Reference Ranges of Reticulocytes in Adults

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Abstract

According to the International Committee for Standardization in Haematology (ICSH), we determined the reference values for reticulocytes using an automated blood cell analyzer Technicon H*3 RTC in 200 healthy adult blood donors, aged between 17 and 60 years, 100 of whom were male and 100 female. The parameters included reticulocyte count, and its corpuscular indices; mean reticulocyte corpuscular volume (MCVr), mean reticulocyte corpuscular hemoglobin concentration (CHCMr), mean reticulocyte hemoglobin content (CHr), reticulocyte distribution width (RDWr), reticulocyte hemoglobin distribution width (HDWr) and reticulocyte corpuscular hemoglobin concentration distribution width (CHDWr). The reference ranges were established by setting the reference limits at two standard deviations from the arithmetic reference mean.

The reticulocyte count is one of the most valuable tests to assess the bone marrow erythropoietic activity. Under physiologically normal conditions, the reticulocytes mature in the bone marrow for about three days and subsequently in the blood circulation for an additional day until they become mature erythrocytes(1). Recent technological advances led the way to the development of blood cell analyzers capable of performing an accurate, precise, and complete analysis of circulating reticulocytes and erythrocytes. In the near future, the clinical importance of reticulocytes and its corpuscular indices will be emphasized by additional studies that will allow enhancement of the spectrum of indications by using these parameters for diagnosis.

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The reference ranges should not be taken from established texts, but derived from a carefully chosen reference population. The univariant reference ranges for a laboratory with a specific automated system will be unique to that laboratory, the system and the local population. In addition, consideration of differences arising from gender or environmental characteristics may also be important in assisting cost-effective clinical decisions[2]. The objective of this study is to establish the reference values for reticulocyte parameters and maturation from reticulocytes to erythrocytes using an automated blood cell analyzer, Technicon H*3 RTC, based on the theory of reference ranges recommended by the International Committee for Standardization in Haematology (ICSH)[3,4]. The reticulocyte parameters included reticulocyte count, and its corpuscular indices; mean reticulocyte corpuscular volume (MCVr), mean reticulocyte corpuscular hemoglobin concentration (CHCMr), mean reticulocyte hemoglobin content (CHr), reticulocyte distribution width (RDWr), and reticulocyte hemoglobin distribution width (HDWr). The erythrocyte parameters included erythrocyte count, and its corpuscular indices; mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), a direct measure of cell hemoglobin concentration mean (CHCM), red blood cell distribution width (RDW), and hemoglobin distribution width (HDW).

MATERIAL AND METHOD

Samples and Subjects:

The 200 target subjects (100 males and 100 females) were chosen from blood donors at the Thai Red Cross from January to May 1997. Age, gender and history of illness of each subject were noted at the time of sample collection. The inclusion criteria were that the subjects should not have any history of illness likely to influence the complete blood count (CBC). All of them had normal results of blood pressure measurements and blood examinations according to the Thai Red Cross criteria for blood donors. No anemia was found using the WHO criteria[5]. The exclusion criteria were that the subjects had abnormal results of blood pressure measurements, blood examinations, anemia or flags on CBC reported by Tech-

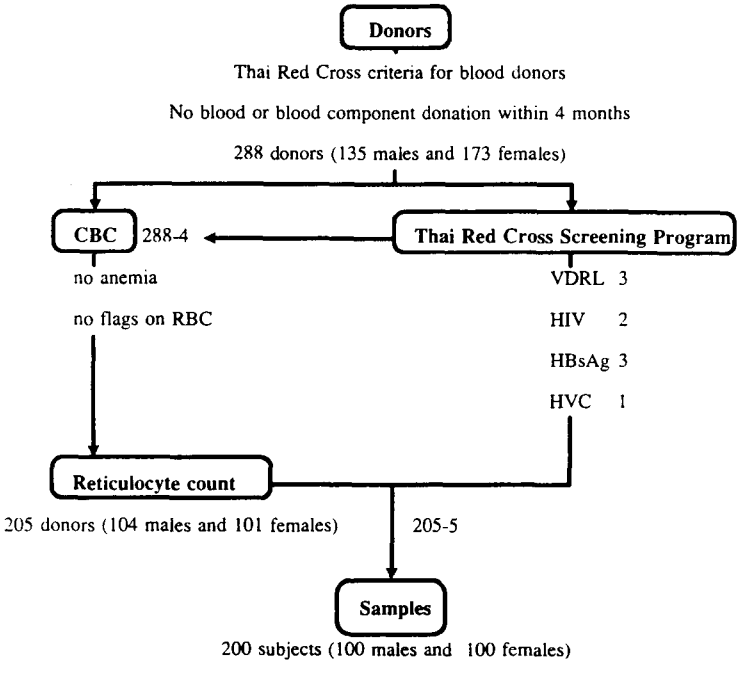

Fig. 1. The diagram of sample collection.

nicon H*3 RTC. Because the current state of erythropoiesis can be assumed to have been in a steady state for the previous 3 months, the subjects who had donated blood within 4 months prior to the study also were excluded in order to avoid any interference from the recent donation. The diagram of the samples selected is shown in Fig. 1.

Blood samples of the healthy adults who had come to donate blood were drawn into tripotassium ethylene diamine tetraacetic acid (K_3EDTA). Using evacuated tubes (Venoject), 3 mL of blood were collected and the ratio of 0.06 mL K_3EDTA (0.235 mol/L, 10.5%) to 3 mL of blood was maintained. Samples were stored at room temperature (20-25°C) and then analyzed by a Technicon H*3 RTC. The system was calibrated and operated in accordance with the manufacturer's operating instructions (Technicon Instruments Corporation)[6]. Storage times in anticoagulant before analysis varied from one to three hours. All experiments were performed at the same room temperature.

Instrument:

The Technicon H*3 RTC is an automated blood cell analyzer that performs CBC and reticulocyte count using an optical method based on the measurement of scatter and absorption of helium-neon laser light, associated with automated peroxidase cytochemical white blood cell differential counting[7]. The Technicon H*3 red blood cell counting method,[8] in particular, is identical to that of previous Technicon H*1 and H*2 systems:[9,10] after isovolumetric red blood cell sphering, measurements of monochromatic light scattered at two different angular intervals are electronically processed to derive their volume and refractive index, which is a linear function of hemoglobin concentration. Thus, in addition to directly measuring the mean corpuscular volume (MCV) and calculating the mean corpuscular hemoglobin concentration (MCHC) and content (MCH), the Technicon H*3 series instruments also provide a direct measure of the cell hemoglobin concentration mean (CHCM), which is compared in each analyzed sample with MCHC for quality control purposes and for detection of red blood cell abnormalities. The Technicon H*3 also provides red blood cell distribution width (RDW), and hemoglobin distribution width (HDW).

The Technicon H*3 reticulocyte method requires a preliminary manual mixing of 3 μL of whole blood with 3 mL of reticulocyte reagent, containing a surfactant, which spheres red blood cells and reticulocytes, and the nucleic acid-binding dye oxazine 750, which selectively stains reticulocytes by complexion with cytoplasmic RNA. After a 15-minute incubation, the prepared sample is aspirated through the Technicon H*3 red blood cell flow cell, where three detectors measure laser light scatter, at low angle (2° to 3°) and high angle (5° to 15°), and absorption. On a two-dimensional cytogram of absorption versus low angle scatter, the stained reticulocytes are separated from unstained erythrocytes, platelets, and leukocytes by appropriate thresholds. Moreover, because the amount of light absorbed by reticulocytes is proportional to the intensity of staining and RNA content, reticulocytes are subdivided into three populations with low, medium, and high RNA content. From the amount of light scattered at two different angles, the Technicon H*3 is capable of separately measuring the mean reticulocyte corpuscular volume (MCVr) in femtoliters, the mean reticulocyte corpuscular hemoglobin concentration (CHCMr) in grams per deciliter, and the mean reticulocyte hemoglobin content (CHr) is calculated from the product of the volume multiplied by the hemoglobin concentration of single cells, reticulocyte distribution width (RDWr), and reticulocyte hemoglobin distribution width (HDWr).

Calculation:

The reference ranges were estimated by 95 per cent ranges (mean±2SD) for all parameters. For each parameter, the mean was analyzed, as well as the reference limits at two standard deviations from the arithmatic mean[11]. Difference between genders was calculated using the unpaired student *t'* test, with $p<0.050$ considered statistically significant.

RESULTS

Reference ranges:

A total of 200 healthy subjects were analyzed, 100 (50%) males and 100 (50%) females, aged between 17 and 60 years, arithmetic mean (X) 38.9 years, standard error 10.20 years. Fig. 2 illustrates the age distribution with sex differentiation.

Table 1 shows the reference ranges (95% ranges) of red blood cells and Table 2 shows the reference ranges (95% ranges) of reticulocyte

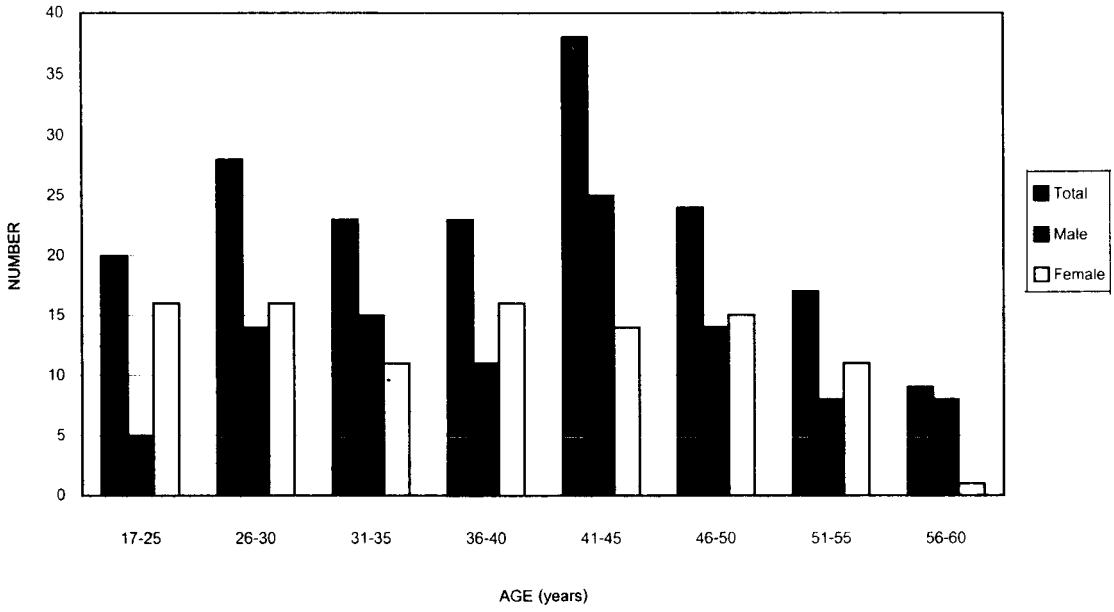

Fig. 2. Histogram of age distribution.

Table 1. The reference ranges (95% ranges) for red cell parameters from 200 healthy subjects.

Parameters	Reference ranges		
	Male (n=100)	female (n=100)	Total (n=200)
RBC ($X10^6/\mu L$)	4.03-5.80	3.75-5.01	3.71-5.58
Hemoglobin (g/dL)	13.0-15.9	12.0-14.0	12.0-15.7
Hematocrit (%)	38.4-48.4	35.4-42.6	34.6-47.5
MCV (fL)	77.4-100.0	79.0-98.5	78.2-99.2
MCH (pg)	25.1-33.8	25.2-33.0	25.1-33.4
MCHC (g/dL)	30.8-35.6	29.4-35.6	30.6-35.3
RDW (%)	12.2-14.6	12.1-14.8	12.1-14.7
HDW (g/dL)	2.0-3.4	1.9-3.2	2.0-3.3

parameters provided by the Technicon H*3 RTC in 200 healthy subjects in total, including sex differentiation. Table 3 demonstrates the average reference parameters of reticulocyte parameters with sex differentiation. With the exception of L-retic (%) H-retic (% and #), MCVr, RDWr and HDWr, we find statistically significant differences between the sexes. The correlation between red blood cells and reticulocytes as to number, MCV, and CHCM is demonstrated in Table 4. The correlation between the counts attained in the mature, in comparison to the immature reticulocyte fraction (maturation index, H-retic + M-retic) is also included in Table 4.

Table 2. The reference ranges (95% ranges) for reticulocyte parameters from 200 healthy subjects.

Parameters	Reference ranges		
	Male (n=100)	female (n=100)	Total (n=200)
Reticulocyte (%)	0.6-2.4	0.5-2.2	0.6-2.4
L-retic (%)	74.0-92.9	75.1-94.4	74.5-93.7
M-retic (%)	6.6-20.6	4.4-20.0	5.4-20.4
H-retic (%)	0.7-8.7	0.6-7.1	0.6-6.8
Reticulocyte ($X10^3/\mu L$)	37.15-144.75	30.90-110.16	33.24-124.68
L-retic ($X10^3/\mu L$)	32.38-115.76	27.18-89.62	29.29-99.76
M-retic ($X10^3/\mu L$)	3.51-26.70	2.12-21.10	2.54-24.66
H-retic ($X10^3/\mu L$)	0.36-9.46	0.28-7.42	0.31-8.44
MCVr (fL)	98.3-121.6	101.6-119.6	99.9-120.7
CHCMr (g/dL)	23.7-28.7	23.2-28.2	23.4-28.5
RDWr (%)	14.5-22.7	14.1-22.3	14.3-22.6
HDWr (g/dL)	2.49-4.08	2.45-3.97	2.38-3.99
CHr (pg)	24.5-31.1	24.2-30.9	24.3-31.2
CHDWr (pg)	3.7-5.5	3.5-5.5	3.6-5.5

Table 3. The reference means for reticulocyte parameters from 200 healthy subjects.

Parameters	Reference means		P value
	Male (n=100)	female (n=100)	
Reticulocyte (%)	1.5	1.4	0.002*
L-retic (%)	83.5	84.8	0.056
M-retic (%)	13.6	12.2	0.005*
H-retic (%)	3.0	3.1	0.755
Reticulocyte ($X10^3/\mu L$)	78.51	61.40	0.000*
L-retic ($X10^3/\mu L$)	64.90	51.62	0.000*
M-retic ($X10^3/\mu L$)	11.06	7.83	0.001*
H-retic ($X10^3/\mu L$)	2.62	1.96	0.059
MCVr (fL)	109.9	110.6	0.296
CHCMr (g/dL)	26.2	25.7	0.001*
RDWr (%)	18.6	18.2	0.121
HDWr (g/dL)	3.20	3.20	0.509
CHr (pg)	28.0	27.5	0.046*
CHDWr (pg)	4.6	4.5	0.021*

*, $P<0.050$ is considered as statistical significance.

Table 4. The correlation between red blood cells and reticulocytes regarding to number, MCV, CHCM and the correlation between reticulocytes and less maturation fraction.

Parameters	Correlation	slope	intercepts
Red blood cell (X 10^6/μL) VS Reticulocyte (%)	-0.06[n]	-0.06	5.01
MCV (fL) VS MCVr (fL)	0.72[m]	0.69	12.77
CHCM (g/dL) VS CHCMr (g/dL)	0.66[m]	0.80	12.12
Reticulocyte (%) VS M-retic + H-retic (%)	0.50[m]	0.05	0.74
Reticulocyte (X 10^3/μL) VS M-retic + H-retic (/μL)	0.95[h]	3.65	28.63

correlation; h, 0.8-1.0 = high correlation
m, 0.5-0.8 = moderate correlation
l, 0.2-0.5 = low correlation
n, 0.0-0.2 = no correlation

DISCUSSION

The manual method using supravital stain (12-14) has its limitations, for example the variable distribution of reticulocytes in the blood smear, the small number of reticulocytes in the blood smear, and the small number of reticulocytes actually counted. In addition, technicians widely vary as to morphologic identification and enumeration of reticulocytes (with coefficiants of variation higher than 25%)(12). It is also time-consuming(14). Automated reticulocyte count gives accurate results since about 20,000 cells are analyzed in each sample. The turnover time is also shorter. Moreover, the automated system provides not only the reticulocyte percentage but also much more useful parameters, i.e., the maturation degrees of reticulocytes and reticulocyte indices. The overall percentage of reticulocytes and reticulocyte maturation index (i.e., a measure of the RNA content of red cells with low RNA content, medium content, or high content) can yield information about the process of erythrocyte formation in bone marrow and thus distinguish patients with disturbances in red blood cell formation. In addition, Technicon H*3 can produce results with an acceptable degree of accuracy. Precision studies of the reticulocyte measurements indicate the CV to be reproducible by less than 10 per cent for normal and increased reticulocyte levels, along with excellent correlations with manual, flow cytometry and Sysmex R-3000 methods(8,15). Comparison of reticulocyte counts obtained with Technicon H*3 with manual counting, flow cytometry (thiazole orange method) and Sysmex R-3000 showed an acceptable correlation between Technicon H*3 and Sysmex R-3000 (correlation coefficient, r = 0.952), Technicon H*3 and flow cytometry (r = 0.922), and Sysmex R-3000 and flow cytometry (r = 0.936). There was no satisfactory correlation between any of the three methods and the values obtained with manual counting of reticulocytes (r = 0.538-0.755), consistent with the well known imprecision of the manual technique(16). According to our reference the mean reticulocyte count amounts to 1.5 per cent with the mean volume of reticulocytes being approximately 24 per cent higher than that of erythrocytes. The volume ratio (MCVr/MCV, 110.3/88.7) of those cell populations is approximately 1.24. The reticulocyte mean corpuscular hemoglobin concentration (CHCMr) is 21 per cent lower than the erythrocyte mean corpuscular hemoglobin concentration (CHCM). The ratio (CHCMr/CHCM, 25.9/32.7) is approximately 0.79. Thus, we can conclude from these preliminary studies that reticulocytes are larger than erythrocytes by about 24 per cent with the hemoglobin concentration being on average 21 per cent lower. From our study, the red blood cell count showed no correlation with the reticulocyte percentage. However, there is a moderate correlation between the erythrocyte mean corpuscular volume, the reticulocyte mean corpus-

cular volume (r, 0.72), the erythrocyte mean corpuscular hemoglobin concentration and the reticulocyte mean corpuscular hemoglobin concentration (r, 0.65). Furthermore, our results demonstrated the high correlation (r, 0.95) between the absolute reticulocyte count and the count attained in the absolutely immature fraction (H-retic + M-retic).

Buttarello M, et al[8] studied 133 Italians using Technicon H*3 and the results are: reticulocyte count (%), 1.35 (0.65-2.30); reticulocyte count ($X10^6/\mu L$), 66.5 (35.1-112.0); MCVr (fL), 104.9 (92.4-120.2); CHCMr (g/dL), 30.5 (26.7-33.0); MCH (pg), 31.1 (27.1-33.9). Lofsness KG, et al[17] studied 118 healthy Americans using flow cytometry and the results are: reticulocyte count (%), 1.56 (±0.54); reticulocyte count ($X10^6/\mu L$), 68.4 (±24.6) for males; 75.7 (±27.2) for females. Our results are shown in Table 2 and 3. The difference of reference values encountered both in absolute and in percentage concentration is due to the different methods of determination and the characteristics of the population under observation.

This study applied the recommendation of the International Committee for Standardization in Haematology (ICSH) to establish the reference ranges for reticulocyte parameters on the flow cytometric system, using Technicon H*3. However, it is impractical to satisfy one of the stringent conditions of standardization laid down by the ICSH which requires technicians to perform venipuncture without using a tourniquet. Indeed, this condition can generally not be met when sampling for routine hematology and is unlikely to be adhered to in general practice. Nevertheless, since reference values are system-specific, they should be established by each laboratory. If a laboratory does not derive its own normal ranges but adopts those of others it is obliged to ascertain not only that the type of population is similar and the appropriate statistical techniques have been applied but also that the blood sampling techniques and laboratory methods, including the methods of calibrating instruments, are identical. Furthermore, hematological variables are affected not only by age, sex, ethnic origin and altitude but also by a number of other biological factors and extraneous influences.

Finally, the reference values for the reticulocyte parameters of this study hopefully will be useful not only as baseline data but also as an efficient tool for hematologists. With these reference values, further studies of pathological conditions could establish the respective pattern which might help specify the bone marrow erythropoiesis of each condition.

ACKNOWLEDGEMENTS

This work was supported by Rachada-Pisek-Sompoch Grant, Faculty of Medicine, Chulalongkorn University.

We wish to thank the staff of the Thai Red Cross and Hematology Unit, Department of Laboratory Medicine for their assistance throughout the program. We also wish to thank Ms Petra Hirsch for editing the manuscript.

(Received for publication on September 25, 1997)

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ค่าอ้างอิงมาตรฐานของเม็ดเลือดแดงเรติคูโลซัยท์ในผู้ใหญ่

นวพรรณ จารุรักษ์, พ.บ., วัชรี ลิมปนสิทธิกุล, ปร.ด.**, นรินทร์ วรวุฒิ, พ.บ.***, ทิพย์มาศ วิโรจน์โภคา, ป. (พยาบาล และผดุงครรภ์)****, ชัยเวช นุชประยูร, พ.บ.***, *****

โดยเกณฑ์ของคณะกรรมการมาตรฐานโลหิตวิทยาสากลนานาชาติในการกำหนดค่าอ้างอิง คณะผู้วิจัยได้ทำการศึกษาค่าอ้างอิงของเม็ดเลือดแดงเรติคูโลซัยท์และดัชนีเม็ดเลือดแดงเรติคูโลซัยท์ด้วยเครื่องตรวจวิเคราะห์เลือดอัตโนมัติ Technicon H*3 RTC โดยใช้ตัวอย่างเลือดจากผู้บริจาคโลหิตจำนวน 200 คน อายุระหว่าง 17–60 ปี เป็นเพศชาย 100 คน และเพศหญิง 100 คน ค่าที่ทำการศึกษาประกอบด้วย ค่านับของเม็ดเลือดแดงเรติคูโลซัยท์ ค่าเฉลี่ยของขนาดของเม็ดเลือดแดงเรติคูโลซัยท์ ค่าเฉลี่ยของฮีโมโกลบินของเม็ดเลือดแดงเรติคูโลซัยท์ /ค่าเฉลี่ยของฮีโมโกลบินของเม็ดเลือดแดงเรติคูโลซัยท์แต่ละเซลล์ ค่าการกระจายของขนาดของเม็ดเลือดแดงเรติคูโลซัยท์ ค่าการกระจายของฮีโมโกลบินของเม็ดเลือดแดงเรติคูโลซัยท์ ค่าการกระจายของฮีโมโกลบินของเม็ดเลือดแดงเรติคูโลซัยท์แต่ละเซลล์ ค่าอ้างอิงกำหนดขึ้นให้ครอบคลุมตัวอย่างเลือดร้อยละ 95 โดยใช้ค่าเฉลี่ยทางคณิตศาสตร์บวกลบด้วยสองค่าเบี่ยงเบนมาตรฐาน

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