

Research Article

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Determination of reference intervals of hemogram with advanced clinical parameters by indirect method on Sysmex XN-1000

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Abstract

Objectives: The reference interval is the primary tool used to interpret laboratory test results. Each laboratory should determine reference intervals (RIs) that reflect their population. In this study, it was aimed to determine the RIs of hemogram routine and advanced clinical test parameters for our hospital and region by indirect method and to compare these calculated RIs with the limits recommended by the current manufacturer and the literature.

Methods: The hemogram results of patients aged 18–65 years who applied to Kastamonu Training and Research Hospital between July 2020 and June 2022, were included in the study. Hemogram analyzes were performed on Sysmex XN-1000 (Kobe, Japan) hematology auto analyzers. The RIs were determined by indirect method from the obtained data using the non-parametric percentage estimation method. Harris-Boyd method was used to decide on subgroup separation based on gender.

Results: All parameters had non-parametric distribution. RBC, HGB, HCT, MCH, MCHC, PLT, RDW-CV, RDW-SD, PCT, monocytes count, eosinophils count, monocytes % and macroR parameters which required gender-specific RIs were determined separately for genders.

Conclusions: When the results are evaluated, it shows that the manufacturer's recommendations together with the studies in the literature do not fully reflect the RIs of our

population. Therefore, it is very important for each laboratory to determine its own RIs due to the differences in population, diet, technical equipment used and reference group. In addition, we think that our study will make a significant contribution to the literature, since there is insufficient data in the literature on RIs for advanced clinical parameters.

Keywords: advanced clinical parameters; hemogram; indirect reference intervals; Sysmex XN-1000

Introduction

Clinical laboratory results play a decisive role in disease diagnosis, treatment, and follow-up. Reference intervals (RIs) reported with test results are indispensable for clinicians in assessing an individual's health or disease status and are highly guiding in the clinical decision process. Therefore, reporting accurate and reliable RIs with test results is crucial for excellent clinical care [1, 2].

The reference interval (RI) consists of reference values obtained using certain statistical methods from the sample reference distribution formed by reference individuals [3]. The International Federation of Clinical Chemistry (IFCC) and the Clinical and Laboratory Standards Institute (CLSI) recommend using the direct method in the determination of reference individuals. The direct method for determining RIs is the selection of individuals from the population by direct sampling according to the criteria determined. In this method, the questionnaire forms prepared according to the defined criteria are filled. The sample is taken after the preparation period to prevent situations that need attention and are thought to affect the measurement result [4, 5].

For the direct method, 120 reference individuals reflecting a healthy population with informed consent are necessary. However, due to the disadvantages, such as the necessity of bringing together 120 healthy individuals for each group's subgroups and the high cost, the direct method could not be used widely and has led many researchers to find more applicable methods. Indirect methods have been developed and many studies have been carried out by

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selecting data per certain rules from the laboratory information management system (LIS) where patient test results are recorded [6–11].

Parametric and non-parametric statistical methods are used to determine RIs, depending on the distribution type, homogeneity, and number of data obtained by direct or indirect methods. While IFCC recommends parametric and non-parametric methods, CLSI recommends the non-parametric method. Non-parametric methods are generally preferred in RI studies [3, 12–17].

Currently, the values determined by the manufacturer and presented in the reagent package inserts are generally used as the RI in laboratories. IFCC and CLSI recommended that each laboratory determine its own RIs due to population, diet, technical equipment, and reference group differences. These international organizations issued a series of guidelines on methods. However, it is a labor-intensive and costly process for each laboratory to determine the RIs for each test. Therefore, many clinical laboratories use the values suggested by the manufacturer or obtained from the literature instead of determining and using their RIs [3, 4].

Clinicians often request hemogram parameters that provide information about the blood cells' number, shape, and structure for diagnosis, follow-up, and screening of many diseases, such as anemia, infection, bleeding disorders, and cancer. Flow cytometric techniques are used in modern hematology analyzers. This technology gives us important information regarding the developmental processes and functional status of erythrocytes, leukocytes, platelets, and their subgroups. Today's hematology auto analyzers contain over 20–30 routine and clinical and research hemogram parameters. These advanced clinical and research parameters have been associated with immunological response and specific diagnoses or immunological response patterns in recent years [18–23]. However, data on the RIs of these parameters are limited and new studies are carried out [24, 25].

The aim of this study was to determine the RIs of the routine parameters (white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), red blood cell distribution width-coefficient of variation (RDW-CV), red blood cell distribution width-standard deviation (RDW-SD), platelet distribution width (PDW), mean platelet volume (MPV), plateletcrit (PCT), neutrophils (# (absolute cell count) and % (percentage of a cell population)), lymphocytes (# and %), monocytes (# and %), eosinophils (# and %), basophils (# and %) and advanced clinical parameters (platelet large cell ratio (P-LCR), nucleated red blood cells

(NRBC # and %), immature granulocytes (IG # and %), microcytic red blood cells (microR) and macrocytic red blood cells (macroR)) of the complete blood count using the non-parametric percentage estimation method using the patient results studied in our laboratory and to compare the results obtained with the current manufacturer's recommended RIs and the literature.

Materials and methods

This study used hemogram results of 68,316 patients aged 18–65 years who applied to Kastamonu Training and Research Hospital outpatient clinics between July 2020 and June 2022. Hemogram data were obtained from LIS records retrospectively. The study did not include data from hospitalized patients and patients from nephrology, hemodialysis, gastroenterology, endocrine, hematology, oncology, radiation oncology, obstetrics, COVID-19 and emergency outpatient clinics. Only the first test results of patients with the same test performed more than once a year were included. Repeated test results of these patients were excluded. The study was approved by Kastamonu University Clinical Research Ethics Committee (approval number: 2022-KAEK-86, date: 08.24.2022).

Hemogram tests were performed on Sysmex XN-1000 (Kobe, Japan) hematology auto analyzers. The analytical process was evaluated with three different levels of internal quality control samples (Sysmex XN Check) daily and external quality assessment programs (KBUEK, Istanbul, Turkey) monthly during the study period, and acceptable results were obtained.

Hemogram data for all parameters were analyzed with Statistical Package for Social Sciences 18.0 (SPSS Inc., Chicago, IL, USA). Values were sorted in ascending order for each parameter and analyzed with the Kolmogorov-Smirnov test. All parameters had non-parametric distribution. Therefore, the log transformation of the original values was performed first; outliers were identified and excluded from the study using stem-leaf and box plots in SPSS. This software first calculates the inter-quartile range (IQR) between the distribution's lower and upper quartiles. Then it indicates the data lying outside the three IQRs from the first (Q1) and third (Q3) quartiles, i.e., outliers. This process was repeated until there were no outliers. The values were then converted to original values with an anti-logarithmic procedure, and extremes were omitted again.

A non-parametric method was used to determine the indirect RIs of each parameter. The lower and upper limits of the reference intervals, i.e., rank numbers of the 2.5th and 97.5th percentiles, were determined using formulas $0.025(n+1)$ and $0.975(n+1)$, respectively. In light of the IFCC's recommendations, 90 % confidence intervals (CIs) were calculated for the lower and upper reference limits.

The Harris-Boyd method was used to determine which parameters required gender-specific RIs. Z values were calculated by substituting the standard deviation (s), mean ($\bar{x}$), and data number (n) in the Harris-Boyd equation (Figure 1). Calculated Z compared to critical Z values.

$$Z_{\text{calculated}} = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \quad \text{and} \quad Z_{\text{critical}} = 3.0 \times \sqrt{\frac{n_1 + n_2}{240}}$$

Figure 1: Harris–Boyd equations ($Z_{\text{calculated}}$ and Z_{critical}).

The fact that the $Z_{\text{calculated}}$ is greater than the Z_{critical} indicates that the means of the groups differ. Z_{critical} is the threshold value determined in response to 120 data from each subgroup. The value $z=3$ in the Z_{critical} formula shows that the difference between subgroups is large/significant enough to separate the groups [26]. Then, separate reference intervals for males and females were determined for the parameters that were determined to be different between the sexes. The methods part of our study was summarized with a flowchart (Figure 2).

Results

In total, 68,316 hemogram results were included in this study according to patient selection criteria. Among 68,316 patients, 41,432 (60.6 %) were female, and 26,884 (39.4 %) were male. The mean age $\pm$ standard deviation (SD) of the patients was 40.9 ± 14 years. All parameters had non-parametric distribution. Therefore, a logarithmic transformation was performed for each parameter, and it was observed that the kurtosis/skewness statistics approached zero. The SPSS explore/outliers step was used until no extreme values remained. Then, the values were converted to normal values by anti-logarithmic transformation, and the same extreme value removal process was repeated. As a result of these procedures, 6.1 % of the total data were excluded from the study. In Table 1, descriptive statistics of hemogram parameters are presented. Gender-specific parameters were stated as female and male.

Harris-Boyd method was used to make decisions for gender-specific RIs, and no difference was found in WBC, MCV, PDW, MPV, P-LCR, NRBC, NRBC %, neutrophils count, lymphocytes count, basophils count, neutrophils %, lymphocytes %, eosinophils %, basophils %, IG, IG % and microR tests. Therefore, common RIs were calculated for these parameters. RBC, HGB, HCT, MCH, MCHC, PLT, RDW-CV,

RDW-SD, PCT, monocyte count, eosinophil count, monocyte %, and macroR parameters which required gender-specific RIs were determined separately as female and male. The CI of the lower and upper reference limits (LRL and URL) and the RIs determined by the indirect non-parametric percentage estimation method were given in Table 2. Median values, upper and lower reference limits of HGB, RBC, and PLT parameters according to gender were presented in Figure 3.

Discussion

In this study, RIs of routine and advanced clinical parameters of hemogram were determined by the indirect method in Kastamonu Training and Research Hospital. Hemogram results of individuals aged 18–65 years, analyzed in the clinical biochemistry laboratory between July 2020 and June 2022, were used. After inpatients and some outpatients were excluded by data filtration, the remaining 68,316 data were used. The disadvantage of this study was the possibility of including a large number of abnormal patient results due to the large number of data. It was very important to exclude the extreme values to minimize the effect of these pathological results on the study and reach the results of only healthy individuals. For this, the SPSS explore/outliers step was used until there were no extreme values for all parameters. The reference intervals of RBC, HGB, HCT, MCH, MCHC, PLT, RDW-CV, RDW-SD, PCT, monocytes count, Eosinophils count, Monocytes %, and MacroR parameters were determined separately as female and male due to the differences between genders. Our results were evaluated together with confidence intervals; there were differences between the literature and the manufacturer's recommended reference limits [24, 25, 27].

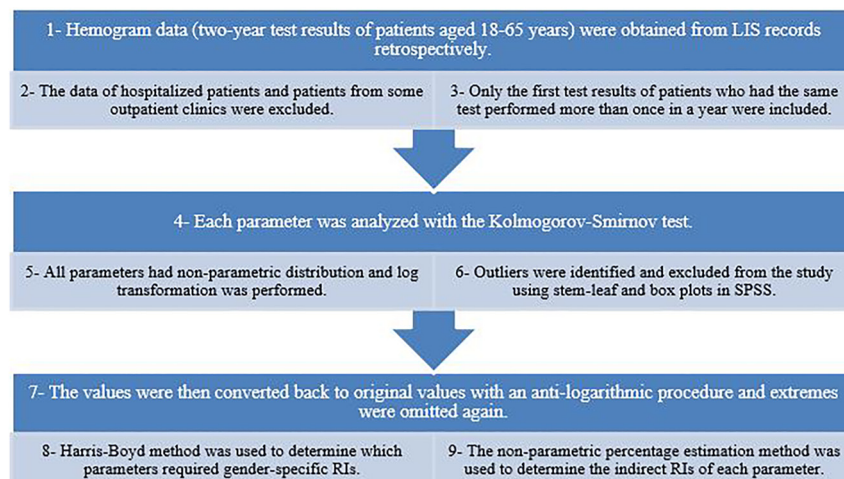


Figure 2: Flowchart of the methodology.

Table 1: Descriptive statistics of hemogram parameters.

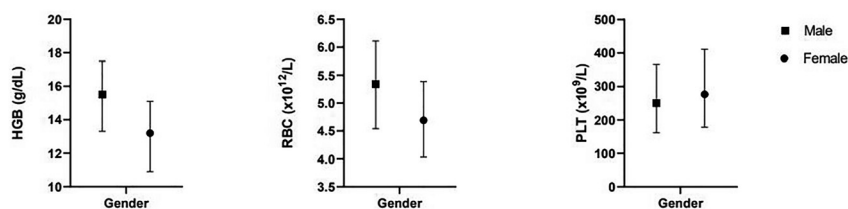
Parameter, unit	Sex	Outliers, n (%)	Included, n (%)	Median	Q1–Q3
WBC, $\times 10^9/L$	Female + Male	2,611 (3.8)	65,705 (96.2)	7.13	6.06–8.37
RBC, $\times 10^{12}/L$	Female	1,136 (2.7)	40,296 (97.3)	4.69	4.46–4.93
	Male	959 (3.6)	25,925 (96.4)	5.34	5.06–5.60
HGB, g/dL	Female	2,197 (5.3)	39,235 (94.7)	13.2	12.4–13.9
	Male	1,322 (4.9)	25,562 (95.1)	15.5	14.7–16.2
HCT, %	Female	1,813 (4.4)	39,619 (95.6)	40.3	38.3–42.2
	Male	1,058 (3.9)	25,826 (96.1)	46.1	44.0–48.1
MCV, fL	Female + Male	3,764 (5.5)	64,552 (94.5)	86.3	83.6–89.0
MCH, pg	Female	3,308 (8.0)	38,124 (92.0)	28.3	27.0–29.4
	Male	1,455 (5.4)	25,429 (94.6)	29.1	28.1–30.0
MCHC, g/dL	Female	1,539 (3.7)	39,893 (96.3)	32.6	31.8–33.3
	Male	777 (2.9)	26,107 (97.1)	33.5	32.8–34.2
PLT, $\times 10^9/L$	Female	1,461 (3.5)	39,971 (96.5)	277	238–321
	Male	1,083 (4.0)	25,801 (96.0)	250	216–289
RDW-SD, fL	Female	1,626 (3.9)	39,806 (96.1)	41.1	39.4–43.2
	Male	1,079 (4.0)	25,805 (96.0)	40.0	38.3–42.0
RDW-CV, %	Female	4,649 (11.2)	36,783 (88.8)	13.0	12.5–13.6
	Male	1,557 (5.8)	25,327 (94.2)	12.7	12.2–13.2
PDW, fL	Female + Male	29,126 (42.6)	39,190 (57.4)	12.3	11.1–13.6
MPV, fL	Female + Male	704 (1.0)	67,612 (99.0)	10.4	9.8–11.1
P-LCR, %	Female + Male	550 (0.8)	67,766 (99.2)	28.6	23.7–34.2
PCT, %	Female	1,388 (3.4)	40,044 (96.6)	0.29	0.26–0.34
	Male	1,019 (3.8)	25,865 (96.2)	0.26	0.23–0.30
NRBC, $\times 10^9/L$	Female	1,863 (4.5)	39,569 (95.5)	0.00	0.00
	Male	1,207 (4.5)	25,677 (95.5)	0.00	0.00
NRBC, %	Female	1,858 (4.5)	39,574 (95.5)	0.00	0.00
	Male	1,207 (4.5)	25,677 (95.5)	0.00	0.00
Neutrophils, $\times 10^9/L$	Female + Male	3,595 (5.3)	64,721 (94.7)	3.98	3.23–4.91
Lymphocytes, $\times 10^9/L$	Female + Male	2,665 (3.9)	65,651 (96.1)	2.26	1.86–2.72
Monocytes, $\times 10^9/L$	Female	1,726 (4.2)	39,706 (95.8)	0.51	0.43–0.61
	Male	1,145 (4.3)	25,739 (95.7)	0.60	0.50–0.72
Eosinophils, $\times 10^9/L$	Female	3,105 (7.5)	38,327 (92.5)	0.11	0.07–0.17
	Male	1,764 (6.6)	25,120 (93.4)	0.14	0.09–0.22
Basophils, $\times 10^9/L$	Female + Male	1,217 (1.8)	67,099 (98.2)	0.04	0.03–0.06
Neutrophils, %	Female + Male	1,738 (2.5)	66,578 (97.2)	57.1	51.6–62.7
Lymphocytes, %	Female + Male	2,713 (4.0)	65,603 (96.0)	32.4	27.4–37.6
Monocytes, %	Female	1,394 (3.4)	40,038 (96.6)	7.3	6.4–8.5
	Male	1,001 (3.7)	25,883 (96.3)	8.0	7.0–9.2
Eosinophils, %	Female + Male	5,225 (7.6)	63,091 (92.4)	1.7	1.1–2.5
Basophils, %	Female + Male	814 (1.2)	67,502 (98.8)	0.6	0.4–0.8
IG, $\times 10^9/L$	Female + Male	5,410 (7.9)	62,906 (92.1)	0.02	0.01–0.03
IG, %	Female + Male	3,325 (4.9)	64,991 (95.1)	0.3	0.2–0.4
MicroR, %	Female + Male	12,212 (17.9)	56,104 (82.1)	1.7	1.1–2.5
MacroR, %	Female	2,385 (5.8)	39,047 (94.2)	3.7	3.5–3.9
	Male	852 (3.2)	26,032 (96.8)	4.1	3.9–4.4

Determination of RIs is a very laborious and challenging process that requires high cost. Today, many laboratories use RIs specified in the literature or suggested by the manufacturer instead of determining their RIs. These RIs may contain methodological and ethnic differences compared to laboratory-specific RIs. Therefore, manufacturers recommend that the laboratory determine its reference values in the kit inserts [3, 4].

Direct and indirect methods select reference individuals who best represent the reference population. According to IFCC, results obtained via the indirect method are approximate, and the direct method is more precise. On the other hand, the indirect method is preferred by laboratories to determine their RIs because it is cost-effective and more practical. In this method, after the laboratory test results are separated into “pathological” and “non-pathological” in

Table 2: Reference intervals and confidence intervals determined in the current study.

Parameter, unit	Sex	Reference interval	LRL (90 % CI)	URL (90 % CI)
WBC, $\times 10^9/L$	Female + Male	4.45–10.95	4.43–4.47	10.93–10.97
RBC, $\times 10^{12}/L$	Female	4.03–5.39	4.025–4.035	5.385–5.395
	Male	4.54–6.11	4.53–4.55	6.10–6.12
HGB, g/dL	Female	10.90–15.10	10.88–10.92	15.08–15.12
	Male	13.30–17.50	13.28–13.32	17.48–17.52
HCT, %	Female	34.40–45.80	34.36–34.44	45.76–45.84
	Male	40.00–51.80	39.95–40.05	51.75–51.85
MCV, fL	Female + Male	78.10–94.10	78.06–78.14	94.06–94.14
MCH, pg	Female	24.40–31.40	24.37–24.43	31.37–31.43
	Male	26.20–31.80	26.18–26.22	31.78–31.82
MCHC, g/dL	Female	30.20–34.60	30.18–30.22	34.58–34.62
	Male	31.40–35.50	31.38–31.42	35.48–35.52
PLT, $\times 10^9/L$	Female	178–411	177–179	410–412
	Male	162–367	161–163	366–368
RDW-SD, fL	Female	36.50–47.30	36.46–36.54	47.26–47.34
	Male	35.40–46.00	35.35–35.45	45.95–46.05
RDW-CV, %	Female	11.80–14.90	11.79–11.81	14.89–14.91
	Male	11.60–14.30	11.59–11.61	14.29–14.31
PDW, fL	Female + Male	9.60–16.40	9.57–9.63	16.37–16.43
MPV, fL	Female + Male	8.90–12.40	8.89–8.91	12.39–12.41
P-LCR, %	Female + Male	16.50–45.60	16.42–16.58	45.52–45.68
PCT, %	Female	0.20–0.43	0.199–0.201	0.429–0.431
	Male	0.18–0.37	0.179–0.181	0.369–0.371
NRBC, $\times 10^9/L$	Female	0.0–0.0	0.00	0.00
	Male	0.0–0.0	0.00	0.00
NRBC, %	Female	0.0–0.0	0.00	0.00
	Male	0.0–0.0	0.00	0.00
Neutrophils, $\times 10^9/L$	Female + Male	2.14–6.83	2.13–2.15	6.82–6.84
Lymphocytes, $\times 10^9/L$	Female + Male	1.26–3.67	1.25–1.27	3.66–3.68
Monocytes, $\times 10^9/L$	Female	0.30–0.81	0.298–0.302	0.808–0.812
	Male	0.35–0.96	0.347–0.353	0.957–0.963
Eosinophils, $\times 10^9/L$	Female	0.01–0.29	0.009–0.011	0.289–0.291
	Male	0.02–0.37	0.018–0.022	0.368–0.372
Basophils, $\times 10^9/L$	Female + Male	0.01–0.09	0.0098–0.0102	0.0898–0.0902
Neutrophils, %	Female + Male	42.10–73.60	42.01–42.19	73.51–73.69
Lymphocytes, %	Female + Male	19.50–47.30	19.42–19.58	47.22–47.38
Monocytes, %	Female	4.80–10.80	4.78–4.82	10.78–10.82
	Male	5.30–11.50	5.27–5.33	11.47–11.53
Eosinophils, %	Female + Male	0.20–4.20	0.19–0.21	4.19–4.21
Basophils, %	Female + Male	0.20–1.20	0.197–0.203	1.197–1.203
IG, $\times 10^9/L$	Female + Male	0.01–0.05	0.0099–0.0101	0.0499–0.0501
	Female + Male	0.10–0.60	0.098–0.102	0.598–0.602
MicroR, %	Female + Male	0.50–4.30	0.49–0.51	4.29–4.31
MacroR, %	Female	3.10–4.30	3.096–3.104	4.296–4.304
	Male	3.50–4.90	3.49–3.51	4.89–4.91

**Figure 3:** Median, upper, and lower reference limits of HGB, RBC, and PLT parameters.

relation to health, indirect RIs are calculated by applying statistical procedures [3, 4, 6]. Healthcare professionals and patients expect inter-laboratory harmonization of test results. Electronic archiving of health records enables the determination of RIs by harmonizing [28, 29]. Therefore, we think that RIs can be determined by the indirect method as an alternative to the direct method when certain conditions are met since it does not require an additional cost and is not difficult to implement.

This study was carried out with hematology auto analyzers that we use in our daily practice, and analytical performance was monitored with regular internal quality control and external quality assessment programs. Since our

internal quality control and external quality assessment results are within acceptable limits, these RIs can be considered as RIs for the patient population admitted to our hospital. Our results may be valuable for laboratories using the same hematology analyzers and serving a similar population.

We compared our current study with RI studies on Sysmex XN devices, including new advanced clinical parameters. In addition, the RIs determined for Sysmex XN analyzers by Mayo Clinic Laboratory [27], a reference laboratory, manufacturer's recommended RIs, and other studies [24, 25] were presented in Table 3. Reference intervals of some parameters were not determined by L. van Pelt et al.

Table 3: Comparison of the current study with other reference interval studies on Sysmex XN auto analyzers.

Parameter, unit	Sex	Current study	Park SH, et al.	L. van Pelt, et al.	Mayo Clinic Lab.	Manufacturer
WBC, $\times 10^9/L$	Female	4.45–10.95	3.89–9.23	3.7–9.2	3.4–9.6	3.39–8.86
	Male					3.80–8.76
RBC, $\times 10^{12}/L$	Female	4.03–5.39	3.83–4.86	4.0–5.2	3.92–5.13	3.91–5.31
	Male	4.54–6.11	4.30–5.58	4.4–5.7	4.35–5.65	4.54–6.00
HGB, g/dL	Female	10.90–15.10	12.13–14.87	11.8–15.2	11.6–15.0	11.1–14.7
	Male	13.30–17.50	13.85–16.67	13.4–17.0	13.2–16.6	13.2–16.6
HCT, %	Female	34.40–45.80	34.79–44.27	37–46	35.5–44.9	36.9–49.1
	Male	40.00–51.80	39.15–51.65	41–50	38.3–48.6	43.2–54.5
MCV, fL	Female	78.10–94.10	81.30–100.12	82.5–97.4	78.2–97.9	87–102.2
	Male					86–100.1
MCH, pg	Female	24.40–31.40	26.04–33.56	26.8–32.6	–	25.6–30.8
	Male	26.20–31.80				25.9–30.8
MCHC, g/dL	Female	30.20–34.60	30.59–33.76	31.1–34.6	–	28.2–31.1
	Male	31.40–35.50	31.45–34.74	31.7–35.2		29.3–31.7
PLT, $\times 10^9/L$	Female	178–411	158.1–387.1	164–369	157–371	171–388
	Male	162–367	165–396.2		135–317	173–360
RDW-SD, fL	Female	36.50–47.30	35.26–48.70	37.9–48.3	–	38.9–50.0
	Male	35.40–46.00				36.3–47.3
RDW-CV, %	Female	11.80–14.90	11.22–15.56	11.8–14.3	12.2–16.1	11.2–14.0
	Male	11.60–14.30			11.8–14.5	11.2–13.4
PDW, fL	Female	9.60–16.40	9.30–16.70	10.0–17.4	–	9.7–15.1
	Male					9.5–15.5
MPV, fL	Female	8.90–12.40	9.10–12.60	9.3–12.7	–	9.2–12.2
	Male					9.2–12.1
P-LCR, %	Female	16.50–45.60	17.21–46.29	19.3–47.1	–	19.5–43.8
	Male					17.9–43.7
PCT, %	Female	0.20–0.43	0.19–0.38	0.2–0.4	–	0.19–0.41
	Male	0.18–0.37	0.12–0.35			0.19–0.36
NRBC, $\times 10^9/L$	Female	0.0–0.0	0.00–0.03	0.00–0.01	–	0.000–0.015
	Male	0.0–0.0				0.000–0.014
NRBC, %	Female	0.0–0.0	0.00–0.50	–	–	0.000–0.030
	Male	0.0–0.0				0.000–0.026
Neutrophils, $\times 10^9/L$	Female	2.14–6.83	1.78–6.04	1.6–5.8	1.56–6.45	1.50–5.00
	Male					1.65–4.97
Lymphocytes, $\times 10^9/L$	Female	1.26–3.67	1.39–3.15	1.1–3.3	0.95–3.07	1.05–2.87
	Male					1.17–3.17
Monocytes, $\times 10^9/L$	Female	0.30–0.81	0.24–0.72	0.3–0.8	0.26–0.81	0.22–0.63
	Male	0.35–0.96	0.29–0.72			0.23–0.68

Table 3: (continued)

Parameter, unit	Sex	Current study	Park SH, et al.	L. van Pelt, et al.	Mayo Clinic Lab.	Manufacturer
Eosinophils, $\times 10^9/L$	Female	0.01–0.29	0.01–0.59	0.05–0.53	0.03–0.48	0.03–0.27
	Male	0.02–0.37	0.04–0.58			0.05–0.32
Basophils, $\times 10^9/L$	Female	0.01–0.09	0.01–0.09	0.02–0.10	0.01–0.08	0.02–0.07
	Male					0.02–0.08
Neutrophils, %	Female	42.10–73.60	40.80–70.39	–	–	40.2–71.4
	Male					40.1–67.0
Lymphocytes, %	Female	19.50–47.30	20.11–46.79	–	–	21.6–49.0
	Male					23.6–48.0
Monocytes, %	Female	4.80–10.80	4.03–10.57	–	–	4.3–9.7
	Male	5.30–11.50	4.17–11.37			4.8–10.2
Eosinophils, %	Female	0.20–4.20	0.24–10.24	–	–	0.6–5.1
	Male		0.73–8.86			0.8–5.5
Basophils, %	Female	0.20–1.20	0.20–1.50	–	–	0.2–1.5
	Male					0.4–1.4
IG, $\times 10^9/L$	Female	0.01–0.05	0.00–0.04	–	–	0.01–0.04
	Male					0.01–0.03
IG, %	Female	0.10–0.60	0.00–0.50	–	–	0.16–0.62
	Male					0.17–0.61
MicroR, %	Female + Male	0.50–4.30	0.14–5.79	0.3–3.3	–	–
MacroR, %	Female	3.10–4.30	1.31–8.48	3.1–4.5	–	–
	Male	3.50–4.90	3.30–5.56			

[24] and Mayo Clinic Laboratory (Table 3). The manufacturers generated RIs of all parameters according to gender, except for MicroR and SH Park et al. [25], RIs of the MCH, RDW-CV, and RDW-SD parameters were not gender-specific. Monocyte count and Eosinophil count did not require gender-specific RIs in L. van Pelt et al. and Mayo Clinic Laboratory studies. Whereas in our study, RBC, HGB, HCT, MCH, MCHC, PLT, RDW-CV, RDW-SD, PCT, monocyte count, eosinophil count, monocytes %, and macroR parameters needed gender-specific RIs. L. van Pelt et al. and Park SH et al. found that RIs of NRBC (# and %) and MacroR, advanced clinical parameters, were not gender-specific. Despite the statistical significance, the differences in RIs were negligible from a clinical point of view.

In our study, the RIs of WBC, neutrophils (# and %), and Lymphocytes (#) parameters were found to be higher than other studies [24, 25, 27] and manufacturer's recommendations, which may affect the clinical decision. This discrepancy can be explained by the fact that as much as one-third of our samples were from primary care units, where inflammatory diseases such as the common flu and urinary tract infections are much more frequent. Our RIs of RBC parameters were higher than in other studies and the manufacturer's recommended RI. This might be due primarily to the altitude of our province (approximately 800 m). The lower limit for hemoglobin concentration in women (10.90 g/dL) was lower than in other studies. Since ferritin,

vitamin B12, and folate were not evaluated in our study, patients with iron deficiency anemia or mild anemia due to vitamin B12/folate deficiency may have been unnoticed when establishing the reference population. The RI of the PLT in our study was found to be slightly higher than in other studies, but this difference was insignificant.

Advanced clinical parameters such as P-LCR, NRBC, IG, microR, and macroR were also examined. P-LCR is an index that expresses the percentage of platelets larger than 12 fL in the blood and identifies more enzymatically and metabolically active platelets. P-LCR is a good marker to evaluate megakaryocyte and platelet activities [30]. The RI we determined for P-LCR was similar to the results of other studies and the manufacturer's recommendations. NRBC in peripheral blood is known to be pathological [31]. NRBC is an immature RBC found in the bone marrow and is not expected to be in peripheral blood in healthy individuals. In our study, the NRBC (# and %) parameters' upper limit was zero in contrast to L. van Pelt et al. and Park SH et al. studies [24, 25, 31]. IG is a hemogram parameter reflecting increased bone marrow activation in peripheral blood and has been shown to increase earlier than WBC and C-reactive protein (CRP), which markers are routinely evaluated in inflammatory diseases [32–34]. Our RIs for IG (# and %) were found to be quite similar to Park SH et al. study and manufacturer's recommendations. The microR and macroR parameters are RBC indices that enable a detailed morphological analysis of

erythrocytes. MicroR is the percentage of microcytic RBC with a volume <60 fL, and macroR with a volume >120 fL is the percentage of macrocytic RBC [35]. In our study, it was observed that there were slight differences in the lower and upper reference limits of the microR and macroR tests compared to other studies. It was thought that these different results were not very different in influencing clinical decisions.

Our study used the Harris-Boyd equation for gender-specific RI, while age specificity was not analyzed for RIs. The RIs were determined for the adult period between 18 and 65. It was observed that age-specific RIs were not mentioned in the studies [24, 25, 27] and the manufacturer's recommendations. However, there may be changes in the RIs of RBC, HGB, HCT, and other related parameters (MCV, RDW, MCH, and MCHC) with the onset of menopause, and amenorrhea, especially in women [36]. Therefore, there was a limitation for the relevant parameters in the studies, and further clinical studies are needed for the post-menopausal period.

Considering these results, the manufacturer's recommendations did not fully reflect our population's RIs. It was thought that some of the differences between our study and other studies conducted in different communities are largely related to the differences in race, socioeconomic level, nutritional behaviors, altitude of the living area, and the statistical method. We believe that more studies should be done on the RI to examine these factors' effects better. In addition, since this study is an indirect study, the variables such as hunger and satiety status, nutritional habits, obesity, and body mass index of the selected individuals could not be controlled. They may have caused different results from other studies. Therefore, each laboratory must determine its reference values suitable for its population [4].

In order to insufficient data in the literature related to RIs for advanced clinical parameters, we think our study will significantly contribute to the literature. In addition, we believe that our results will be precious for studies on these advanced clinical parameters in our region and our country.

The reference interval is a hot topic due to the development of new tests and measurement methods. Although much progress has been made over the years, there is a need for new studies on standardization and the methods used. Issues such as differences between the inclusion of reference individuals, exclusion criteria, extreme value exclusion, subgrouping in terms of gender and age, and the statistical methods used in calculating the reference interval have existed among studies. We think the indirect method will replace the direct method with the development of laboratory information systems and further studies.

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References

1. Siest G, Henny J, Gräsbeck R, Wilding P, Petitclerc C, Queralto JM, et al. The theory of reference values: an unfinished symphony. *Clin Chem Lab Med* 2013;51:47–64.
2. Horowitz GL. Establishment and use of reference values. In: Burtis CA, Ashwood ER, editors. *Tietz fundamentals of clinical chemistry and molecular diagnostics*, 5th ed. Missouri: Saunders; 2011:60–70 pp.
3. National Committee for Clinical Laboratory Standards. How to define and determine reference intervals in the clinical laboratory; approved guideline, NCCLS document C28-A2. 1-56238-406-6. Wayne, Pennsylvania, USA: NCCLS; 2000.
4. Clinical and Laboratory Standards Institute. Defining, establishing, and verifying reference intervals in the clinical laboratory. In: *Approved guideline CLSI document C28-A3*, 4th ed. Pennsylvania, USA: CLSI; 2008.
5. Horowitz GL. Establishment and use of reference values. In: Burtis CA, Ashwood ER, Burns DE, editors. *Tietz clinical chemistry and molecular diagnostics*, 5th ed. St. Louis: Elsevier Saunders Pub.; 2012:95–118 pp.
6. Ferré-Masferrer M, Fuentes-Arderiu X, Puchal-Ané R. Indirect reference limits estimated from patients' results by three mathematical procedures. *Clin Chim Acta* 1999;279:97–105.
7. Baadenhuijsen H, Smit JC. Indirect estimation of clinical chemical reference intervals from total hospital patient data: application of a modified Bhattacharya procedure. *J Clin Chem Clin Biochem* 1985;23: 829–39.
8. Kouri T, Kairisto V, Virtanen A, Uusipaikka E, Rajamäki A, Finneman H, et al. Reference intervals developed from data for hospitalized patients: computerized method based on combination of laboratory and diagnostic data. *Clin Chem* 1994;40:2209–15.
9. Solberg HE. Using a hospitalized population to establish reference intervals: pros and cons. *Clin Chem* 1994;40:2205–6.
10. Enli Y, Aslan D, Akalin N, Aydın Y, Can Yılmaztürk G, Göçhan İ, et al. Determination of reference intervals for 18–40 years old people living in denizli by using different methods. *Turk J Biochem* 2003;28: 228–45.
11. Kalafat H. The evaluation of reference intervals of clinical biochemistry tests by indirect method in Mersin province. [thesis for specialist]. Mersin: Mersin University, Faculty of Medicine, Department of Medical Biochemistry; 2008.
12. Solberg HE. Approved recommendation (1986) on the theory of reference values. Part 1. The concept of reference values. *J Clin Chem Clin Biochem* 1987;25:337–42.

13. Gowans EMS, Petersen PH, Blaabjerg O, Hørder M. Analytical goals for the acceptance of common reference intervals for laboratories throughout a geographical area. *Scand J Clin Lab Invest* 1998;48:757–64.
14. PetitClerc C, Solberg HE. Approved recommendation on the theory of reference values: Part 2. Selection of individuals for the production of reference values. *J Clin Chem Clin Biochem* 1987;25:639–44.
15. Solberg HE, PetitClerc C. Approved recommendation on the theory of reference values: Part 3. Preparation of individuals and collection of specimens for the production of reference values. *J Clin Chem Clin Biochem* 1988;26:593–8.
16. Solberg HE, Stamm D. Approved recommendation on the theory of reference values: Part 4. Control of analytical variation in the production, transfer and application of reference values. *Eur J Clin Chem Clin Biochem* 1991;29:531–5.
17. Solberg HE. Approved recommendation on the theory of reference values: Part 5. Statistical treatment of collected reference values. Determination of reference limits. *J Clin Chem Clin Biochem* 1987;25: 645–56.
18. Di Luise D, Giannotta JA, Ammirabile M, De Zordi V, Torricelli S, Bottalico S, et al. Cell population data NE-WX, NE-FSC, LY-Y of Sysmex XN-9000 can provide additional information to differentiate macrocytic anaemia from myelodysplastic syndrome: a preliminary study. *Int J Lab Hematol* 2021;44:e40–3.
19. Furundarena JR, Uranga A, Sainz MR, Gonzalez C, Uresandi N, Argoitia N, et al. Usefulness of the lymphocyte positional parameters in the Sysmex XN haematology analyser in lymphoproliferative disorders and mononucleosis syndrome. *Int J Lab Hematol* 2018;40:41–8.
20. Park SH, Kim HH, Kim IS, Yi J, Chang CL, Lee EY. Cell population data NE-SFL and MO-WX from Sysmex XN-3000 can provide additional information for exclusion of acute promyelocytic leukemia from other acute myeloid leukemias: a preliminary study. *Ann Lab Med* 2016;36: 607–10.
21. Linssen J, Ermens A, Berrevoets M, Seghezzi M, Previtali G, van der Sar-van der Brugge S, et al. A novel haemocytometric COVID-19 prognostic score developed and validated in an observational multicentre European hospital-based study. *Elife* 2020;9:1–28.
22. Kabore B, Post A, Berendsen MLT, Diallo S, Lompo P, Derra K, et al. Red blood cell homeostasis in children and adults with and without asymptomatic malaria infection in Burkina Faso. *PLoS One* 2020;15: e0242507.
23. Introcaso G, Bonomi A, Salvini L, D'Errico T, Cattaneo A, Assanelli E, et al. High immature platelet fraction with reduced platelet count on hospital admission. Can it be useful for COVID-19 diagnosis? *Int J Lab Hematol* 2021;43:1319–24.
24. van Pelt JL, Klatte S, Hwandih T, Barcaru A, Riphagen IJ, Linssen J, et al. Reference intervals for Sysmex XN hematological parameters as assessed in the Dutch Lifelines cohort. *Clin Chem Lab Med* 2022;60: 907–20.
25. Park SH, Park CJ, Lee BR, Kim MJ, Han MY, Cho YU, et al. Establishment of age- and gender-specific reference ranges for 36 routine and 57 cell population data items in a new automated blood cell analyzer, Sysmex XN-2000. *Ann Lab Med* 2016;36:244–9.
26. Harris EK, Boyd JC. On dividing reference data into subgroups to produce separate reference ranges. *Clin Chem* 1990;36:265–70.
27. <https://www.mayocliniclabs.com/test-catalog/overview/9109#Overview> [Accessed 24 Aug 2022].
28. Plebani M. Harmonization in laboratory medicine: the complete picture. *Clin Chem Lab Med* 2013;51:741–51.
29. Tate JR, Sikaris KA, Jones GR, Yen T, Koerbin G, Ryan J, et al. Harmonising adult and paediatric reference intervals in Australia and New Zealand: an evidence-based approach for establishing a first panel of chemistry analytes. *Clin Biochem Rev.* 2014;35:213–35.
30. Bessman JD, Williams LJ, Gilmer Jr PR. Mean platelet volume. The inverse relation of platelet size and count in normal subjects, and an artifact of other particles. *Am J Clin Pathol* 1981;76:289–93.
31. Stachon A, Sebbers E, Holland-Letz T, Kempf R, Herinf S, Krieg M. Nucleated red blood cells in the blood of medical intensive care patients indicate increased mortality risk: a prospective cohort study. *Crit Care* 2007;11:R62.
32. Senthilnayagam B, Kumar T, Sukumaran J, Jeya M, Rao KR. Automated measurement of immature granulocytes: performance characteristics and utility in routine clinical practice. *Pathol Res Int* 2012;2012:483670.
33. Ansari-Lari MA, Kickler TS, Borowitz MJ. Immature granulocyte measurement using the Sysmex XE-2100. Relationship to infection and sepsis. *Am J Clin Pathol* 2003;120:795–9.
34. Park BH, Kang YA, Park MS, Jung WJ, Lee SH, Lee SK, et al. Delta neutrophil index as an early marker of disease severity in critically ill patients with sepsis. *BMC Infect Dis* 2011;11:299.
35. Urrechaga E, Borque L, Escanero JF. Potential utility of the new Sysmex XE 5000 red blood cell extended parameters in the study of disorders of iron metabolism. *Clin Chem Lab Med* 2009;47:1411–6.
36. Minkin MJ. Menopause: hormones, lifestyle, and optimizing aging. *Obstet Gynecol Clin North Am* 2019;46:501–14.