and without applying it and listed the results in Table 5. The ratio of ΔLL to SD_{BI} was well above the critical level of 0.25 for RBC, Hb, Hct, MCV, MCH and MCHC while the ratio of ΔUL to SD_{BI} was above the critical level for RDW, UIBC, TIBC and ferritin as shown in Table 5. Therefore, for these parameters we judged to use RIs with applying the LAVE method. As no appreciable changes in the RI limits occurred to other parameters (WBC, Neu, Neu%, Lym, Lym%, Mon, Mon%, Bas, Bas%, PLT and MPV) not primarily related to the status of latent anaemia (Table 5), for these parameters we recommended to use the RIs without the LAVE method. Accordingly, the effect of the LAVE method was conspicuously observed with raised LLs for RBC, Hb, Hct, MCV, MCH and MCHC as shown in Figure 2.

Discussion

This nationwide study involving 12 laboratories in 7 geographical regions of Turkey aimed to establish well-defined RIs for haematology parameters with high precision from a large number of volunteers even after partitioning by region, gender, or manufacturer, if relevant. Gender was a significant factor influencing RVs for Hb, Hct, RBC, ferritin, UIBC, and Fe, respectively. With confirmation of no analyser-dependent bias and lack of regional differences, RIs were derived for nationwide use as 'common RIs' for WBC, Neu, Neu%, Mon, Mon%, Lym, Lym%, Eos, Eos%, MCH, MCV, PLT, and Fe. 'Manufacturer-specific RIs' were derived for Bas, Bas%, MCHC, RDW and MPV. With the observation of regional differences, despite the lack of analyser-dependent bias, 'Region-specific RIs' were derived for RBC, Hb, Hct, UIBC, and TIBC.

As pre-analytical errors are estimated to account for up to 70% of all mistakes made in laboratory diagnostics and the standardization of the pre-analytical phase is an important prerequisite of a multicentre study (18), all the participating laboratories followed the common protocol adopted in the IFCC global multicentre study on reference values and used the same SOPs for harmonizing the pre-analytical phase (2). We encouraged the use of the same manufacturer and model of tubes for standardization. K_2EDTA was the preferred anticoagulant for haematology measurements because K_3 EDTA can adversely affect some antibodies or assays (19).

The RIs established by a multicentre study are expected to be in a wider range than those established by a single laboratory due to the inclusion of between-laboratory variation, which is composed of analytical bias and/or regional bias (8). In this study, different haematology analysers from different manufacturers were used in the laboratories. Therefore, when between-laboratory differ-

Table 5. Reference intervals derived with the parametric method for hematological parameters in all subgroups

Parameter	Unit	Area	LAVE	Age: 18 – 65 years											Ratio of ΔLL or ΔUL to $SDRI_{BI}$				
				Males + Females				Males				Females				ΔLL by LAVE			ΔUL by LAVE		
				N	Me	LL	UL	N	Me	LL	UL	N	Me	LL	UL	Males + Females	Males	Females	Males + Females	Males	Females
WBC	x 10^9/L	All	(-)	2862	7.16	4.39	11.59	1365	7.35	4.55	11.68	1496	7.02	4.35	11.56	0.05	0.02	0.03	0.07	0.13	0.12
			(+)	2390	7.16	4.48	11.46	1141	7.34	4.59	11.45	1246	6.99	4.40	11.34						
Neu	x 10^9/L	All	(-)	2849	4.04	2.04	7.54	1360	4.07	2.14	7.46	1495	4.01	2.02	7.55	0.04	0.07	0.01	0.10	0.06	0.09
			(+)	2393	4.03	2.10	7.41	1140	4.04	2.23	7.54	1247	4.00	2.03	7.43						
Neu	%	All	(-)	2863	57	40	74	1368	56	39	73	1492	58	41	74	0.02	0.03	0.01	0.03	0.09	0.01
			(+)	2394	57	40	73	1145	56	39	72	1249	58	41	74						
Lym	x 10^9/L	All	(-)	2863	2.28	1.21	3.77	1370	2.36	1.22	3.83	1498	2.22	1.20	3.70	0.02	0.02	0.02	0.00	0.03	0.03
			(+)	2393	2.29	1.22	3.77	1143	2.36	1.23	3.81	1252	2.22	1.21	3.72						
Lym	%	All	(-)	2878	32	17	47	1373	32	17	47	1503	32	17	47	0.05	0.12	0.01	0.02	0.02	0.03
			(+)	2404	32	17	47	1146	33	18	47	1256	32	17	47						
Mon	x 10^9/L	All	(-)	2864	0.53	0.26	0.94	1366	0.57	0.29	1.00	1493	0.50	0.25	0.87	0.06	0.00	0.07	0.06	0.11	0.13
			(+)	2391	0.52	0.27	0.93	1141	0.56	0.29	0.98	1250	0.49	0.26	0.85						
Mon	%	All	(-)	2864	7.4	4.2	11.7	1366	7.7	4.4	12.0	1484	7.0	4.1	11.5	0.01	0.02	0.05	0.04	0.01	0.09
			(+)	2391	7.3	4.1	11.6	1143	7.7	4.5	12.0	1281	7.0	4.0	11.3						
Eos	x 10^9/L	All	(-)	2849	0.14	0.02	0.50	1362	0.17	0.03	0.57	1485	0.12	0.01	0.44	0.00	0.00	0.00	0.08	0.14	0.09
			(+)	2381	0.14	0.02	0.51	1137	0.17	0.03	0.59	1241	0.12	0.01	0.45						
Eos	%	All	(-)	2851	2	0.3	6.3	1365	2.3	0.0	6.6	1486	1.7	0.0	5.8	0.01	0.01	0.01	0.03	0.04	0.11
			(+)	2383	2	0.3	6.4	1141	2.3	0.0	6.6	1242	1.8	0.0	5.9						
Baso	x 10^9/L	All	(-)	2851	0.04	0.01	0.12	1365	0.04	0.01	0.13	1483	0.04	0.01	0.11	0.00	0.00	0.00	0.00	0.00	0.00
			(+)	2385	0.04	0.01	0.12	1143	0.04	0.01	0.13	1241	0.04	0.01	0.11						
		BC	(-)	1548	0.03	0.01	0.09	715	0.03	0.01	0.09	828	0.03	0.01	0.09	0.00	0.00	0.00	0.00	0.00	0.00
			(+)	1258	0.03	0.01	0.09	598	0.03	0.01	0.09	663	0.03	0.01	0.09						
		A	(-)	981	0.06	0.01	0.13	487	0.07	0.02	0.14	490	0.06	0.01	0.12	0.00	0.00	0.00	0.00	0.00	0.00
			(+)	831	0.06	0.01	0.13	408	0.07	0.02	0.14	425	0.06	0.01	0.12						
		S	(-)	322	0.03	0.01	0.07	156	0.03	0.01	0.08	167	0.03	0.01	0.07	0.00	0.00	0.00	0.00	0.00	0.00
			(+)	287	0.03	0.01	0.07	138	0.03	0.01	0.08	149	0.03	0.01	0.07						

Table 5. Reference intervals derived with the parametric method for hematological parameters in all subgroups (continued)

Baso	%	All	(-)	2855	0.6	0.1	1.5	1365	0.6	0.2	1.5	1494	0.6	0.1	1.4	0.03	0.00	0.06	0.03	0.06	0.03
			(+)	2383	0.6	0.2	1.5	1138	0.6	0.2	1.5	1250	0.6	0.1	1.4						
		BC	(-)	1552	0.5	0.1	1.0	709	0.5	0.2	1.0	839	0.5	0.1	1.1	0.18	0.05	0.08	0.05	0.05	0.04
			(+)	1261	0.5	0.2	1.0	595	0.5	0.2	1.0	666	0.5	0.1	1.1						
		A	(-)	978	0.8	0.2	1.7	488	0.9	0.2	1.7	490	0.8	0.2	1.7	0.00	0.03	0.00	0.11	0.14	0.11
			(+)	834	0.8	0.2	1.7	409	0.9	0.2	1.7	425	0.8	0.2	1.7						
		S	(-)	325	0.4	0.1	1.1	158	0.4	0.1	1.1	166	0.4	0.1	1.0	0.08	0.04	0.08	0.04	0.04	0.08
			(+)	289	0.4	0.1	1.1	140	0.4	0.1	1.1	149	0.4	0.1	1.0						
RBC*	x 10^{12}/L	All	(-)	2862	4.87	3.97	6.05	1365	5.21	4.31	6.17	1487	4.60	3.88	5.39	0.14	0.27	0.23	0.16	0.24	0.23
			(+)	2446	4.86	4.04	5.97	1164	5.20	4.43	6.07	1250	4.60	3.96	5.31						
		M	(-)	165	4.70	3.92	5.49	61	4.99	4.23	5.56	103	4.55	3.81	5.16	0.36	0.22	0.73	0.06	0.20	0.07
			(+)	139	4.67	4.05	5.47	53	4.99	4.30	5.50	85	4.52	4.02	5.14						
		A	(-)	401	4.68	3.68	5.63	171	4.94	3.88	5.86	231	4.50	3.65	5.27	0.33	0.50	0.30	0.02	0.19	0.13
			(+)	336	4.67	3.83	5.62	145	4.92	4.12	5.78	190	4.50	3.76	5.22						
		MED	(-)	324	4.99	4.08	6.18	158	5.35	4.57	6.22	163	4.68	3.97	5.38	0.06	0.32	0.03	0.20	0.46	0.15
			(+)	288	4.99	4.11	6.08	138	5.36	4.69	6.06	147	4.68	3.98	5.33						
		BS	(-)	448	4.67	3.88	5.70	227	4.98	4.23	5.73	221	4.40	3.80	5.07	0.21	0.22	0.39	0.05	0.15	0.18
			(+)	391	4.66	3.97	5.68	204	4.99	4.31	5.68	187	4.38	3.91	5.02						
		CEA	(-)	604	4.83	4.05	5.86	271	5.15	4.35	5.91	329	4.60	4.00	5.39	0.14	0.51	0.18	0.09	0.09	0.15
			(+)	499	4.84	4.11	5.82	229	5.14	4.55	5.88	265	4.59	4.06	5.34						
		EA	(-)	450	5.00	4.13	6.07	213	5.35	4.69	6.15	235	4.71	4.02	5.44	0.17	0.28	0.38	0.04	0.15	0.22
			(+)	383	5.00	4.21	6.05	184	5.33	4.79	6.10	198	4.71	4.14	5.37						
		SEA	(-)	475	5.19	4.18	6.44	264	5.51	4.60	6.51	210	4.81	4.05	5.61	0.14	0.18	0.28	0.04	0.00	0.17
			(+)	410	5.19	4.26	6.46	230	5.52	4.69	6.51	180	4.82	4.15	5.55						
Hb*	g/L	All	(-)	2872	141	107	173	1368	153	126	175	1494	131	102	153	0.40	0.40	0.76	0.02	0.00	0.08
			(+)	2498	142	113	173	1200	153	131	175	1308	132	110	152						
		M	(-)	167	135	105	161	61	147	125	163	105	129	102	149	0.18	0.02	0.44	0.04	0.04	0.10
			(+)	147	136	107	160	55	147	125	164	90	131	107	148						
		A	(-)	403	132	96	164	172	144	111	169	231	125	91	146	0.55	0.54	0.94	0.05	0.01	0.04
			(+)	352	133	105	165	151	145	119	169	200	126	102	146						
		MED	(-)	325	142	108	176	158	156	131	175	166	130	104	150	0.19	0.37	0.44	0.02	0.04	0.06
			(+)	298	142	111	176	146	156	135	175	151	131	109	149						

Table 5. Reference intervals derived with the parametric method for hematological parameters in all subgroups (continued)

Hb*	g/L	BS	(-)	449	139	112	168	227	150	129	167	221	130	108	146	0.33	0.01	0.87	0.10	0.02	0.07
			(+)	409	139	117	170	212	150	129	167	196	130	115	147						
		CEA	(-)	607	141	110	169	274	151	130	169	330	133	106	154	0.68	0.60	1.03	0.04	0.10	0.01
			(+)	516	142	118	169	237	152	136	170	278	134	116	154						
		EA	(-)	451	148	116	179	214	160	139	179	235	138	110	158	0.57	0.23	1.19	0.20	0.05	0.09
			(+)	403	147	124	182	193	160	141	178	210	138	122	159						
		SEA	(-)	473	145	109	180	262	156	133	181	207	132	103	154	0.51	0.17	0.84	0.00	0.09	0.06
			(+)	415	146	117	180	232	157	135	180	183	133	112	155						
Hct*	L/L	All	(-)	2875	0.422	0.330	0.516	1370	0.456	0.378	0.521	1497	0.396	0.316	0.460	0.35	0.38	0.67	0.04	0.04	0.04
			(+)	2502	0.424	0.345	0.518	1195	0.456	0.392	0.522	1305	0.398	0.337	0.461						
		M	(-)	167	0.403	0.318	0.472	61	0.434	0.373	0.484	104	0.384	0.318	0.442	0.23	0.06	0.25	0.00	0.09	0.13
			(+)	146	0.403	0.327	0.472	54	0.434	0.372	0.482	91	0.386	0.326	0.438						
		A	(-)	403	0.396	0.295	0.485	172	0.429	0.341	0.489	231	0.375	0.285	0.436	0.54	0.50	0.78	0.07	0.03	0.05
			(+)	350	0.399	0.318	0.488	151	0.431	0.360	0.498	198	0.380	0.310	0.434						
		MED	(-)	326	0.414	0.332	0.498	159	0.445	0.385	0.502	167	0.387	0.324	0.439	0.16	0.44	0.23	0.05	0.14	0.04
			(+)	297	0.415	0.339	0.500	145	0.446	0.398	0.505	151	0.388	0.330	0.440						
		BS	(-)	448	0.414	0.341	0.500	226	0.445	0.383	0.499	221	0.389	0.330	0.437	0.24	0.00	0.71	0.12	0.03	0.06
			(+)	407	0.415	0.351	0.505	211	0.444	0.383	0.498	194	0.390	0.346	0.439						
		CEA	(-)	608	0.425	0.338	0.508	273	0.456	0.396	0.508	331	0.402	0.329	0.467	0.60	0.61	0.87	0.03	0.07	0.08
			(+)	514	0.428	0.361	0.509	234	0.457	0.414	0.510	278	0.405	0.354	0.470						
		EA	(-)	451	0.438	0.348	0.529	214	0.472	0.415	0.528	235	0.410	0.332	0.469	0.57	0.15	1.16	0.23	0.01	0.12
			(+)	398	0.437	0.372	0.538	191	0.473	0.419	0.528	206	0.411	0.364	0.472						
		SEA	(-)	475	0.450	0.354	0.541	264	0.477	0.405	0.545	210	0.416	0.340	0.486	0.40	0.31	0.62	0.05	0.10	0.05
			(+)	416	0.452	0.371	0.543	231	0.478	0.416	0.548	184	0.421	0.360	0.485						
MCV*	fL	All	(-)	2639	87.4	75.1	95.7	1254	88.2	78.2	96.0	1388	86.7	72.9	95.5	0.46	0.36	0.68	0.00	0.08	0.00
			(+)	2235	87.7	77.2	95.7	1054	88.2	79.9	96.4	1175	87.1	76.2	95.6						
MCH*	pg	All	(-)	2851	29.1	23.8	32.4	1345	29.5	25.6	32.4	1494	28.7	22.9	32.2	0.79	0.39	0.87	0.07	0.03	0.07
			(+)	2383	29.3	25.2	32.2	1131	29.6	26.3	32.4	1249	28.9	24.5	32.1						
MCHC*	g/L	All	(-)	2849	334	306	353	1365	336	310	356	1490	332	304	350	0.29	0.22	0.22	0.00	0.07	0.02
			(+)	2380	335	309	353	1140	336	313	356	1247	333	305	350						
		BC	(-)	1561	335	316	350	722	337	319	351	838	333	313	349	0.48	0.16	0.59	0.05	0.00	0.06
			(+)	1283	335	319	350	606	337	321	351	677	334	318	349						

Table 5. Reference intervals derived with the parametric method for hematological parameters in all subgroups (continued)

RDW-CV*	%	All	(-)	2849	13.6	11.8	17.7	1354	13.4	11.6	16.4	1479	13.9	12.1	18.5	0.02	0.05	0.05	0.83	0.57	0.96
			(+)	2379	13.5	11.8	16.6	1137	13.3	11.5	15.8	1235	13.7	12.0	17.2						
		BC + S	(-)	1884	13.6	12.2	17.6	867	13.3	12.2	16.0	1003	13.8	12.3	18.5	0.00	0.00	0.00	1.24	0.87	1.45
			(+)	1562	13.5	12.2	16.3	739	13.3	12.2	15.3	816	13.6	12.3	16.9						
PLT	x 10^9/L	All	(-)	2860	250	152	383	1366	240	147	365	1492	260	157	392	0.03	0.01	0.02	0.07	0.04	0.09
			(+)	2390	148	151	378	1141	239	146	363	1249	258	155	387						
MPV*	fL	All	(-)	2868	8.8	6.3	11.8	1372	8.6	6.1	11.6	1499	8.9	6.5	12.1	0.02	0.01	0.03	0.00	0.01	0.03
			(+)	2403	8.8	6.2	11.8	1148	8.6	6.0	11.6	1252	8.9	6.5	12.1						
		BC	(-)	1565	8.8	7.0	11.1	723	8.6	6.9	11.0	839	8.9	7.2	11.2	0.01	0.02	0.01	0.04	0.01	0.06
			(+)	1286	8.8	7.0	11.1	607	8.7	6.9	11.0	678	8.9	7.1	11.3						
		A	(-)	978	8.1	5.8	11.9	488	7.9	5.6	11.7	490	8.3	6.0	12.2	0.02	0.02	0.01	0.06	0.27	0.09
			(+)	834	8.0	5.7	11.8	407	7.8	5.5	11.3	424	8.2	6.1	12.4						
		S	(-)	325	10.6	9.0	12.7	158	10.4	8.9	12.4	167	10.8	9.1	13.0	0.01	0.03	0.04	0.01	0.08	0.00
			(+)	290	10.6	8.9	12.7	140	10.4	8.9	12.3	150	10.8	9.0	13.0						
Fe	µmol/L	All	(-)	2878	14.4	3.8	29.6	1368	16.5	5.9	31.6	1496	12.4	3.5	27.8	0.19	0.23	0.16	0.01	0.03	0.12
			(+)	2460	14.8	5.0	29.6	1178	16.8	7.4	31.8	1271	12.9	4.4	27.1						
UIBC*	µmol/L	All	(-)	2867	46.5	23.8	78.9	1367	42.6	20.1	69.6	1501	50.8	27.8	82.4	0.04	0.13	0.04	0.41	0.44	0.34
			(+)	2546	45.8	24.2	73.7	1198	42.0	21.5	64.7	1307	49.9	28.3	78.1						
		M	(-)	165	40.1	21.9	65.6	61	36.4	21.6	62.6	102	41.8	25.5	68.1	0.07	0.27	0.00	0.36	0.30	0.51
			(+)	146	40.0	21.2	61.9	57	36.5	19.0	59.6	89	41.4	25.4	63.1						
		A	(-)	404	49.2	25.9	78.1	170	45.1	25.1	74.6	232	52.5	28.6	79.6	0.02	0.10	0.03	0.47	0.61	0.40
			(+)	347	48.1	25.7	72.5	150	43.8	24.1	67.8	197	51.4	29.0	74.9						
		MED	(-)	326	44.6	23.3	79.1	153	40.2	22.7	69.7	164	49.1	29.1	85.2	0.07	0.18	0.06	0.30	0.45	0.21
			(+)	301	43.9	24.2	75.1	145	39.6	20.9	64.3	155	48.2	29.9	82.4						
		BS	(-)	448	47.4	24.6	69.5	226	45.0	24.1	64.9	222	50.0	30.8	73.2	0.06	0.14	0.09	0.25	0.08	0.44
			(+)	409	47.0	27.0	67.0	213	44.9	25.5	64.2	196	49.3	29.9	68.8						
		CEA	(-)	606	49.3	27.9	86.3	273	45.0	27.3	82.7	330	53.1	31.6	89.0	0.00	0.01	0.05	0.39	0.66	0.48
			(+)	528	48.1	28.0	81.1	242	44.2	27.4	74.8	284	51.6	31.0	82.7						
		EA	(-)	452	48.2	16.2	86.1	215	40.5	12.1	75.3	237	55.4	26.2	89.9	0.03	0.08	0.09	0.20	0.42	0.17
			(+)	394	47.3	16.7	82.7	189	39.6	13.3	69.3	205	54.5	27.5	87.3						
		SEA	(-)	473	43.2	24.2	69.1	266	40.3	22.0	61.2	210	48.1	25.2	73.5	0.02	0.02	0.31	0.61	0.65	0.49
			(+)	421	42.8	24.0	63.1	237	39.8	21.8	55.6	184	46.7	28.4	68.5						

Table 5. Reference intervals derived with the parametric method for hematological parameters in all subgroups (continued)

		All	(-)	2681	61.7	44.5	89.7	1179	59.0	43.1	85.7	1498	64.0	46.1	90.8	0.05	0.09	0.07	0.24	0.36	0.18
			(+)	2329	61.1	45.0	87.1	1004	58.6	44.0	82.2	1300	63.3	46.8	88.9						
		M	(-)	166	55.3	40.9	72.8	62	54.4	41.3	72.9	104	55.7	42.1	74.6	0.21	0.10	0.10	0.10	0.09	0.28
			(+)	144	55.4	42.5	72.0	54	54.8	42.1	72.2	89	55.7	42.8	72.5						
		A	(-)	402	62.7	44.5	85.7	166	60.0	43.6	85.8	230	64.4	46.9	85.5	0.00	0.09	0.17	0.47	0.47	0.43
			(+)	343	61.8	44.5	81.3	149	59.3	42.8	81.2	195	63.6	48.4	81.8						
		MED	(-)	325	60.5	45.4	87.0	157	58.3	45.4	83.2	164	62.3	47.5	90.2	0.03	0.01	0.01	0.19	0.28	0.15
			(+)	398	60.1	45.7	85.0	142	58.0	45.5	80.7	152	62.0	47.6	88.6						
TIBC*	µmol/L	BS	(-)	259	61.8	49.8	81.3	46	56.8	47.6	73.2	221	62.9	50.8	82.3	0.07	0.07	0.07	0.36	0.13	0.46
			(+)	228	61.5	49.2	78.6	40	56.4	47.1	72.4	189	62.4	50.3	79.0						
		CEA	(-)	607	64.4	47.8	97.8	275	61.8	46.8	98.8	320	66.6	51.7	98.8	0.04	0.10	0.02	0.29	0.58	0.30
			(+)	522	63.7	48.3	94.4	242	61.1	47.9	92.3	276	65.8	51.5	95.5						
		EA	(-)	450	64.6	39.8	99.2	215	59.4	35.2	91.3	237	69.4	46.6	101.7	0.04	0.01	0.10	0.16	0.35	0.02
			(+)	389	63.8	40.3	96.8	188	58.4	35.3	86.6	206	69.4	48.0	101.4						
		SEA	(-)	475	58.8	42.9	77.1	265	57.2	43.1	73.8	211	61.3	43.3	80.1	0.16	0.26	0.16	0.35	0.37	0.32
			(+)	421	58.4	44.1	74.3	237	56.8	45.0	71.3	184	60.4	44.6	77.4						
ferritin	µg/L	All	(-)	2548	41.2	4.1	258	1217	71	10.4	297	1328	21.5	3.8	148	0.01	0.04	0.03	0.16	0.41	0.36
			(+)	2172	41.0	5.0	248	1035	74	13.0	270	1119	21.9	4.7	136						
< 45 years																					
ferritin	µg/L	All	(-)	-	-	-	-	-	-	-	-	838	17.0	3.5	98	-	-	0.04	-	-	0.31
			(+)									704	17.3	4.3	91						
≥ 45 years																					
ferritin	µg/L	All	(-)	-	-	-	-	-	-	-	-	587	38.1	4.9	191	-	-	0.02	-	-	0.37
			(+)									489	38.3	5.9	175						

LAVE - latent abnormal values exclusion method. (-) – LAVE not applied. (+) – LAVE applied. Me – median of the reference interval. LL - lower limit of the reference interval. UL - upper limit of the of the reference interval.
M: Marmara; A: Aegean; MED: Mediterranean; BS: Black Sea; CEA: Central Anatolia; EA: East Anatolia; SEA: South East Anatolia.
*RIs were derived after applying the LAVE method in a mode allowing a single abnormal result in analytes chosen as exclusion criteria: HB, HCT, MCV, Fe, UIBC, TIBC and ferritin. The choice between the two reference intervals was made by the ratio of the difference in the two LLs (or ULs) to the SD comprising the RIs which correspond to between-individual SD (SDR_{BI}). The critical value for ΔLL (or ΔUL) ratio was set as 0.25.

Table 6. The list of RIs derived

Test item	Unit	RIs		N	SDR-gender	Males + Females			Males			Females		
						LL	Me	UL	LL	Me	UL	LL	Me	UL
WBC	10^9/L	**C**	**All**	2862	0.11	4.39	7.16	11.59	-	-	-	-	-	-
Neu	10^9/L	**C**	**All**	2849	0.00	2.04	4.04	7.54	-	-	-	-	-	-
Neu%	%	**C**	**All**	2863	0.10	0.40	0.57	0.74	-	-	-	-	-	-
Lym	10^9/L	**C**	**All**	2863	0.10	1.21	2.28	3.77	-	-	-	-	-	-
Lym%	%	**C**	**All**	2878	0.00	0.17	0.32	0.47	-	-	-	-	-	-
Mon	10^9/L	**C**	**All**	2864	0.31	0.26	0.53	0.94	-	-	-	-	-	-
Mon%	%	**C**	**All**	2853	0.23	0.04	0.07	0.12	-	-	-	-	-	-
Eos	10^9/L	**C**	**All**	2849	0.25	0.02	0.14	0.50	-	-	-	-	-	-
Eos%	%	**C**	**All**	2851	0.23	0.00	0.02	0.06	-	-	-	-	-	-
Bas	10^9/L	**MS**	**A**	981	0.04	0.01	0.06	0.13	-	-	-	-	-	-
			BC	1548		0.01	0.03	0.09	-	-	-	-	-	-
			S	322		0.01	0.03	0.07	-	-	-	-	-	-
Bas%	%	MS	A	978	0.00	0.0018	0.0084	0,017	-	-	-	-	-	-
			BC	1552		0.0013	0.0048	0.0101	-	-	-	-	-	-
			S	325		0.0009	0.0040	0.0110	-	-	-	-	-	-
RBC*	10^{12}/L	**RS**	**All**	2446	1.00	-	-	-	4.43	5.20	6.07	3.96	4.60	5.31
			M	139		-	-	-	4.30	4.99	5.50	4.02	4.52	5.14
			MED	288		-	-	-	4.69	5.36	6.06	3.98	4.68	5.33
			BS	391		-	-	-	4.31	4.99	5.68	3.91	4.38	5.02
			A	336		-	-	-	4.12	4.92	5.78	3.76	4.50	5.22
			SEA	410		-	-	-	4.69	5.52	6.51	4.15	4.82	5.55
			CEA	499		-	-	-	4.55	5.14	5.88	4.06	4.59	5.34
			EA	383		-	-	-	4.79	5.33	6.10	4.14	4.71	5.37
Hb*	g/L	**RS**	**All**	2498	1.26	-	-	-	131	153	175	110	132	152
			M	147		-	-	-	125	147	164	107	131	148
			MED	298		-	-	-	135	156	175	109	131	149
			BS	367		-	-	-	129	150	167	115	130	147
			A	352		-	-	-	119	145	169	102	126	144
			SEA	415		-	-	-	135	157	180	112	133	155
			CEA	516		-	-	-	136	152	170	116	134	154
			EA	403		-	-	-	141	160	178	122	138	159
Hct*	L/L	**RS**	**All**	2502	1.20	-	-	-	0.392	0.456	0.522	0.337	0.398	0.461
			M	146		-	-	-	0.372	0.434	0.482	0.326	0.386	0.438
			MED	271		-	-	-	0.398	0.446	0.505	0.330	0.388	0.440
			BS	407		-	-	-	0.383	0.444	0.498	0.346	0.390	0.439
			A	350		-	-	-	0.360	0.431	0.498	0.310	0.380	0.434
			SEA	416		-	-	-	0.416	0.478	0.548	0.360	0.421	0.485
			CEA	514		-	-	-	0.414	0.457	0.510	0.354	0.405	0.470
			EA	398		-	-	-	0.419	0.473	0.528	0.364	0.411	0.472

MCV*	fL	**C**	**All**	2235	0.17	77.2	87.7	95.7	-	-	-	-	-	-
MCH*	pg	**C**	**All**	2383	0.27	25.2	29.3	32.2	-	-	-	-	-	-
MCHC*	g/L	**MS**	**BC**	1283	0.27	319	335	350	-	-	-	-	-	-
RDW-CV	%	**MS**	**BC+S**	1562	0.21	12.2	13.5	16.3	-	-	-	-	-	-
PLT	10^9/L	**C**	**All**	2869	0.23	152	250	383	-	-	-	-	-	-
MPV*	fL	**MS**	**A**	978	0.01	5.8	8.1	11.9	-	-	-	-	-	-
			BC	1565		7.0	8.8	11.8	-	-	-	-	-	-
			S	325		9.0	10.6	12.7	-	-	-	-	-	-
Fe	µmol/L	**C**	**All**	2878	0.40	-	-	-	5.9	16.5	31.6	3.5	12.4	27.8
UIBC*	µmol/L	**RS**	**All**	2546	0.43	-	-	-	21.5	42	64.7	28.3	49.9	78.1
			M	146		-	-	-	19.0	36.5	59.6	25.4	41.4	63.1
			MED	301		-	-	-	20.9	39.6	64.3	29.9	48.2	82.4
			BS	409		-	-	-	25.5	44.9	64.2	29.9	49.3	68.8
			A	347		-	-	-	24.1	43.8	67.8	29.0	51.4	74.9
			SEA	421		-	-	-	21.8	39.8	55.6	28.4	46.7	68.5
			CEA	528		-	-	-	27.4	44.2	74.8	31.0	51.6	82.7
			EA	394		-	-	-	13.3	39.6	69.3	27.5	54.5	87.3
TIBC*	µmol/L	**RS**	**All**	2329	0.29	45.0	58.6	82,2	44.0	58.6	82.2	46.8	63.3	88.9
			M	144		42.5	55.4	72.0	42.1	54.8	72.2	42.8	55.7	72.7
			MED	298		45.7	60.1	85.0	45.5	58.0	80.7	47.6	62.0	88.6
			BS	228		49.2	61.5	78.6	47.1	56.4	72.4	50.3	62.4	79.0
			A	318		44.5	61.8	81.3	42.8	59.3	81.2	48.4	63.6	81.8
			SEA	421		44.1	58.4	74.3	45.0	56.8	71.3	44.6	60,4	77.4
			CEA	522		48.3	63.7	94.4	47.9	61.1	92.8	51.5	65.8	95.5
			EA	398		40.3	63.8	96.8	35.3	58.4	86.6	48.0	69.4	101.4
Ferritin*	µg/L	**C**	**All**	2172	0.84	-	-	-	13	74	276	4.7	21.0	136
			< 45 y			-	-	-				4.3	17.3	91
			≥ 45 y			-	-	-				5.9	38.3	175

RI - reference interval. LL - lower limit of the RI. Me – median. UL - upper limit of the RI. C – common. MS - manufacturer-specific. RS - region-specific. SDR - standard deviation ratio. A – Abbott. BC - Beckman Coulter. S - Sysmex.
*RIs were derived after applying the LAVE method in a mode allowing a single abnormal result in analytes chosen as exclusion criteria: HB, HCT, MCV, Fe, UIBC, TIBC and ferritin.
Regions (altitude above sea levels in meters): M - Marmara (100); MED - Mediterranean (295); BS - Black Sea (395); A - Aegean (500); SEA - South East Anatolia (745); CEA - Central Anatolia (1000); EA - East Anatolia (745).

ences in the results were observed, it was not clear whether these differences were attributable to regional factors or to analyser-dependent bias, so the panel of whole blood samples was prepared to detect between-laboratory bias more clearly (3). As far as we know, this is the first attempt to employ a panel of whole blood samples in a nationwide multicentre study to manage analytical bias in determining RIs of haematological parameters.

The test results of the blood panel revealed large between-laboratory differences (SDR_{BL1} > 0.6) in values for Bas, Bas%, RDW, MCHC, and MPV, which were apparently dependent on the manufacturers of the analysers. The between-manufacturer bias in test results for MCHC, and MPV have been re-

ported and attributed to the difference in the assay principle (20).

As a problem of using the blood panel for assessing between-laboratory bias, we found that the SDR_{BL1} tended to be larger than SDR_{BL2} for Mon, Mon%, Bas and Bas%. This appears to be due to the instability of those leukocyte sub-fractions during transportation and storage. The actual time required from sampling (at 8 am) to measurement (at a unified time of 11 pm) was 15 hours. The temperature during transportation and storage was maintained at 10 – 20°C. This low temperature may also have been responsible for the instability of the leukocyte sub-fractions (21). Therefore, the instability of Mon, Mon%, Bas and Bas% during transportation and storage is the limitation of the study.

A number of factors may contribute to differences between reference intervals reported in different studies; these include characteristics of the studied volunteers, number of studied participants, inclusion criteria, the analytical methods and used analysers and the manner in which reference intervals were calculated.

Similar to other studies, we found that the RIs of RBC, Hb and Hct required partition by gender and calculated the RIs of RBC, Hb and Hct separately (6,22). Anaemia was defined according to the WHO criteria as a haemoglobin concentration lower than 120 g/L in females and 130 g/L in males (23). The LL for Hb before application of the LAVE method was 126 g/L in males, and 102 g/L in females, but with LAVE the value was 131 g/L in males, and 110 g/L in females. The LL for males matches with the WHO decision limit, but for females, it is lower than the decision limit, though appreciably raised by the LAVE method with reduced influence of latent anaemia. The LL of Fe was determined as 5.9 µmol/L for males and 3.5 µmol/L for females. These values are comparable to the reported values for adult Turkish males (7.3 µmol/L) and females (5.0 µmol/L), but much lower than the values for males and females (9.2 µmol/L) living in Nordic countries (24,25). Iron deficiency usually manifests as a falling MCV accompanied by a rising RDW (26). In the present study, although the LL of the RI for MCV in females was raised from 72.9 to 76.2 fL by the application of the LAVE method (in reference to the results of Hb, Hct, Fe, UIBC, TIBC, and ferritin), it is still lower than that found in the Nordic Reference Interval Project (82 fL) and reported in the recent study from Canada (82.5 fL) (6,8). However, ferritin values of < 17.8 µg/L have been reported to be generally associated with depleted iron stores (23). In the present study, the LL of ferritin for males and females was 13.8 µg /L and 4.7 µg /L, respectively. Taken together, the current study showed that many Turkish females have mild iron deficiency anaemia.

Many studies have addressed the effect of high altitude on Hb, erythropoietin, Hct and PLT (11,27). In the present study, judged from the results of MRA, the association of the altitude was significant for Hb, Hct and ferritin in males and RBC, Hb, Hct, and TIBC in females, but not for WBC, WBC subfractions, and PLT. There was a noticeable increase in RIs of Hb and Hct with increasing altitude. For example, in the Marmara region, which is approximately 100 m above sea level, the RIs for Hb and Hct were 125 - 164 g/L and 0.372 - 0.482 in males, respectively, whereas in East Anatolia, which is approximately 1800 m above sea level and the highest region in the study, the RIs for Hb and Hct were 141 - 178 g/L and 0.419 - 0.528 in males. However, the SDR_{BR} computed by ANOVA after sub-grouping results from the 12 laboratories into 7 regions were appreciably higher in East Anatolia for RBC, Hb, Hct, UIBC, and TIBC, with the SDR_{BR} ranging from 0.34 to 0.54. These findings indicate a need for regional RIs for RBC, Hb, Hct, UIBC, and TIBC instead of common RIs.

The observed RIs for WBC and sub-fractions of WBC in both sexes are in good accordance with the values reported in previous studies (6,9,22). Although males had slightly higher values for Mon, Mon%, Eos, and Eos%, SDR_{gender} was at or below the critical level. Therefore, separate RIs were not set by gender for WBC and its sub-fractions. The RI derived for eosinophil counts (0.02-0.50x10^9/L) was very similar to the reported RIs for five different haematology analysers (20). However, the upper reference limit (URL) of the RI for eosinophil

count was lower than those reported in Africa (28), but higher than those in Canada (6).

It is well known that cigarette smoking is associated with elevated levels of some haematological parameters (*e.g.* RBC, Hb, Hct, WBC) (29). The results of the MRA in this study supported that cigarette smoking was positively associated with the value of WBC in males. However, the association was not very strong, with r_p between 0.20 and 0.25. Therefore, we did not set different RIs for smokers and non-smokers. It has been reported that reference values of RBC, Hb and Hct decrease with age in males (30). In the present study, age was found to be negatively related to the values of RBC, Hb and Hct by MRA in males. However, in terms of SDR_{age}, the levels of these major parameters were all well below 0.30. Therefore, we did not adopt the age-related RIs except for RVs of ferritin in females, which showed prominent increase after the time around menopause.

In conclusion, this nationwide multicentre study established well-defined RIs of haematological parameters for the Turkish population with high precision from a large number of reference subjects. With the novel use of a freshly prepared blood panel, we clearly detected analytical bias in values for Bas, Bas%, MCHC, RDW and MPV which depended on the manufacturers of haematology analysers, requiring manufacturer-specific RIs for those. Regional differences in values of RBC, Hb, Hct, and UIBC were observed among the 7 major geographical regions of Turkey, which may be attributed to nutritional or environmental factors including altitude.

Acknowledgments

This study was supported by the Research Fund of Uludag Universty (UAP(T)-2011/48), Abbott Diagnostics (Abbott Laboratories, IL, USA) and Becton Dickinson (BD Diagnostics, Oxford, England). We wish to thank Dr. David Armbruster from Abbott Diagnostics (Abbott Laboratories, IL, USA) for his kind support for realization of the study. We especially thank all the volunteers for their participation in this study. Finally, we are grateful to Professor Andrew Myron Johnson for his detailed scientific editing of this manuscript.

Potential conflict of interest

None declared.

References

1. *Ceriotti F, Henny J, Queraltó J, Ziyu S, Ozarda Y, Chen B, et al. IFCC Committee on Reference Intervals and Decision Limits (C-RIDL); Committee on Reference Systems for Enzymes (C-RSE). Common reference intervals for aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyl transferase (GGT) in serum: results from an IFCC multicenter study. Clin Chem Lab Med 2010;48:1593–601. https://doi.org/10.1515/CCLM.2010.315*
2. *Ozarda Y, Ichihara K, Barth JH, Klee G, on behalf of the Committee on Reference Intervals and Decision Limits (C-RIDL), International Federation for Clinical Chemistry and Laboratory Medicine. Protocol and standard operating procedures for common use in wordwide multicenter study on reference values. Clin Chem Lab Med 2013;51: 1027–40. https://doi.org/10.1515/cclm-2013-0249*
3. *Ichihara K, Ozarda Y, Klee G, Straseski J, Barth JH, Baumann N, Ishikura K. Utility of panel of sera for alignment of test results in the worldwide multicenter study on reference values. Clin Chem Lab Med 2013;51:1007–20. https://doi.org/10.1515/cclm-2013-0248*
4. *Ichihara K, Ozarda Y, Barth JH, Klee G, Qui L, Erasmus R, et al. A global multicenter study on reference values: 1. Assessment of methods for derivation and comparison of reference intervals. Clin Chim Acta 2017;467:70-82. https://doi.org/10.1016/j.cca.2016.09.016*
5. *Ozarda Y, Ichihara K, Aslan D, Aybek H, Ari Z, Taneli F, et al. A multicenter nationwide reference intervals study for common biochemical analytes in Turkey using Abbott analyzers. Clin Chem Lab Med 2014;52:1823–33. https://doi.org/10.1515/cclm-2014-0228*
6. *Adeli K, Raizman JE, Chen Y, Higgins V, Nieuwesteeg M, Abdelhaleem M, et al. Complex Biological Profile of Hematologic Markers across Pediatric, Adult, and Geriatric Ages: Establishment of Robust Pediatric and Adult Reference Intervals on the Basis of the Canadian Health Measures Survey. Clin Chem 2015;61:1075–86. https://doi.org/10.1373/clinchem.2015.240531*
7. *International Organization for Standardization. ISO 15189:2012: Medical laboratories -- Requirements for quality and competence, 3rd ed. ISO, 2012.*

8. Ozarda Y. Reference intervals: current status, recent developments and future considerations. Biochem Med (Zagreb) 2016;26:5–16. https://doi.org/10.11613/BM.2016.001
9. Nordin G, Mårtensson A, Swolin B, Sandberg S, Cristensen NJ, Thorsteins et al. A multicenter study of reference intervals for haemoglobin, basic cell counts and erythrocyte indices in the adult population in Nordic countries. Scand J Clin Lab Invest 2004;64:385–98. https://doi.org/10.1080/00365510410002797
10. Sincleir L, Hall S, Badrick T. A survey of Australian haematology reference intervals. Pathology 2014;46:538–43. https://doi.org/10.1097/PAT.0000000000000148
11. Republic of Turkey, Ministry of Health. Health Statistics Yearbook 2014. Available at: http://sbu.saglik.gov.tr/Ekutuphane/kitaplar/EN%20YILLIK.pdf. Accessed January 5th 2015.
12. Akdag R, Energin VM, Kalayci AG, Karakelleoglu C. Reference limits for routine hematological measurements in 7-14 year-old children living at intermediate altitude (1869m, Erzurum, Turkey). Scand J Clin Lab Invest 1996;56:103–9. https://doi.org/10.3109/00365519609088595
13. CLSI and IFCC. EP28-A3C document; Defining, establishing and verifying reference intervals in the clinical laboratory: Approved guideline - 3rd edition. CLSI, 2010.
14. Westgard QC. Desirable specifications for total error, imprecision, and bias, derived from intra- and inter-individual biological variation. Available at: http://www.westgard.com/biodatabase1.htm#11. Accessed January 5th 2014.
15. Ichihara K. Statistical considerations for harmonization of the global multicenter study on reference values. Clin Chim Acta 2014;432:108–18. https://doi.org/10.1016/j.cca.2014.01.025
16. Ichihara K, Itoh Y, Lam CW, Poon PM, Kim JH, Kyono H, et al. Sources of variation of commonly measured serum analytes among 6 Asian cities and consideration of common reference intervals. Clin Chem 2008; 54:356–65. https://doi.org/10.1373/clinchem.2007.091843
17. Borai A, Ichihara K, Al Masaud A, Tamimi W, Bahijri S, Armbuster D et al. Establishment of reference intervals of clinical chemistry analytes for the adult population in Saudi Arabia: a study conducted as a part of the IFCC global study on reference values. Clin Chem Lab Med 2016; 54:843–55. https://doi.org/10.1515/cclm-2015-0490
18. Simundic AM, Cornes MP, Grankvist K, Lippi G, Nybo M, Ceriotti F, et al. Colour coding for blood collection tube closures - a call for harmonisation. Clin Chem Lab Med 2015;5:371-6. https://doi.org/10.1515/cclm-2014-0927
19. International Council for Standardization in Haematology (ICSH). Expert panel on cytometry recommendations of the International Council for Standardization in Haematology for ethylene-diamine-tetraacetic acid anticoagulation of blood for blood cell counting and sizing. Am J Clin Pathol1993;100:371–2. https://doi.org/10.1093/ajcp/100.4.371
20. Van den Bossche J, Deevreese K, Malfrait R, Van De Vyvere M, Neels H, De Schouwer P. Reference intervals for a complete blood count determined on different automated haematology analysers: Abx Pentra 120 Retic, Coulter Gen-S, Sysmex SE 9500, Abbott Cell Dyn 4000 and Bayer Advia 120. Clin Chem Lab Med 2002;40:69–73.
21. Lippi G, Salvagno GL, Solero GP, Franchini M, and Guidi GC. Stability of blood cell counts, hematological parameters and reticulocytes indexes on the Advia A120 hematological analyzer. J Lab Clin Med 2005;146:333-40. https://doi.org/10.1016/j.lab.2005.08.004
22. Ambayya A, Su AT, Osman NH, Nik-Samsudin NR, Khalid K, Chang KM, et al. Haematological reference intervals in a multiethnic population. PLoS One 2014;9:e91968. https://doi.org/10.1371/journal.pone.0091968
23. Guyatt GH, Oxman AD, Ali M, Willan A, McIlroy W, Patterson C. Laboratory diagnosis of iron-deficiency anemia: an overview. J Gen Intern Med 1992;7:145–53. https://doi.org/10.1007/BF02598003
24. Ozarda Ilcol Y, Aslan D. Use of total patient data for indirect data for indirect estimation of reference intervals for clinical chemical analytes in Turkey. Clin Chem Lab Med 2006;44;867–76. https://doi.org/10.1515/CCLM.2006.139
25. Rustad P, Felding P, Franzson L, Kairisto V, Lahti A, Martensson A, et al. The Nordic Reference Interval Project 2000: recommended reference intervals for 25 common biochemical properties. Scand J Clin Lab Invest 2004;64:271–84. https://doi.org/10.1080/00365510410006324
26. Aslan D, Gumruk F, Gurgey A, Altay C. Importance of RDW value in differential diagnosis of hypochrome anemias. Am J Hematol 2002;69:31–3. https://doi.org/10.1002/ajh.10011
27. Hartmann S, Krafft A, Huch R, Breymann C. Effect of altitude on thrombopoietin and the platelet count in healthy volunteers. Thromb Haemost 2005;93:115–7.
28. Karita E, Ketter N, Price MA, Kayitenkore K, Kaleebu P, Nanvubya A et al. CLSI-derived hematology and biochemistry reference intervals for healthy adults in Eastern and Southern Africa. PLoS One 2009;4:e4401. https://doi.org/10.1371/journal.pone.0004401
29. Asif M, Karim S, Umar Z, Malik A, Ismail T, Chaudhary A, et al. Effect of cigarette smoking based on hematological parameters: comparison between male smokers and nonsmokers. Turk J Biochem 2013;38;75–80. https://doi.org/10.5505/tjb.2013.68077
30. Ittermann T, Roser M, Wood G, Preez H, Ludemann J, Volzke H, Nauck M. Reference intervals for eight measurands of the blood count in a large population based study. Clin Lab 2010;56:9–19.