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

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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 km² 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 (K2 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 °C 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 K2EDTA 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<sub>a</sub> data

| Centre     | Analytical system | CVA, % | WBC | RBC | Hct | MCV | MCH | MCHC | RDW | PLT | MPV | Fe | Fer |
|------------|-------------------|--------|-----|-----|-----|-----|-----|------|-----|-----|-----|----|-----|
| Bursa      | Architect i2000   |        |     |     |     |     |     |      |     |     |     |    |     |
|            | Cell Dynne 16000  |        |     |     |     |     |     |      |     |     |     |    |     |
| Izmir      | Unicell AU 5800   |        |     |     |     |     |     |      |     |     |     |    |     |
|            | (BC) DX 8000 (BC) |        |     |     |     |     |     |      |     |     |     |    |     |
| Antalya    | Architect i2000   |        |     |     |     |     |     |      |     |     |     |    |     |
|            | Cell Dynne 16000  |        |     |     |     |     |     |      |     |     |     |    |     |
| Ankara     | Unicell AU 5800   |        |     |     |     |     |     |      |     |     |     |    |     |
|            | (BC) DX 8000 (BC) |        |     |     |     |     |     |      |     |     |     |    |     |
| Diyarbakir | Abbott Rubi      |        |     |     |     |     |     |      |     |     |     |    |     |
|            | 16000 (A)         |        |     |     |     |     |     |      |     |     |     |    |     |
| Mersin     | Beckman Coulter  |        |     |     |     |     |     |      |     |     |     |    |     |
|            | LH780             |        |     |     |     |     |     |      |     |     |     |    |     |
|            | (BC) AU 6800      |        |     |     |     |     |     |      |     |     |     |    |     |
|            | (BC) DX 8000 (BC) |        |     |     |     |     |     |      |     |     |     |    |     |
| Urfa       | Abbott Rubi      |        |     |     |     |     |     |      |     |     |     |    |     |
|            | 16000 (A)         |        |     |     |     |     |     |      |     |     |     |    |     |
| Erzurum    | Beckman Coulter  |        |     |     |     |     |     |      |     |     |     |    |     |
|            | LH780             |        |     |     |     |     |     |      |     |     |     |    |     |
|            | (BC) AU 5800      |        |     |     |     |     |     |      |     |     |     |    |     |
|            | (BC) DX 8000 (BC) |        |     |     |     |     |     |      |     |     |     |    |     |
| Konya      | Beckman Coulter  |        |     |     |     |     |     |      |     |     |     |    |     |
|            | LH780             |        |     |     |     |     |     |      |     |     |     |    |     |
|            | (BC) AU 5800      |        |     |     |     |     |     |      |     |     |     |    |     |
| Ordu       | Beckman Coulter  |        |     |     |     |     |     |      |     |     |     |    |     |
|            | LH780             |        |     |     |     |     |     |      |     |     |     |    |     |
|            | (BC) AU 5800      |        |     |     |     |     |     |      |     |     |     |    |     |
|            | (BC) DX 8000 (BC) |        |     |     |     |     |     |      |     |     |     |    |     |

The desirable limits for CV<sub>a</sub> were set as half of within-individual within-subject biologic variation (CV<sub>I</sub>) as reported on the Westgard website; https://www.westgard.com/biodatabase1.html. WBC = 11.4, RBC = 3.2, Hb = 2.85, Hct = 1.14, MCV = 1.4, MCH = 1.4, MCHC = 1.06, RDW = 1.34, PLT = 3.1. CV<sub>a</sub> – 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) 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 CVs were set as a half of the within-individual CV (CVI) reported on the Westgard website (14). The within- and between-day CVs 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 (SDBL) was computed based on one-way ANOVA. The relative magnitude of SDBL to that of residual SD (or net between-individual SD: SDBI) was computed as the SD ratio (SDR): SDRBL = SDBL / SDBI. For detailed analysis of sources of variation of RVs, SDRs for between-gender (SDRgender), between-age subgroup differences (SDRage) and between-region (SDRR), 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 (SDT) 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 (SDRBL1) 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 SDRBL1. If SDRBL1 remained > 0.30, we then checked for the consistency of the findings in volunteers’ test results (SDRBL2) 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 (SDRBL1 ≤ 0.3), SDRBL2 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 (SDRBM > 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 (SDBI), as follows (17):
\[ \Delta L\text{LL} = \left| LL_+ - LL_- \right| / \left( UL_+ - LL_+ \right) / 3.92 \]
\[ \Delta U\text{UL} = \left| UL_+ - UL_- \right| / \left( UL_+ - LL_+ \right) / 3.92 \]

where \( LL_+ \), \( LL_- \), \( UL_+ \), and \( UL_- \) represent LL (or UL) determined with/without the LAVE method, respectively. We set the critical value for \( \Delta L\text{LL} \) (or \( \Delta U\text{UL} \)) ratio as 0.25 in analogy to the theory of acceptable analytical bias in laboratory tests since the numerator of \( \Delta L\text{LL} \) (or \( \Delta U\text{UL} \)) ratio is a bias by the choice of derivation method and the denominator corresponds to \( SDBI \) (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...
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.
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[Diagram showing reference intervals for various haematology parameters for males and females, with different SDR values.]
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* between-lab SDR after limiting labs using BC and Sy analyzer

[Image of graphs and tables showing hematological parameters and SDR values, with indications of male and female data, and order by manufacturer for each parameter.]
to a reduction in SDRBL1 from 0.44 to 0.15. After removal of the biased test results, SDRBL2 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 (SDRBL1 of 0.54 and 0.62, respectively) with a tendency of analyser dependent bias. However, SDRBL2 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 (SDRBL1 of 1.08 and 1.15, respectively) and in the volunteers’ test results (SDRBL2 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 (SDRBM) of Bas and Bas% were com-

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**Figure 1 (continued).** Between-laboratory differences computed as SD ratio (SDR) were denoted as SDRBL1 for the panel test results and as SDRBL2 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.
### 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<sub>BL1</sub> | SDR<sub>-gender</sub> | SDR<sub>-age</sub> | SDR<sub>BL2</sub> | SDR<sub>BR</sub> | SDR<sub>BM</sub> | Scheme for deriving RIs |
|           | All centres | Aft excl | (M, F) | (M, F) | RI from all labs’ results |
| WBC       | 0.24        | -        | 0.11   | 0.00  | 0.25  | -         | -  | RI from all labs’ results |
| Neu       | 0.48        | 0.26<sup>a</sup> | 0.00   | 0.05  | 0.20  | -         | -  | RI from all labs’ results |
| %Neu      | 0.60        | 0.00<sup>a</sup> | 0.10   | 0.10  | 0.15  | -         | -  | RI from all labs’ results |
| Lym       | 0.18        | -        | 0.10   | 0.07  | 0.18  | -         | -  | RI from all labs’ results |
| %Lym      | 0.06        | -        | 0.00   | 0.14  | 0.07  | -         | -  | RI from all labs’ results |
| Mon       | 0.54        | -        | 0.31   | 0.14  | 0.12  | -         | -  | RI from all labs’ results |
| %Mon      | 0.62        | -        | 0.23   | 0.07  | 0.07  | -         | -  | RI from all labs’ results |
| Eos       | 0.00        | -        | 0.25   | 0.03  | 0.15  | -         | -  | RI from all labs’ results |
| %Eos      | 0.00        | -        | 0.23   | 0.05  | 0.16  | -         | -  | RI from all labs’ results |
| Bas       | 1.08        | -        | 0.04   | 0.17  | 0.61  | 0.71      | -  | RIs for 3 manufacturers |
| %Bas      | 1.15        | -        | 0.00   | 0.00  | 0.62  | 0.76      | -  | RIs for 3 manufacturers |
| RBC       | 0.26        | -        | 1.00   | 0.16  | 0.49  | 0.45      | -  | RIs for 7 regions for each sex |
| Hb        | 0.11        | -        | 1.26   | 0.19  | 0.41  | 0.39      | -  | RIs for 7 regions for each sex |
| Hct       | 0.30        | -        | 1.20   | 0.11  | 0.53  | 0.50      | -  | RIs for 7 regions for each sex |
| MCV       | 0.44        | 0.15<sup>†</sup> | 0.17   | 0.07  | 0.36  | 0.31      | -  | RI from all labs’ results |
| MCH       | 0.29        | -        | 0.27   | 0.00  | 0.30  | 0.29      | -  | RI from all labs’ results |
| MCHC      | 1.00        | 0.08<sup>‡</sup> | 0.27   | 0.00  | 0.66  | 0.36      | -  | RI for BC |
| RDW       | 1.28        | 0.00<sup>‡</sup> | 0.21   | 0.13  | 0.50  | 0.69      | -  | RI for BC + Sy |
| PLT       | 0.23        | -        | 0.23   | 0.10  | 0.24  | 0.28      | -  | RI from all labs’ results |

<sup>a</sup> RI from all labs’ results
<sup>†</sup> RIs for 3 manufacturers
<sup>‡</sup> RIs for 7 regions for each sex
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MPV 0.75 - 0.00 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) - - 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. SDRBL1 - between laboratory SDR based on panel test results. Aft excl - after exclusion. SDRBL2 - between laboratory SDR based on volunteers’ test results. SDRBR - between region SDR. SDRBM - 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 (SDRBL1) than the volunteers’ results (SDRBL2). 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 SDRBL1 of 1.00, 1.28 and 0.75, respectively. Similar between-laboratory differences were also observed in volunteers’ test results as indicated by SDRBL2 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 SDRBM (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 SDRBL1 ≤ 0.3, we examined between-laboratory differences in volunteers’ results by computing SDRBL2 as shown in Column 6 of Table 3. The following findings were obtained.

No obvious between-laboratory difference was observed with SDRBL2 ≤ 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 SDRBL2 > 0.3 were observed for RBC, Hb, Hct, UIBC, TIBC, and ferritin. For ferritin, the high SDRBL2 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
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 ($rp$) $\geq$ 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 | 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  |
| Neu       | 1526| 0.240| 0.119    | 0.098| 0.020| 0.001  | 0.189  | -0.029  |
| %Neu      | 1526| 0.179| 0.100    | 0.149| -0.064| 0.018  | 0.051  | -0.002  |
| Lym       | 1526| 0.218| 0.052    | -0.112| 0.150| -0.029 | 0.138  | -0.002  |
| %Lym      | 1526| 0.186| -0.041   | -0.177| 0.086| -0.009 | -0.064| 0.005   |
| Mon       | 1526| 0.161| -0.037   | 0.065| 0.000| 0.064  | 0.118  | -0.046  |
| Eos       | 1526| 0.187| -0.023   | 0.046| 0.080| -0.058 | 0.158  | 0.033   |
| %Eos      | 1526| 0.138| -0.062   | 0.034| 0.055| -0.057 | 0.091  | 0.041   |
| Bas*      | 876 | 0.130| 0.040    | -0.001| 0.048| -0.064 | 0.085  | -0.027  |
| %Bas*     | 876 | 0.066| 0.006    | 0.003| 0.010| -0.056 | 0.016  | -0.018  |
| RBC       | 1526| 0.337| -0.284   | 0.103| 0.048| 0.050  | -0.008 |         |
| Hb        | 1526| 0.379| 0.228    | -0.273| 0.101| -0.014 | 0.129  | -0.006  |
| Hct       | 1526| 0.390| 0.279    | -0.222| 0.090| -0.062 | 0.135  | 0.013   |
| MCV†      | 1428| 0.181| 0.059    | 0.150| -0.040| 0.021  | 0.106  | 0.015   |
| MCH       | 1526| 0.107| 0.038    | 0.058| -0.27 | 0.046  | 0.075  | 0.002   |
| MCHC‡     | 705 | 0.155| -0.019   | -0.152| 0.075| 0.004  | -0.007 | -0.048  |
| RDW*      | 876 | 0.297|         | 0.111| 0.079| 0.036  | 0.011  | 0.023   |
| PLT       | 1526| 0.162| 0.067    | -0.115| 0.073| -0.081 | 0.018  | 0.002   |
| MPV§      | 705 | 0.151| 0.099    | -0.026| 0.014| 0.090  | 0.070  | 0.037   |
| Fe        | 1365| 0.188| 0.086    | -0.114| 0.056| 0.067  | 0.045  | -0.015  |
| UIBC      | 1366| 0.150| 0.001    | 0.035| 0.109| -0.045 | 0.059  | 0.009   |
| TIBC      | 1181| 0.154| 0.079    | -0.007| 0.121| 0.019  | 0.043  | 0.006   |
| Ferritin§ | 1224| 0.231| 0.224    | 0.039| 0.082| 0.033  | 0.015  | 0.008   |

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 $r_p \geq 0.394$ 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 anemia.

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 $\Delta LL$ to $SD_{bl}$ was well above the critical level of 0.25 for RBC, Hb, Hct, MCV, MCH and MCHC while the ratio of $\Delta UL$ 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. $K_2$EDTA 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 | Males + Females | Males | Females | Males + Females | Males | Females | Ratio of ΔLL or ΔUL to SDRI<sub>UL</sub> |
|-----------|------|------|------|---------------|-------|---------|---------------|-------|---------|----------------------------------|
| 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 | 0.04 0.07 0.01 0.10 0.06 0.09 |
| 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 | 0.02 0.03 0.01 0.09 0.01 0.01 |
|           |      |      | (+)  | 2393 4.03 2.10 7.41 | 1140 4.04 2.23 7.54 | 1247 4.00 2.03 7.43 | 0.02 0.03 0.01 0.09 0.01 0.01 |
| Neu %     | All  | (-)  | 2863 57 40 74 | 1368 56 39 73 | 1492 58 41 74 | 0.02 0.03 0.01 0.09 0.01 0.01 |
|           |      | (+)  | 2394 57 40 73 | 1145 56 39 72 | 1249 58 41 74 | 0.02 0.03 0.01 0.09 0.01 0.01 |
| 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 0.03 0.09 0.00 |
|           |      |      | (+)  | 2393 2.29 1.22 3.77 | 1143 2.36 1.23 3.81 | 1252 2.22 1.21 3.72 | 0.02 0.02 0.02 0.03 0.09 0.00 |
| 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 | 0.05 0.12 0.01 0.02 0.02 0.03 |
| 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 | 0.06 0.00 0.07 0.06 0.11 0.13 |
| Mon %     | All  | (-)  | 2864 7.4 4.2 11.7 | 1366 7.7 4.5 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 | 0.01 0.02 0.05 0.04 0.01 0.09 |
| 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 | 0.00 0.00 0.00 0.08 0.14 0.09 |
| 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 | 0.01 0.01 0.01 0.03 0.04 0.11 |
| Baso      | x 10^9/L | All  | (-)  | 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 | 0.00 0.00 0.00 0.00 0.00 0.00 |
| A         | x 10^9/L | All  | (-)  | 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 | 0.00 0.00 0.00 0.00 0.00 0.00 |
| S         | x 10^9/L | All  | (-)  | 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 | 0.00 0.00 0.00 0.00 0.00 0.00 |

Age: 18 – 65 years
| Parameter | Subgroup | Mean | Standard Deviation | Reference Interval |
|-----------|----------|------|--------------------|--------------------|
| Baso (%)  | All      | 2862 | 141                | 2855 - 2872        |
|           | BC       | 2646 | 125                | 2637 - 2657        |
|           | A        | 336  | 188                | 325 - 348          |
| RBC* x 10^{12}/L | All | 2862 | 141                | 2855 - 2872        |
|           | BC       | 2646 | 125                | 2637 - 2657        |
|           | A        | 336  | 188                | 325 - 348          |
| Hb* g/L   | All      | 2872 | 141                | 2855 - 2872        |
|           | BC       | 2646 | 125                | 2637 - 2657        |
|           | A        | 336  | 188                | 325 - 348          |
| Parameter | Subgroup | Method | Reference Interval |
|-----------|----------|--------|-------------------|
| Hb*       | All      | (-)    | 112 - 168         |
|           |          | (+)    | 112 - 168         |
|           | M        | (-)    | 141 - 168         |
|           |          | (+)    | 141 - 168         |
|           | A        | (-)    | 148 - 168         |
|           |          | (+)    | 148 - 168         |
|           | MED      | (-)    | 142 - 168         |
|           |          | (+)    | 142 - 168         |
|           | BS       | (-)    | 145 - 168         |
|           |          | (+)    | 145 - 168         |
|           | CEA      | (-)    | 142 - 168         |
|           |          | (+)    | 142 - 168         |
|           | EA       | (-)    | 147 - 168         |
|           |          | (+)    | 147 - 168         |
|           | SEA      | (-)    | 147 - 168         |
|           |          | (+)    | 147 - 168         |
|           | All      | (-)    | 2875 - 1126       |
|           |          | (+)    | 2875 - 1126       |
| Hct*      | All      | (-)    | 306 - 350         |
|           |          | (+)    | 306 - 350         |
| MCV*      | All      | (-)    | 87 - 95.7         |
|           |          | (+)    | 87 - 95.7         |
| MCH*      | All      | (-)    | 23.8 - 32.4       |
|           |          | (+)    | 23.8 - 32.4       |
| MCHC*     | All      | (-)    | 313 - 350         |
|           |          | (+)    | 313 - 350         |
| BC        | All      | (-)    | 338 - 350         |
|           |          | (+)    | 338 - 350         |

References intervals derived with the parametric method for hematological parameters in all subgroups (continued).
| Parameter | Subgroup | Reference Interval (-) | Reference Interval (+) | Difference | p-value |
|-----------|----------|------------------------|------------------------|------------|---------|
| RDW-CV*   | All      | 13.6 11.8 17.7         | 13.4 11.6 16.4         | 0.2        | 0.02    |
|           | BC       | 13.5 11.8 16.6         | 13.3 11.5 15.8         | 0.2        | 0.00    |
|           | S        | 13.6 11.8 17.7         | 13.4 11.6 16.4         | 0.2        | 0.02    |
| PLT       | All      | 250 152 383            | 147 135 214            | 0.5        | 0.04    |
| MPV*      | All      | 8.8 6.3 11.8           | 8.6 6.0 11.6           | 0.2        | 0.03    |
|           | BC       | 8.8 7.0 11.1           | 8.6 6.9 11.0           | 0.2        | 0.02    |
|           | S        | 8.8 6.3 11.8           | 8.6 6.0 11.6           | 0.2        | 0.03    |
| Fe        | All      | 14.4 3.8 29.6          | 16.5 5.9 31.6          | 0.12       | 0.03    |
|           | BC       | 14.8 5.0 29.6          | 16.8 7.4 31.8          | 0.2        | 0.03    |
| UIBC*     | All      | 46.5 23.8 78.9         | 42.6 20.1 69.6         | 0.4        | 0.02    |
|           | BC       | 45.8 24.2 73.7         | 42.0 21.5 64.7         | 0.4        | 0.04    |
|           | S        | 46.5 23.8 78.9         | 42.6 20.1 69.6         | 0.4        | 0.02    |
| UIBC*     | All      | 44.6 23.3 79.1         | 40.2 22.7 69.7         | 0.4        | 0.04    |
|           | BC       | 43.9 24.2 75.1         | 39.6 20.9 64.3         | 0.4        | 0.07    |
|           | S        | 44.6 23.3 79.1         | 40.2 22.7 69.7         | 0.4        | 0.04    |
| CEA       | All      | 47.4 24.6 69.5         | 45.0 24.1 64.9         | 0.6        | 0.06    |
|           | BC       | 47.0 27.0 67.0         | 44.9 25.5 64.2         | 0.6        | 0.04    |
|           | S        | 47.4 24.6 69.5         | 45.0 24.1 64.9         | 0.6        | 0.06    |
| EA        | All      | 49.3 27.9 86.3         | 45.0 27.3 82.7         | 0.6        | 0.03    |
|           | BC       | 48.1 28.0 81.1         | 44.2 27.4 74.8         | 0.6        | 0.06    |
|           | S        | 49.3 27.9 86.3         | 45.0 27.3 82.7         | 0.6        | 0.06    |
| SEA       | All      | 43.2 24.2 69.1         | 40.3 22.0 61.2         | 0.6        | 0.02    |
|           | BC       | 42.8 24.0 63.1         | 39.8 21.8 55.6         | 0.6        | 0.02    |
|           | S        | 43.2 24.2 69.1         | 40.3 22.0 61.2         | 0.6        | 0.02    |

*RDW-CV: Red Cell Distribution Width Coefficient of Variation, MPV: Mean Platelet Volume, Fe: Iron, UIBC: Uric Acid, CEA: Carcinoembryonic Antigen, EA: Alpha-Fetoprotein, SEA: Serum Iron.*
| Parameter | Units | All | M | A | MED | BS | CEA | EA | SEA | < 45 years | ≥ 45 years |
|-----------|-------|-----|----|----|-----|----|-----|----|-----|------------|------------|
| TIBC*     | μmol/L| (-) 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 |                |
|           |       | (-) 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 |                |
|           |       | (-) 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 |                |
|           |       | (-) 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 |                |
|           |       | (-) 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 |                |
|           |       | (-) 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 |                |
|           |       | (-) 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 |                |
|           |       | (-) 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  | (-) 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 |                |

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 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 Rs which correspond to between-individual SD (SDRI). The critical value for ΔLL (or ΔUL) ratio was set as 0.25.
| Test item | Unit | RIs | N | SDR- gender | Males + Females | Males | Females |
|-----------|------|-----|---|-------------|----------------|-------|---------|
|          |      |     |   |             | LL  | Me  | UL  | LL  | Me  | UL  | LL  | Me  | UL  |
| WBC      | 10⁹/L | C   |   |             | 2862 | 0.11 | 4.39 | 7.16 | 11.59 | -   | -   | -   | -   | -   |
| Neu      | 10⁹/L | C   |   |             | 2849 | 0.00 | 2.04 | 4.04 | 7.54 | -   | -   | -   | -   | -   |
| Neu%     | %    | C   |   |             | 2863 | 0.10 | 0.40 | 0.57 | 0.74 | -   | -   | -   | -   | -   |
| Lym      | 10⁹/L | C   |   |             | 2863 | 0.10 | 1.21 | 2.28 | 3.77 | -   | -   | -   | -   | -   |
| Lym%     | %    | C   |   |             | 2878 | 0.00 | 0.17 | 0.32 | 0.47 | -   | -   | -   | -   | -   |
| Mon      | 10⁹/L | C   |   |             | 2864 | 0.31 | 0.26 | 0.53 | 0.94 | -   | -   | -   | -   | -   |
| Mon%     | %    | C   |   |             | 2853 | 0.23 | 0.04 | 0.07 | 0.12 | -   | -   | -   | -   | -   |
| Eos      | 10⁹/L | C   |   |             | 2849 | 0.25 | 0.02 | 0.14 | 0.50 | -   | -   | -   | -   | -   |
| Eos%     | %    | C   |   |             | 2851 | 0.23 | 0.00 | 0.02 | 0.06 | -   | -   | -   | -   | -   |
| Bas      | 10⁹/L | MS  |   |             | 981  | 0.04 | 0.01 | 0.06 | 0.13 | -   | -   | -   | -   | -   |
|          |      |     |   |             | 1548 | 0.00 | 0.01 | 0.03 | 0.09 | -   | -   | -   | -   | -   |
|          |      |     |   |             | 322  | 0.00 | 0.01 | 0.03 | 0.07 | -   | -   | -   | -   | -   |
| Bas%     | %    | MS  |   |             | 978  | 0.00 | 0.00 | 0.00 | 0.00 | -   | -   | -   | -   | -   |
|          |      |     |   |             | 1552 | 0.00 | 0.00 | 0.00 | 0.00 | -   | -   | -   | -   | -   |
| RBC*     | 10¹²/L | RS  |   |             | 2446 | 1.00 | 4.39 | 5.20 | 6.07 | 3.96 | 4.60 | 5.31 |
|          |      |     |   |             | 139  | 1.00 | 4.30 | 4.99 | 5.50 | 4.02 | 4.52 | 5.14 |
|          |      |     |   |             | 288  | 1.00 | 4.69 | 5.36 | 6.06 | 3.98 | 4.68 | 5.33 |
|          |      |     |   |             | 391  | 1.00 | 4.31 | 4.99 | 5.68 | 3.91 | 4.38 | 5.02 |
|          |      |     |   |             | 336  | 1.00 | 4.12 | 4.92 | 5.78 | 3.76 | 4.50 | 5.22 |
|          |      |     |   |             | 410  | 1.00 | 4.69 | 5.52 | 6.51 | 4.15 | 4.82 | 5.55 |
|          |      |     |   |             | 499  | 1.00 | 4.55 | 5.14 | 5.88 | 4.06 | 4.59 | 5.34 |
|          |      |     |   |             | 383  | 1.00 | 4.79 | 5.33 | 6.10 | 4.14 | 4.71 | 5.37 |
| Hb*      | g/L  | RS  |   |             | 2498 | 1.26 | 0.392| 0.456| 0.522| 0.337| 0.398| 0.461|
|          |      |     |   |             | 139  | 1.26 | 0.372| 0.434| 0.482| 0.326| 0.386| 0.438|
|          |      |     |   |             | 298  | 1.26 | 0.398| 0.466| 0.505| 0.330| 0.388| 0.440|
|          |      |     |   |             | 367  | 1.26 | 0.383| 0.444| 0.498| 0.346| 0.390| 0.439|
|          |      |     |   |             | 352  | 1.26 | 0.360| 0.431| 0.498| 0.310| 0.380| 0.434|
|          |      |     |   |             | 415  | 1.26 | 0.416| 0.478| 0.548| 0.360| 0.421| 0.485|
|          |      |     |   |             | 516  | 1.26 | 0.414| 0.457| 0.510| 0.354| 0.405| 0.470|
|          |      |     |   |             | 403  | 1.26 | 0.419| 0.473| 0.528| 0.364| 0.411| 0.472|
| Hct*     | L/L  | RS  |   |             | 2502 | 1.20 | -    | -    | -    | 0.0392| 0.456| 0.522| 0.337| 0.398| 0.461|
|          |      |     |   |             | 146  | 1.20 | -    | -    | -    | 0.372| 0.434| 0.482| 0.326| 0.386| 0.438|
|          |      |     |   |             | 271  | 1.20 | -    | -    | -    | 0.398| 0.446| 0.505| 0.330| 0.388| 0.440|
|          |      |     |   |             | 407  | 1.20 | -    | -    | -    | 0.383| 0.444| 0.498| 0.346| 0.390| 0.439|
|          |      |     |   |             | 350  | 1.20 | -    | -    | -    | 0.360| 0.431| 0.498| 0.310| 0.380| 0.434|
|          |      |     |   |             | 416  | 1.20 | -    | -    | -    | 0.416| 0.478| 0.548| 0.360| 0.421| 0.485|
|          |      |     |   |             | 514  | 1.20 | -    | -    | -    | 0.414| 0.457| 0.510| 0.354| 0.405| 0.470|
|          |      |     |   |             | 398  | 1.20 | -    | -    | -    | 0.419| 0.473| 0.528| 0.364| 0.411| 0.472|
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| Parameter | Unit | C | All | M | MED | BS | A | SEA | CEA | EA |
|-----------|------|---|-----|---|-----|----|---|-----|-----|----|
| **MCV**   | fL   |   | 2235| 0.17 | 77.2 | 87.7 | 95.7 | -   | -   | -   | -   |
| **MCH**   | pg   |   | 2383| 0.27 | 25.2 | 29.3 | 32.2 | -   | -   | -   | -   |
| **MCHC**  | g/L  | MS | 1283| 0.27 | 319  | 335  | 350  | -   | -   | -   | -   |
| **RDW-CV**| %    | MS | 1562| 0.21 | 12.2 | 13.5 | 16.3 | -   | -   | -   | -   |
| **PLT**   | 10^9/L| C | 2869| 0.23 | 152  | 250  | 383  | -   | -   | -   | -   |
| **MPV**   | fL   | MS | 1565| 0.01 | 7.0  | 8.8  | 11.8 | -   | -   | -   | -   |
| **Fe**    | μmol/L| C | 2878| 0.40 | 5.9  | 16.5 | 31.6 | 3.5 | 12.4| 27.8 |
| **UIBC**  | μmol/L| RS| 2546| -    | -    | -    | -    | 21.5| 42  | 64.7 | 28.3 | 49.9 | 78.1 |
| **TIBC**  | μmol/L| RS| 2329| -    | -    | -    | 45.0 | 58.6| 82.2| 44.0 | 58.6| 82.2 | 46.8 | 63.3 | 88.9 |
| **Ferritin**| μg/L | C | 2172| < 45 y | -   | -   | 4.7  | 21.0| 136 | -   | -   | -   | -   | -   | -   |
|           |      | ≥ 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.

*RIIs 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).

Inces 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 sub-fractions, 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 SDRage, 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.

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Potential conflict of interest

None declared.

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