Establishing local reference intervals for full blood count and white blood cell differential counts in Cape Town, South Africa

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Background. Accurate laboratory reference intervals (RIs) are essential to differentiate between health and disease. There are variations in haematological indices within populations relating to gender, age, ethnicity and environment. Iron deficiency is common, has a wide range of clinical morbidities and affects red cell indices. Locally derived RIs for full blood count (FBC) parameters are needed for the Western Cape region of South Africa, after the exclusion of iron deficiency. In addition, information regarding the prevalence of iron deficiency in first-time blood donors would inform blood transfusion services regarding policies to screen for and treat iron deficiency.
Objectives. To establish locally derived RIs for FBC and white blood cell (WBC) differential count parameters in healthy adults in the Cape Town area, by including first-time blood donors and excluding those with iron deficiency and thalassaemic indices. These new locally established RIs could update those in use by the local National Health Laboratory Service. A secondary objective was to establish the prevalence of iron deficiency in first-time blood donors. This would inform blood donation policies regarding screening and appropriate iron supplementation in high-risk groups prior to blood donation.
Methods. This was a prospective, descriptive study with direct convenience sampling. Participants were prospective voluntary blood donors aged between 18 and 60 years, presenting for first-time blood donation. Ethnicity was self-identified. Participants who tested positive for HIV or hepatitis B and/or C viruses were excluded. Prospective participants with iron deficiency, defined by serum ferritin levels below the RI, and those with red cell indices suggestive of an underlying thalassaemia trait were excluded. FBC samples were analysed using a Sysmex XN-1000 cell counter. Statistical non-parametric methods were used to calculate the RIs, according to international guidelines.
Results. Of the 774 participants screened, 82 (11%) had iron deficiency and were excluded. Six hundred and sixty-two patients were included for analysis, 409 (62%) female and 253 (38%) male. The majority of the participants, 348 (53%), were between 20 and 29 years of age, with a mean age of 29 years for females and 28 years for males. Participants comprised a mix of the various ethnic groups residing in Western Cape Province. The mean haemoglobin concentration for females was lower than that for males ($p<0.0001$). There were significant gender differences for total WBC count, absolute neutrophil count and platelet count, with females having higher counts than males.
Conclusions. Locally established, population-specific RIs are essential for the accurate interpretation of haematological indices. This study established locally derived gender-specific RIs for the Cape Town region, after exclusion of iron deficiency. These new RIs have implications for the accurate diagnoses of cytopenias, cytoses and other blood count abnormalities. Iron deficiency is common in first-time blood donors, and screening for iron deficiency using point-of-care testing should be considered.

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Accurate laboratory reference intervals (RIs) are essential for the correct interpretation of laboratory results such as cytopenias and cytoses and guide clinical management. RIs are defined as the central 95% of the reference values obtained from the reference sample group.[1,2] There is a need for locally established full blood count (FBC) and white blood cell (WBC) differential count RIs using modern automated technology for the local populations residing in the Cape Town region. Laboratories in African countries usually use RIs obtained from European and North American populations, which is problematic because of diversity of biological indices in populations due to geographical, ethnic and environmental factors.[2] Current RIs in use by the National Health Laboratory Service (NHLS) in the Cape Town region of South Africa (SA) are derived partly from a previous study in Gauteng Province[3] and partly from international sources. The reference sample group used to derive RIs should represent the local population demographics and include adequate numbers of participants.[1,2]

Statistical procedures used to calculate accurate RIs must be performed according to international guidelines, and outlying results indicating a lack of good health should be excluded.[1-3] Iron deficiency is common in Western Cape Province, with a prevalence of 9.8% reported in healthy adults, the majority female.[4] Iron deficiency leads to a hypochromic microcytic anaemia with a reduced red cell count (RCC). Thalassaemia trait is a genetic disorder of haemoglobin (Hb) that may also cause a hypochromic microcytic anaemia, or the combination of microcytosis with an elevated RCC in the absence of anaemia. Historical studies from the Cape Town area have shown that α-thalassaemia trait is relatively common, occurring in up to 3.8%

of healthy blood donors.[5] Iron deficiency and thalassaemia trait are therefore common local conditions occurring in otherwise healthy individuals and require exclusion in the calculation of accurate local RIs. A recent study by Smit *et al.*[6] reported FBC RIs for the Western Cape population without excluding iron-deficient participants. They reported a lower Hb RI for men than women, which is discrepant with current RIs in use and those published in international and local literature.[2,7] Implementation of the RIs of Smit *et al.*[6] would lead to significant underdiagnosis of anaemia in men.

Objectives

To establish locally derived RIs for FBC and WBC differential count parameters in healthy adults in the Cape Town area by including first-time blood donors and excluding those with iron deficiency and thalassaemic indices. These new locally established RIs would update those in current use by the NHLS in the Cape Town region. Establishing the prevalence of iron deficiency in first-time blood donors would be informative for local blood donation policies regarding possible screening for iron deficiency. Screening of high-risk groups prior to blood donation would identify potential donors who may benefit from iron supplementation.

Methods

Study design

This was a prospective, descriptive study with direct convenience sampling. Samples were collected from healthy first-time blood donors presenting voluntarily for blood donation to the Western Cape Blood Service (WCBS). Ethnicity was self-identified by participants on the blood donor questionnaire. Samples were collected between November 2016 and October 2017 at WCBS blood donation clinics in the Cape Town region.

Ethical considerations

The University of Cape Town (UCT) Human Research and Ethics Committee approved the study (ref. no. 132/2016 e), and voluntary informed written consent was obtained from all participants prior to inclusion in the study.

Participants and measurements

Participants were first-time prospective blood donors between 18 and 60 years of age. Each participant completed the blood donor screening and questionnaire (available as a supplementary file at http://samj.org.za/public/sup/15313-q.pdf) and had to meet all routine blood donation criteria for inclusion into the study, apart from point-of-care fingerprick Hb testing. Participants were excluded if any of their responses on the questionnaire or baseline vital signs including blood pressure or pulse rate precluded blood donation. The following laboratory exclusion criteria were applied: (*i*) testing positive for HIV, hepatitis B and C viruses (HBV and HCV) and/or syphilis; (*ii*) confirmed iron deficiency, defined as a serum ferritin level below the local reference limit of <13 µg/L for females and <30 µg/L for males; and (*iii*) red cell indices and RCCs suggestive of α- or β-thalassaemia trait according to the Mentzer index,[8] which uses a cut-off of mean cell volume (MCV)/RCC <13.

Laboratory methods

Blood samples were collected with minimum stasis in accordance with international recommendations.[1,2] Venous blood FBC samples were collected into 4 mL ethylenediamine tetra-acetic acid (EDTA BD Vacutainer Systems, UK) and stored at a mean of 20°C prior to analysis, which was performed within 24 hours of collection. Automated cell counting was performed on a XN-1000 instrument (Sysmex Corp., Japan) in a laboratory accredited by the South African National Accreditation System, which adheres to international quality standards. Screening for HIV, HBV and HCV was performed using standard serological and nucleic acid testing. The following FBC parameters were collected: Hb, RCC, haematocrit (Hct), MCV, mean cell Hb, mean cell Hb concentration, platelet count, total WBC count and 5-part automated WBC differential count, including neutrophils, lymphocytes, monocytes, eosinophils and basophils. Serum ferritin specimens were collected in 4 mL, Z-Serum Clot Activator tubes (Fisher Scientific, USA) and centrifuged, with the serum refrigerated at 2 - 6°C prior to processing using the Abbott AxSYM Ferritin kit (Abbott Laboratories, USA).

Statistical analysis

Data were analysed using MATLAB (version 9.8.0, R2020a; MathWorks Inc., USA) and RStudio (2015, Integrated Development for R, RStudio Inc., USA). Following the Clinical and Laboratory Standards Institute guidelines,[1] the non-parametric percentile method was used for the RI calculations. The non-parametric method expresses the central 95% of the data, calculating the 2.5th and 97.5th percentiles. The lower and upper reference limits of the RIs are reported with their respective 90% confidence intervals (CIs). Outliers were identified following the method of Horn *et al.*[3] The Mann-Whitney *U*-test was employed for comparisons between male and female participants. An alpha level <0.05 was deemed statistically significant.

Results

For inclusion into the study, participants had to meet the standard donor acceptance criteria using the WCBS blood donor questionnaire (http://samj.org.za/public/sup/15313-q.pdf). Of the 774 participants who met the donor questionnaire criteria, 70 of the 491 females (14%) and 12 of the 283 males (4%) were iron deficient and therefore excluded. Five participants with positive HIV serology were excluded, and 2 of these were subsequently confirmed positive on nucleic acid testing. Nineteen participants with a positive Mentzer index[8] indicating a likely underlying thalassaemia trait were excluded. Nine patients aged >60 years were excluded, in view of the effect of ageing on Hb, RCC, Hct and red cell indices.[9] In the final analysis, 662 participants were included, 409 (62%) female and 253 (38%) male. The study flow chart is presented in Fig. 1. Participants comprised a mix of the various ethnic groups residing in the Western Cape, with 257 (37%) self-identifying as coloured, 201 (30%) as black, 189 (29%) as white and 25 (4%) as Asian. The majority of the participants (n=348; 53%) were aged 20 - 29 years, with a mean age of 29 years for females and 28 years for males; 22% of participants were aged 30 - 39 years (Fig. 2). The derived RIs for females and males are provided in Table 1 with 90% CIs. Table 2 presents a comparison of study RIs including local studies from the past 15 years and current NHLS RIs at sea level. Fig. 3 describes the variation in serum ferritin between males and females in the study population, after exclusion of iron deficiency.

Discussion

There are recognised variations in FBC and WBC parameters relating to gender, age, ethnicity, environment and lifestyle factors such as smoking and exercise. For example, smokers have increased Hb and WBC counts, and black African males have been shown to have lower total WBC, neutrophil and platelet counts than populations of Caucasian origin.[10-12] The RIs in current use by the NHLS in the

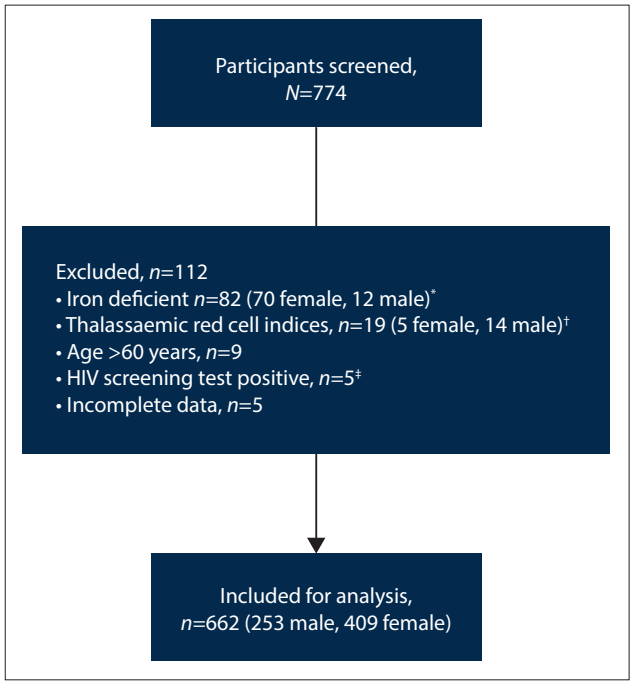

*Fig. 1. Study cohort diagram (note: some participants met more than one exclusion criterion). (*Serum ferritin <13 µg/L for females, <30 µg/L for males; †Positive Mentzer index:[8] mean cell volume/red cell count <13; ‡Of 5 donors with a positive HIV serology test, 2 were confirmed positive on nucleic acid testing.)*

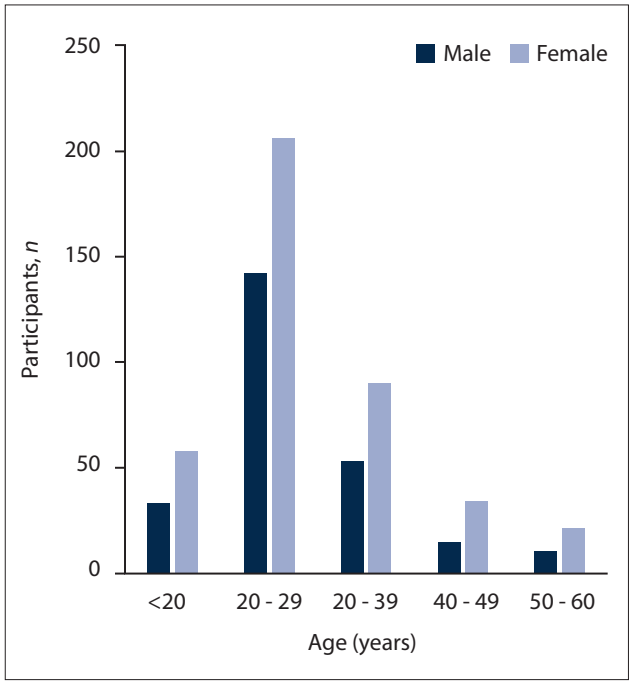

Fig. 2. Age distribution of the study participants.

Western Cape are not locally derived, so this study aimed to provide local FBC and WBC differential counts for healthy adults.

Hb, RCC and red cell indices

The mean Hb, lower reference limit (LRL) and upper reference limit (URL) of the Hb RIs were lower for females than males, despite the exclusion of 70 females with iron deficiency. This finding refutes the

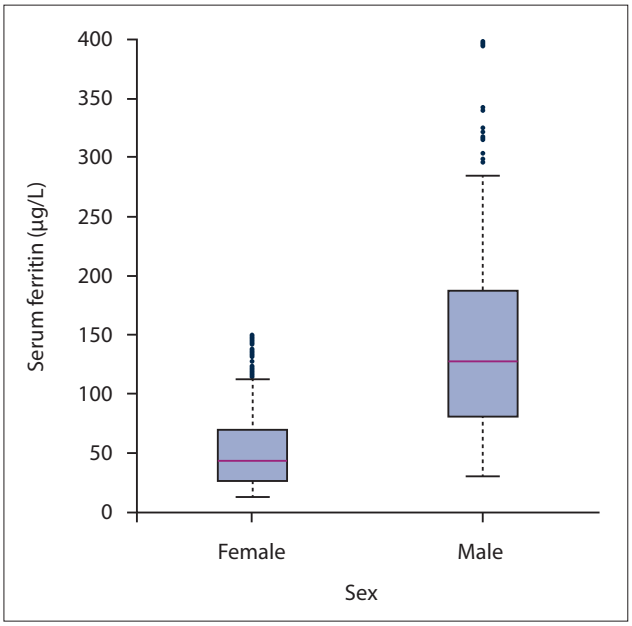

Fig. 3. Variation in serum ferritin between males and females in the study population, after exclusion of iron deficiency.

hypothesis that gender differences in Hb can largely be explained by unrecognised iron deficiency, and suggests that other factors such as higher testosterone and total body muscle mass in males may contribute to this gender-based difference.[13] Our findings contradict the recent study by Smit *et al.*,[6] which reported a lower Hb RI for males than females, and align with local and international studies, which have reported a higher Hb RI and higher LRL for males than females.[7,14] We found the Hb LRL for healthy iron-replete women was 11.7 g/dL, slightly lower than the NHLS current LRL of 12 g/dL, which was unexpected in view of the exclusion of iron deficiency. Our Hb LRL for males was slightly higher than current NHLS values at 13.6 g/dL. The study findings that males had a higher LRL, and females a lower LRL than those in use by the NHLS imply that using current RIs could lead to anaemia being underdiagnosed in males and overdiagnosed in females. Although these new Hb LRLs are unlikely to significantly affect clinical decision-making, they do inform our local blood service regarding Hb cut-offs for donation, as the current Hb cut-off for donation is 12.5 g/dL for females and 13.5 g/dL for males using the HemoCue 301 device (HemoCue, Sweden) and capillary blood samples from fingerprick testing. The findings from this study may therefore lead to a lower Hb cut-off for donation and increase the donor pool.

The URLs of Hb for males and females from our study at 17.6 g/dL and 15.3 g/dL, respectively, are higher than those in current use (Table 1), suggesting that polycythaemia is overdiagnosed in males using the current NHLS coastal URL of 17 g/dL in our local male population. Our new Hb URLs have clinical significance, as the work-up of polycythaemia often includes specialist referral and expensive further testing. Since Hb rises with altitude, it is surprising that our male Hb URL matches that of Gauteng. Notably, the Gauteng study included fewer male participants and no coloured males (Table 2).[7] Our finding of a high male Hb URL may be due to a high rate of smoking in our male study population group; however, smoking history was not collected, so this cannot be confirmed. The young age of our study population compared with the Gauteng study may also have contributed to these findings, since the RCC lowers with ageing. In addition, the Gauteng study used a Beckman Coulter analyser,

Table 1. RIs for males and females

Parameter	Study RIs	90% CI for upper and lower limits	*p*-value*	NHLS current RIs
RCC (10^{12}/L)			<0.0001	
Males	4.66 - 6.04	(4.61 - 4.71) (5.95 - 6.21)		4.5 - 5.5
Females	3.98 - 5.41	(3.94 - 4.10) (5.31 - 5.51)		3.8 - 4.8
Hb (g/dL)			<0.0001	
Males	13.60 - 17.57	(13.5 - 13.9) (17.3 - 17.7)		13 - 17
Females	11.70 - 15.30	(11.50 - 12.00) (15.10 - 15.50)		12 - 15
Hct (L/L)			<0.0001	
Males	0.41 - 0.52	(0.40 - 0.42) (0.52 - 0.53)		0.40 - 0.50
Females	0.36 - 0.47	(0.36 - 0.37) (0.46 - 0.48)		0.36 - 0.46
Hct (%)			<0.0001	
Males	41.33 - 52.39	(40.4 - 41.9) (51.8 - 53.3)		40 - 50
Females	36.30 - 47.10	(36.0 - 36.6) (46.1 - 47.9)		36 - 46
MCV (fL)			<0.0001	
Males	80.28 - 94.10	(79.5 - 81.6) (93.6 - 95.0)		83.1 - 101.6
Females	80.49 - 96.32	(79.1 - 81.2) (95.9 - 97.6)		79.1 - 98.9
MCH (pg)			0.1639	
Males	26.00 - 32.10	(25.5 - 26.7) (31.7 - 32.7)		27.8 - 34.8
Females	25.30 - 32.20	(25.1 - 25.8) (31.7 - 32.6)		26.1 - 33.5
MCHC (g/dL)			<0.0001	
Males	31.72 - 35.30	(31.2 - 31.9) (35.1 - 35.7)		33.0 - 35.0
Females	30.61 - 34.40	(30.4 - 30.9) (34.2 - 34.8)		32.7 - 34.9
Platelets (10^9/L)			<0.0001	
Males	175.18 - 374.83	(152 - 191) (366 - 434)		171 - 388
Females	193.00 - 458.8	(180 - 200) (443 - 496)		186 - 454
WBC (10^9/L)			<0.0001	
Males	4.31 - 12.39	(4.13 - 4.48) (10.97 - 13.33)		3.92 - 10.40
Females	4.71 - 13.20	(4.31 - 4.94) (12.52 - 13.74)		3.90 - 12.60
Neutrophils (10^9/L)			<0.0001	
Males	1.60 - 7.43	(1.49 - 1.93) (6.84 - 8.63)		1.60 - 6.98
Females	2.01 - 8.39	(1.90 - 2.21) (8.00 - 9.26)		1.60 - 8.30
Lymphocytes (10^9/L)			<0.0001	
Males	1.50 - 4.33	(1.43 - 1.54) (3.90 - 5.10)		1.4 - 4.2
Females	1.61 - 4.55	(1.54 - 1.78) (4.38 - 5.09)		1.4 - 4.5
Monocytes (10^9/L)			0.3821	
Males	0.33 - 0.97	(0.31 - 0.35) (0.92 - 1.07)		0.3 - 0.8
Females	0.30 - 1.05	(0.29 - 0.33) (0.92 - 1.12)		0.2 - 0.8
Eosinophils (10^9/L)			0.3222	
Males	0.02 - 0.64	(0.01 - 0.04) (0.61 - 0.74)		0 - 0.95
Females	0.03 - 0.67	(0.03 - 0.04) (0.59 - 0.75)		0 - 0.4
Basophils (10^9/L)			0.7021	
Males	0.01 - 0.1	(0.01 - 0.02) (0.09 - 0.11)		0 - 0.1
Females	0.01 - 0.1	(0.01 - 0.02) (0.09 - 0.12)		0 - 0.1

RIs = reference intervals; CI = confidence interval; NHLS = National Health Laboratory Service; RCC = red cell count; Hb = haemoglobin; Hct = haematocrit; MCV = mean cell volume; MCH = mean cell haemoglobin; MCHC = mean cell haemoglobin concentration; WBC = white blood cell count.
*Mann-Whitney *U*-test comparing ranges for men and women, with level of statistical significance at $p<0.05$.

while a Sysmex analyser was used in this study. The URLs and LRLs of the RCCs in our study were both significantly higher for males and females than the current NHLS ranges in use, despite exclusion of thalassaemic indices using the Mentzer index. Since routine genetic testing was not part of this study, there may have been individuals with thalassaemia trait with high RCCs who were still included in the reference group. The URL of MCV for males in this study was 96 fL rather than the 101.6 fL in current use from the Gauteng study. This difference may also be related to differences in analysers used and the method of determining the MCV. Mild macrocytosis will be missed using the current ranges, which is important since macrocytosis may herald pathologies such as megaloblastic anaemia, hypothyroidism and liver dysfunction.

WBC and platelet counts

Mean total WBC and neutrophil counts and their respective RIs were significantly higher in females than males. This gender-based difference is known, although not fully understood, and may be due to hormonal factors and/or menstruation. The RIs for WBC and neutrophil counts in the present study were somewhat higher for both sexes compared with those in current use. We also found a higher URL for both lymphocyte and monocyte counts for both

Table 2. A comparison of present study RIs with similar South African studies from the past 20 years, and current NHLS coastal RIs

	Present study	Western Cape (Smit *et al.*,[6] 2019)	Gauteng (Lawrie *et al.*,[7] 2009)	NHLS current coastal RIs
Participants included, *n*				
Males	253	248	88	-
Females	409	463	631	-
RCC (10^{12}/L)				
Males	4.66 - 6.04	3.68 - 5.40	4.19 - 5.85	4.5 - 5.5
Females	3.98 - 5.41	3.77 - 5.62	3.93 - 5.40	3.8 - 4.8
Hb (g/dL)				
Males	13.60 - 17.57	10.06 - 15.90	13.4 - 17.5	13 - 17
Females	11.70 - 15.30	11.00 - 16.54	11.6 - 16.4	12 - 15
Hct (L/L)				
Males	0.41 - 0.52	0.32 - 0.47	0.39 - 0.51	0.40 - 0.50
Females	0.36 - 0.47	0.33 - 0.49	0.34 - 0.48	0.36 - 0.46
MCV (fL)		Not provided		
Males	80.28 - 94.10	-	83.1- 101.6	83.1 - 101.6
Females	80.49 - 96.32	-	78.9 - 98.5	79.1 - 98.9
MCH (pg)		Not provided		
Males	26.00 - 32.10	-	27.8 - 34.8	27.8 - 34.8
Females	25.30 - 32.20	-	26.1 - 33.5	26.1 - 33.5
MCHC (g/dL)				
Males	31.72 - 35.30	31.6 - 35.00	33.0 - 35.0	33.0 - 35.0
Females	30.61 - 34.40	32.00 - 35.00	32.7 - 34.9	32.7 - 34.9
Platelets (10^9/L)				
Males	175.18 - 374.83	139 - 427	171 - 388	171 - 388
Females	193.00 - 458.8	151 - 418	186 - 454	186 - 454
WBC (10^9/L)		Not provided		
Males	4.31 - 12.39	-	3.92 - 10.4	3.92 - 10.40
Females	4.71 - 13.20	-	3.90 - 12.60	3.90 - 12.60
Neutrophils (10^9/L)		Absolute counts not provided		
Males	1.60 - 7.43	-	1.6 - 6.98	1.60 - 6.98
Females	2.01 - 8.39	-	1.6 - 8.3	1.60 - 8.30
Lymphocytes (10^9/L)				
Males	1.50 - 4.33	1.2 - 3.4	1.4 - 4.2	1.4 - 4.2
Females	1.61 - 4.55	1.2 - 3.4	1.4 - 4.5	1.4 - 4.5
Monocytes (10^9/L)		Not provided		
Males	0.33 - 0.97	-	0.3 - 0.8	0.3 - 0.8
Females	0.30 - 1.05	-	0.2 - 0.8	0.2 - 0.8
Eosinophils (10^9/L)				
Males	0.02 - 0.64	0.05 - 0.49	0 - 0.95	0 - 0.95
Females	0.03 - 0.67	0.05 - 0.59	0 - 0.4	0 - 0.4
Basophils (10^9/L)		Not provided		
Males	0.01 - 0.1	-	0 - 0.1	0 - 0.1
Females	0.01 - 0.1	-	0 - 0.1	0 - 0.1

RIs = reference intervals; NHLS = National Health Laboratory Service; RCC = red cell count; Hb = haemoglobin; Hct = haematocrit; MCV = mean cell volume; MCH = mean cell haemoglobin; MCHC = mean cell haemoglobin concentration; WBC = white blood cell count.

sexes than the currently used URLs. The URL for eosinophils for both males and females is discrepant with the currently used URLs. We interpret these differences in WBC counts as being of minor clinical significance. As expected, platelet counts were higher in females than males, and aligned with current ranges (Table 2).

Iron deficiency

Individuals with serum ferritin levels below the LRL were excluded in this study, in an effort to remove the influence of iron deficiency on red cell parameters. To our knowledge, this is the first SA study to exclude the effect of iron deficiency on FBC RI calculation by routine testing of the serum ferritin. Of the 774 prospective donors initially screened for inclusion in this study, 11% had confirmed iron deficiency, the majority female. This finding indicates that female blood donors would benefit from screening programmes for iron deficiency and subsequent iron supplementation as part of blood donation drives. Serum ferritin testing can now be performed from a drop of fingerprick blood as a point-of-care test.[15] Since iron deficiency is associated with considerable morbidity, including fatigue, poor concentration and poor work productivity,[16] routine

screening for iron deficiency in prospective blood donors and more widely in primary healthcare settings should be considered. After removal of iron-deficient participants, our study found a significant difference in mean serum ferritin between the genders, with higher levels for males than females.

Study limitations

Since ferritin is an acute-phase protein and raised in inflammatory states, there is a possibility that a proportion of subjects with iron deficiency, but a serum ferritin level within the RI, may have been included in our data set. However, considering the young age and good health of these voluntary blood donors, the potential effect of this is regarded as minimal. Our reference population was young, with more than half being <30 years of age, and none >60 years of age. The ageing population shows slight decreases in Hb and red cell indices,[9,17] which need to be taken into consideration when applying our study RIs to older individuals. Participants were not restricted regarding exercise, alcohol or smoking prior to donation, which ideally should be controlled for in a study of this nature. Details regarding smoking in participants were not captured and would have been helpful in interpretation of the Hb, RCC and red cell indices. Furthermore, there are challenges with comparing RIs established in different settings, since there are several important variables, including instrumentation and lifestyle factors, which may not be available for review when analysing data. A follow-up verification of the RIs established in this study with a wider age group and more exclusion criteria that are known to impact on haematology results, including smoking, alcohol and medications, may provide valuable information. Participants with thalassaemic red cell parameters were removed using the Mentzer index[8] formula; however, genetic testing was not performed. Consequently, some individuals with a thalassaemia trait may have been included. Since genetic testing for thalassaemia in the Western Cape was last studied in 1987,[5] this is an area of further study that would be helpful in establishing current local patterns and incidence of thalassaemia.

Conclusions

Our results confirm the importance of gender- and location-specific RIs for the accurate determination of blood counts. This study clearly demonstrates significant differences between RIs previously established in Gauteng and those currently in use by the NHLS in coastal areas of SA. Many variables contribute to these differences in RIs, including geographical factors, demographics and laboratory instrumentation. The new RIs are clinically relevant and may affect diagnostic and management decisions. Strengths of this study include a healthy and ethnically diverse cohort, the use of standardised testing methodology, adequate sample sizes and exclusion of iron deficiency. The study confirms that males have a higher Hb than females, and refutes the findings of a recent Western Cape study by Smit *et al.*[6] Iron deficiency is common in otherwise healthy females presenting for first-time blood donation. Screening female blood donors for iron deficiency using point-of-care testing would be of value. Clinical staff should be educated on the interpretation and limitations of RIs, which provide comparison data for the interpretation of patients' laboratory results. Interpretation of FBCs and other laboratory results should take place in the particular clinical context, with an emphasis on following trends, rather than individual values.

Declaration. The research for this study was done in partial fulfilment of the requirements for AdK's MMed (Haem Path) degree at the University of Cape Town.

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