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The indirect method in the establishment of reference intervals for blood cell parameters in elderly population: A retrospective study

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ABSTRACT

Introduction: Reference intervals (RIs) of blood parameters vary between gender and age. Therefore, it is important to establish specific RIs for the population served. To interpret the reports of elderly population, laboratories use RIs of adults (>18 years old). In calculation of RIs, healthy population is used in direct method while patient data is used in indirect method. This study was carried out to establish RIs for blood cell parameters for elderly population using indirect method.

Methods: A retrospective study was carried out using complete blood count (CBC) data of patients >65 years of age. Calculated RIs were compared with RIs defined for adults and with RIs defined for elderly population in five other countries.

Results: Data sets in all CBC parameters showed Gaussian distribution thus, parametric approach was adopted to calculate RIs. Calculated RIs of all the CBC parameters for elderly population were similar to those of other countries. RIs calculated for red blood cell count (RBC), haemoglobin (Hb) and packed cell volume (PCV) showed clinically significantly lower values when compared with adult RIs while RIs for other CBC parameters did not show such difference.

Conclusions: Patient data can be used to calculate RIs for CBC parameters for elderly population. Elderly population shows clinically significantly lower RBC, Hb, and PCV compared to the adults thus, adult RIs for RBC, Hb, and PCV cannot be used for elderly population.

Keywords: *Complete blood count, Elderly, Indirect method, Patient data, Reference intervals.*

Introduction

Reference intervals (RIs) are used to interpret laboratory results of patients and are very important on decision making in clinical practice. Thus, reliability of RIs are of paramount importance in routine patient care.

In elderly people, laboratory tests are carried out frequently due to the age associated declining of health. Complete blood count (CBC) is frequently requested in routine care of most of the patients. A CBC report includes a combination of parameters of red blood cells, white blood cells and platelets which help in the diagnosis and management of many different diseases. In standard practice, defining age specific RIs or verifying RIs for the population served is a requirement as age specific differences are described in literature for the CBC parameters (1). When RIs specific for the elderly population are not available, CBC data of them

are interpreted using RIs defined for healthy adults of above 18 years of age. Often, laboratories use RIs provided by the manufacturers of the analysers or RIs extracted from literature. This practice challenges appropriate decision making in clinical care as there are age-related variations in CBC.

Clearly defined healthy and homogenous reference populations are used when generating RIs by traditional direct method. Central 95% of the reference population with its 2.5^{th} and 97.5^{th} percentiles are taken as the lower and the upper limits of RIs respectively (1). Finding a healthy, elderly reference population is challenging since defining ''health' in old age is difficult. The increased prevalence of comorbid conditions and physiological changes associated with advancing age makes the issue more complicated. Literature shows success in indirect method of RI calculation by mining patient databases in modern laboratories where routine patient testing is carried out. The indirect method is proved to be less costly, convenient and complement to the direct method (2, 3).

Therefore, this study was carried out to establish RIs for measurands in CBC for elderly population using a database of elderly patients in an accredited hospital laboratory. The results were compared with published RIs in five countries and with the RIs for adults which are used by the laboratory to interpret the CBC of elderly patients.

Methods

From the database of a hospital laboratory situated at sea level, all the reports of CBC of elderly patients (defined as age >65 years) generated over a one-year period were selected for the study. The CBC reports had been generated by an automated analyser BC 5150 Mindray (China). The laboratory was accredited as per ISO 15189:2012 standard. After partitioning the study sample according to the gender, the RIs were calculated for the elderly (>65 years) in the subgroups of young old (65-74 years) and very old (>74 years)(4).

The reports without age and gender, reports of inpatients, reports of repeated samples and reports with flagging for any parameter were excluded from the study. The reports with red cell distribution width (RDW CV%) >14.5, absolute lymphocyte count <1 and >4.8 10^9 /L and mean platelet volume (MPV) <8.6 and >15.5 fL were also excluded from the study. Using Shine and Lal index, probable beta thalassemia trait patients were identified and excluded(5).

Outliers in each parameter were identified and excluded using Tukey's method (criterion based on the central 50% of the distribution) and obtained a refined data set. When using this method, interquartile ranges (IQR: Q3–Q1; Q1: lower quartile, Q3: upper quartile) were used at levels of <Q1–1.5 IQR and/or >Q3 + 1.5 IQR to identify outliers and they were excluded during calculation of reference intervals(1).

For all the refined data, distribution plots and box plots were drawn, and they were visually inspected to assess their normality of distribution. Descriptive statistics were used to calculate means, standard deviations and reference intervals. Since the data were normally distributed, RIs were defined as mean ± 2SD to represent the central 95% of the reference population.

The generated RIs were compared with the RIs of the adults (>18 years) which were used by the laboratory to interpret CBC of elderly patients and with the RIs defined in five other countries. IBM *SPSS* statistics version 20 was used to perform statistical analysis.

Ethical approval for the study was obtained from the Ethical Review Committee of the institution. Permission to access CBC database was obtained from the hospital management where the study was carried out.

Results

There was a total of 3094 CBC reports of patients >65 years of age. Of those, only 1604 (51.8%) fulfilled the inclusion criteria. Of the 1604, 704 (43.9%) were males and 900 (56.1%) were females. Of the 704 males, 434 (61.7%) were between 66 - 74 years and 270 (38.3%) were >74 years of age. Of the 900 females, 558 (62%) were between 66 - 74 years and 342 (38%) were >75 years of age.

The distribution plots of the data of all the parameters in both male and female groups of >65 years (Figures 1 and 2), 66-74 years and >74 years

showed Gaussian distribution. The generated RIs of CBC parameters of patients in each group and the RIs for adults (>18y) are presented in Table 1.

The generated RIs of all the groups and the RI defined in five other different countries using physically independent elderly populations are given in Table 2.

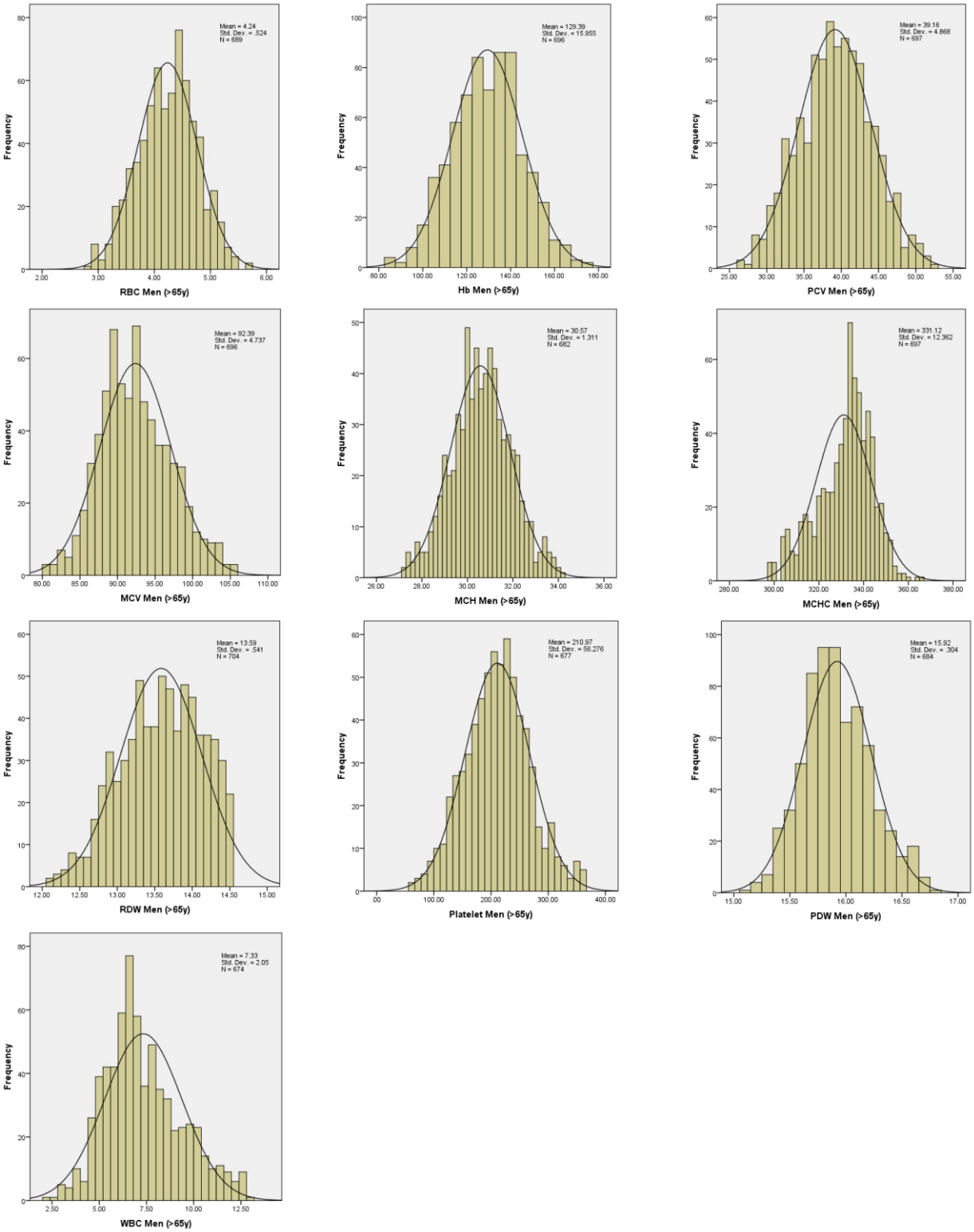

Figure 1: Frequency distribution charts of CBC parameters of male patients >65 years.

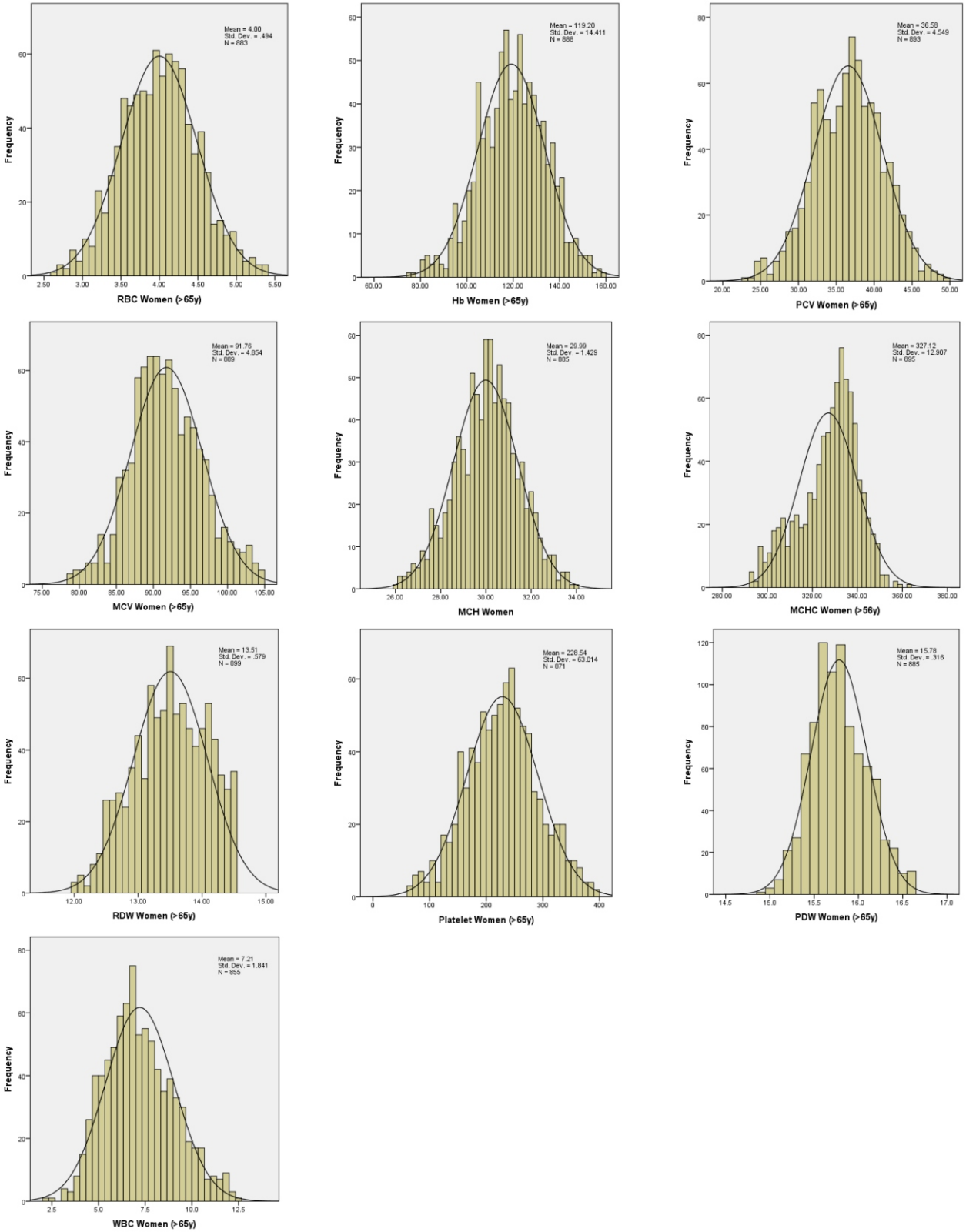

Figure 2: Frequency distribution charts of CBC parameters of female patients >65 years.

Table 1: Calculated RI of the study and the manufacturer's RIs for CBC parameters

CBC parameter	>65y Mean (RI)				66-74y Mean (RI)				>74y Mean (RI)				Laboratory (>18 years) (Combined)
	N	Male	N	Female	N	Male	N	Female	N	Male	N	Female	
RBC x 10^{12} (L)	689	4.23 (3.2-5.2)	883	3.99 (3.0-4.9)	424	4.32 (3.3-5.3)	545	4.0 (3.0 - 4.9)	264	4.09 (3.0-5.1)	336	3.92 (2.9-4.9)	4.3 - 5.8
Hb (g/L)	696	129 (97-161)	888	119 (90-148)	429	131 (101-162)	555	120 (90-149)	265	125 (94-156)	335	118 (90-146)	130 - 175
PCV (%)	697	39.1 (29.5-48.7)	893	36.5 (27.5-45.5)	430	39.8 (30.2-49.4)	556	36.8 (27.8-45.8)	266	37.9 (28.5-47.3)	336	36.2 (27.2-45.2)	40.0 - 50.0
MCV (fL)	696	92.3 (83-101.8)	889	91.7 (82.1-101.3)	432	92.3 (82.7-101.9)	552	91.3 (81.5-101.1)	265	92.5 (83.1-101.9)	339	92.4 (83.2-101.6)	82.0 - 100.0
MCH (pg)	682	30.5 (28-33.2)	885	29.9 (27.1-32.7)	421	30.5 (28.1-32.9)	550	29.8 (26.8-32.8)	264	30.6 (27.8-33.4)	337	30.2 (27.6-32.8)	27.0 - 34.0
MCHC (g/dl)	697	33.1 (30.6-35.5)	895	32.7 (30.1-35.3)	424	33.1 (30.7-35.5)	539	32.8 (30.4-35.2)	265	33 (30.6-35.4)	342	32.6 (30.0-35.2)	31.6 - 35.4
RDW CV%	704	13.5 (12.5-14.5)	899	13.5 (12.3-14.6)	434	13.5 (12.5-14.5)	558	13.4 (12.4-14.4)	270	13.6 (12.6-14.6)	342	13.5 (12.5-14.5)	-
PLT x 10^9 (L)	677	210 (98-323)	871	228 (102-354)	416	214 (106-321)	547	227 (99-354)	256	203 (90-316)	334	226 (90-362)	125 - 350
PLT DW	684	15.9 (15.3-16.5)	885	15.7 (15.1-16.3)	421	15.9 (15.3-16.5)	551	15.7 (15.1-16.3)	267	15.9 (15.3-16.5)	338	15.8 (15.2-16.4)	-
WBC x 10^9 (L)	674	7.3 (3.8-12.0)	855	7.2 (3.6-10.8)	417	7.4 (3.6-11.2)	528	7.2 (3.6-10.4)	249	6.9 (3.1-10.7)	332	7.4 (3.4-11.4)	3.5 - 9.5
Neutrophil x 10^9 (L)	655	4.2 (1.0-7.4)	841	4 (1.2-6.8)	414	4.4 (1.0-7.8)	517	3.8 (1.2-6.8)	247	3.9 (1.0-6.9)	331	4.4 (1.0-7.8)	1.8 - 6.3
Lymphocyte x 10^9 (L)	699	2.1 (0.9-3.3)	890	2.3 (0.9-3.7)	434	2.1 (0.7-3.5)	558	2.3 (0.8-3.9)	268	2.1 (0.7-3.4)	334	2.2 (0.9-3.4)	1.1 - 3.3
Eosinophil x 10^9 (L)	650	0.2 (0.0-0.6)	805	0.2 (0.0-0.5)	401	0.2 (0.0-0.6)	501	0.2 (0.0-0.5)	254	0.2 (0.0-0.6)	305	0.2 (0.0-0.5)	0.02 - 0.50
Basophil x 10^9 (L)	691	0.03 (0.0-0.07)	871	0.02 (0.0-0.04)	425	0.03 (0.0-0.06)	543	0.03 (0.0-0.06)	266	0.02 (0.0-0.56)	326	0.03 (0.0-0.06)	0.0 - 0.06
Monocyte x10^9 (L)	658	0.4 (0.1-0.7)	862	0.4 (0.1 - 0.7)	414	0.4 (0.1-0.7)	532	0.4 (0.1 - 0.6)	246	0.4 (0.1-0.7)	327	0.4 (0.1 - 0.7)	0.1 - 0.6
Neutrophil %	704	58.6 (34.6-82.6)	893	57.3 (34.9-79.7)	434	58.9 (33.9-83.9)	542	55.9 (34.9-76.9)	270	58 (35.2-80.8)	342	58.8 (36.4-81.2)	40 - 75
Lymphocyte %	704	29.9 (9.9-49.9)	895	32.1 (12.3-51.9)	434	29.7 (9.3-50.0)	552	34.4 (13.6-53.2)	270	30.4 (10.6-50.2)	340	30.3 (11.7-48.9)	20 - 50
Eosinophil %	644	3.6 (0.0-8.6)	621	3.1 (0.0-7.3)	405	3.8 (0.0-9.3)	514	3.3 (0.0-7.9)	244	3.4 (0.0-7.8)	310	2.9 (0.0-6.9)	0.4 - 8.0
Basophil %	698	0.3 (0.0-0.7)	896	0.3 (0.0-0.9)	433	0.3 (0.0-0.9)	556	0.3 (0.0-0.9)	267	0.3 (0.0-0.8)	341	0.3 (0.0-0.9)	0.0 - 1.0
Monocyte %	691	6.3 (2.9-9.7)	874	5.7 (2.5-8.9)	427	6.2 (2.8-9.6)	536	5.5 (2.5-8.5)	265	6.4 (3.0-9.8)	332	5.9 (2.8-9.1)	3.0 - 10.0

RI - Reference Interval, CBC - Complete Blood Count, RBC - Red Blood Cell Count, Hb - Haemoglobin, PCV – Packed cell volume, MCV – Mean Corpuscular Volume, MCH – Mean Corpuscular Haemoglobin, RDW – Red Cell Distribution Width, WBC - White Blood Cell Count, PLT – Platelet count, PDW – Platelet Distribution Width.

Table 2: Comparison of RI of CBC parameters of the study with the RI of different countries

CBC parameter		Reference interval										
		Study (>65y)	**Study (66-74y)**	**Study (>74y)**	**Germany (60-69y)**	**Germany (70-79y)**	**Germany (80-89y)**	**Japan (65-74y)**	**Japan (>74y)**	**Uganda (>50y)**	**China (elderly)**	**Brazil (>60y)**
RBC	Male	3.2 – 5.2	3.3 – 5.3	3.0 – 5.1	3.7 – 5.4	3.3 – 5.3	3.0 – 4.8	3.7 – 5.3	3.2 – 5.2	3.5 – 5.8	3.7 – 5.4	4.0 – 5.6
10^{12} (L)	Female	3.0 – 4.9	3.0 – 4.9	2.9 – 4.9	3.4 – 5.1	3.3 – 5.0	3.0 – 5.0			3.6 – 5.3	3.7 – 5.5	3.8 -5.1
Hb	Male	97 – 161	101 – 162	94 – 156	117 – 163	99 – 160	96 – 149	109 – 173	99 – 163	97 – 155	109 – 167	123 -168
(g/dl)	Female	90 – 148	90 – 149	90 – 146	105 – 153	102 – 148	93 – 149			108 – 150	109 – 168	113 -151
PCV	Male	29.5 – 48.7	30.2 – 49.4	28.5 – 47.3	34 – 48	29 – 46	27 – 44	32.8 – 49.2	29.9 – 47.5	29.6 – 48.1	36.0 - 51.8	38.0 – 51.4
(%)	Female	27.5 – 45.5	27.8 – 45.8	27.2 – 45.2	31 – 45	29 – 44	26 – 45			32 – 45	35.7 – 51.8	35.1 – 46.7
MCV	Male	83 – 101.8	82.7 – 101.9	83.1 – 101.9	79.9 – 100 .1	81.4 – 97.2	82.0 – 100.7	81.9 – 100.3	80.2 – 103.4	71 – 100	86 – 105	83.6 – 101.8
(fL)	Female	82.1 – 101.3	81.5 – 101.1	83.2 – 101.6	80.9 – 97.3	80.3 – 97.8	80.4 – 97.6			77 – 97	86 – 106	81.2 – 100.7
MCH	Male	28 – 33.2	28.1 – 32.9	27.8 – 33.4	27.7 – 34.2	27.5 – 33.9	26.9 – 35.9	26.7 – 35.5	25.8 – 36 2	23.2 – 33.8	26.4 - 33.6	27.6 – 33.1
(pg)	Female	27.1 – 32.7	26.8 – 32.8	27.6 – 32.8	27.6 – 33.5	26.9 – 34.1	27.0 – 33.7			25.6 – 32.7	26.4 – 33.8	26.3 – 32.6
MCHC	Male	30.6 – 35.5	30.7 – 35.5	30.6 – 35.4				32 – 36.4	30.8 – 36.8	31.4 – 35.0	29.3 – 33.3	30.6 – 34.5
(g/dl)	Female	30.1 – 35.3	30.4 – 35.2	30.0 – 35.2						32.1 – 35.3	29.1 – 33.5	30.5 – 34.3
RDW CV	Male	12.5 – 14.5	12.5 – 14.5	12.6 – 14.6							11.7 – 15.1	12.1 – 16.0
%	Female	12.3 – 14.6	12.4 – 14.4	12.5 – 14.5							11.1 – 15.2	12.2 – 15.7
PLT	Male	98 – 323	106 – 321	90 – 316	123 – 374	82 – 385	104 – 307	127 – 311	70 – 326	73 – 334	122 – 355	128 – 283
10^9 (L)	Female	102 – 354	99 – 354	90 – 362	146 – 385	122 – 443	125 – 388			78 – 359	122 - 350	126 – 331
PLT DW	Male	15.3 – 16.5	15.3 – 16.5	15.3 – 16.5								
	Female	15.1 – 16.3	15.1 – 16.3	15.2 – 16.4								
WBC	Male	3.8 – 12.0	3.6 – 11.2	3.1 – 10.7	3.9 – 11.8	4.1 – 11.1	4.2 - 10.5	2.2 – 10.2	2.3 – 8.7	2.9 – 8.0		2.8 – 9.6
10^9 (L)	Female	3.6 – 10 8	3.6 – 10.4	3.4 – 11.4	3.2 – 10.2	4.5 – 10.9	4.2 - 11.0			3.0 – 7.0		2.9 – 10.0
Neutrophil	Male	1.0 – 7.4	1.0 – 7.8	1.0 – 6.9						1.0 – 3.8		0.7 – 6.3
10^9 (L)	Female	1.2 – 6.8	1.2 – 6.8	1.0 – 7.8						1.0 – 4.1		0.7 – 6.0
Lymphocyte	Male	0.9 – 3.3	0.7 – 3.5	0.7 – 3.4				0.6 – 1.2	0.5 – 2.5	1.1 – 3.8		0.5 – 3.0
10^9 (L)	Female	0.9 – 3.7	0.8 – 3.9	0.9 – 3.4						1.0 – 3.4		0.7 – 3.3
Eosinophil	Male	0.0 – 0.6	0.0 – 0.6	0.0 – 0.6						0.04 – 0.88		0.0 – 0.7
10^9 (L)	Female	0.0 – 0.5	0.0 – 0.5	0.0 – 0.5						0.0 – 1.0		0.0 – 0.5
Monocyte	Male	0.1 – 0.7	0.0 – 0.06	0.0 – 0.56						0.19 – 0.67		0.02 – 0.84
10^9 (L)	Female	0.1 – 0.7	0.0 – 0.06	0.0 – 0.06						0.17 – 0.57		0.04 – 0.7
Basophil	Male	0.0 – 0.07	0.1 – 0.7	0.1 – 0.7						0.01 – 0.08		0.00 – 0.06
10^9 (L)	Female	0.0 – 0.07	0.1 – 0.6	0.1 – 0.7						0.02 – 0.09		0.00 – 0.06

RI - Reference Interval, CBC - Complete Blood Count, RBC - Red Blood Cell Count, Hb - Haemoglobin, PCV - Packed cell volume, MCV - Mean Corpuscular Volume, MCH - Mean Corpuscular Haemoglobin, RDW - Red Cell Distribution Width, BC - White Blood Cell Count, PLT - Platelet count, PDW - Platelet Distribution Width.

Discussion

Calculation or verification of RIs should be performed by each laboratory to standardize clinical decision making. In the process of RIs calculation, the reference population should be as comparable as possible to the population the laboratory is serving. In this study, we calculated RIs for CBC parameters for a laboratory using the outpatient database of the same laboratory and compared those with the RIs routinely used to interpret CBC reports. As the laboratory is accredited for CBC, reliability of sample collection procedures and the test process could be assured.

Although, the sample size for direct method of RIs calculation is defined as 120 (standard method defined by CLSI, USA), it is not defined for the indirect method. Since more data gives more strength to the statistical analysis, we used more than 500 CBC reports to calculate RIs for each CBC parameter.

While optimum working conditions are adopted in the direct method, in the indirect method, the sample collection procedure, pre-analytical factors such as patient preparation and sample handling reflect the routine conditions practiced in the laboratory. Thus, indirect method is expected to generate results applicable to test results generated under routine conditions of a laboratory.

In our mixed cohort of elderly patients, all the CBC parameters showed Gaussian distribution. Thus, we determined the RIs by mean ± 2SD as it includes central 95% of the reference population. The number of significant numerical figures in the RIs can markedly influence clinical decisions. Since there are no guidelines to determine the number of significant numerical figures that should appear in RI limits, we rounded the RIs limits to the nearest single decimal place.

If there are no significant differences between the calculated RIs and RIs used in the laboratory, the same RIs can be used to the population served. When there is a difference, we have to assess whether these differences are clinically significant or not. For analytes with a Gaussian distribution, Fraser and others have determined optimal, desirable and minimum clinically significant limits which can be expressed as 0.03, 0.06 and 0.09 x the width of the RIs respectively (6). According to this, the RIs generated for all the CBC parameters in both males and females in >65 years, 65-74 years and >74 years groups showed no clinically significant difference. RIs generated for all the CBC parameters except for RBC, Hb and PCV of all the study groups were also not clinically significantly different from the RIs for adults which were used by the laboratory to interpret CBC of elderly population. Interestingly, both upper and lower limits of calculated RIs of RBC, Hb and PCV of elderly population were clinically significantly lower than those of the adults.

Since it is important to get an understanding of RIs defined for elderly population in other communities, we compared RIs generated in our study with the RIs established for elderly population in five other countries. The RIs of each parameter in CBC vary with the measurand, the analytical method, and the characteristics of the reference population. However, despite using patient data, the RIs of all CBC parameters of elderly population in our study had no clinically significant differences from the RIs of other countries defined using elderly patients as well as healthy elderly people. This shows that biological parameters can be similar in populations irrespective of ethnicity, geography or socioeconomic status thus, RIs defined for one population can be adopted by another after verification (7-11).

Surprisingly, the lower limit of reference interval for Hb in our study and the other five studies fell below the anaemia cut off value for both adult males and females defined by the WHO (12). As per the literature, lower Hb levels in older population may be linked to the age related depletion of haematopoietic stem cells, finite number of cell division (13), age related defects in progenitor cell proliferation (14), insufficient mobilization of progenitors (15) and lack of hormonal stimulation (16).

Similar to the observations in the studies carried out in Germany (7) and Japan (8), our study too showed that the RIs of RBC, Hb and PCV were lower in the >74 years group than in the 65-74 years group.

The findings of our study highlight the need to revisit the diagnostic criteria for anaemia in the elderly population and challenge validity of use of

adult reference intervals in interpreting CBC reports of very old population.

Conclusions

With careful refining of data using standard statistical techniques, a large database of elderly patients can be used as a representative cohort for defining RIs for CBC parameters. Except for RBC, Hb, and PCV, RIs for all the parameters of CBC for adults can be used for elderly population. Age specific RIs should be calculated for RBC, Hb, and PCV in elderly and the diagnostic criteria for anaemia for elderly should be revisited.

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