Local reference ranges for full blood count and CD4 lymphocyte count testing

Denise Lawrie, Lindi Marie Coetzee, Piet Becker, Johnny Mahlangu, Wendy Stevens, Deborah Kim Glencross

Objective. Recent advances in full blood count and CD4 technology, coupled with the changing population demographics of the Gauteng region, have necessitated re-evaluation of the reference ranges currently in use.

Methods. A cross-sectional study of 631 female and 88 male HIV-negative participants from the Gauteng region was performed. Full blood count, automated differential and CD4 count analyses were done using the latest internationally accepted technology. Reference ranges were compiled from the 2.5th and 97.5th percentiles for both male and female participant groups, and gender and ethnic comparisons calculated by non-parametric tests.

Results. Results of 41 females were removed from the statistical analysis because their results were suggestive of possible anaemia. Full blood count reference interval comparison confirmed gender-specific differences in red blood cell and platelet parameters. Ethnic-specific differences were found for some red blood cell parameters in the black female cohort. In addition, black males and females both generally had lower neutrophil and higher lymphocyte counts than a combined Asian/Caucasian/coloured ethnic group.

Conclusion. Comparison of the currently calculated reference ranges with published data and reference values in use indicated that a separate ethnic-specific reference range should be introduced for the percentage/absolute neutrophil count and percentage lymphocytes. In addition, locally derived reference ranges for red cell distribution width (RDW) and CD4 percentage of lymphocytes should be implemented for routine diagnostic testing.

S Afr Med J 2009; 99: 243-248.

Reference intervals are critically important for the correct interpretation of diagnostic tests. The full blood count and CD4 count are two frequently performed tests in HIV-endemic settings such as Gauteng province, South Africa. Haemoglobin concentration is an independent prognostic indicator in HIV, while CD4 count determines initiation and response to antiretroviral (ARV) treatment.[1] The South African National Health Laboratory Services (NHLS) establishes reference ranges with the implementation of new tests, and periodically re-assesses reference intervals to accommodate changes in population demographics as well as technological advancements (ISO 15189:2003 guidelines Point 5.5.5).[2] As differences in altitude, endemic disease and ethnic differences influence reference intervals, other reference intervals are not necessarily pertinent to the Gauteng region.

Department of Molecular Medicine and Haematology, Faculty of Health Sciences, University of the Witwatersrand, and National Health Laboratory Service, Johannesburg
Denise Lawrie, ND Med Tech, MSc
Lindi Marie Coetzee, BSc, BSc (Hons), MSc, PhD
Johnny Mahlangu, BSc, MB BCh, MMed, FCPath, Cert Clin Haem
Wendy Stevens, MB BCh, MMed
Deborah Kim Glencross, MB BCh, MMed

Department of Biostatistics, Medical Research Council, Pretoria
Piet Becker, BSc, MSc, PhD

Corresponding author: *D Lawrie (denise.lawrie@nhls.ac.za)*

Few studies from southern Africa address the region's ethnic and geographical diversity.[3-5] The results of three published full blood count reference ranges since 1980 are summarised in Table I. The studies were performed on clinically healthy, predominantly black subjects. Although largely similar, there were some differences between these reference ranges and those obtained in Caucasian subjects, such as the reference ranges by Mendelow *et al*. (unpublished data, 1985) that are currently used in the NHLS, Gauteng region. Although haematology reference ranges were established for the black population of the Witwatersrand in 1987,[3] these were never implemented by the NHLS (previously the South African Institute for Medical Research) because of constraints in the laboratory information system at the time.

Full blood count analyser technologies have developed rapidly over the last 20 years. For example, platelet counts were not routinely available and were done on a separate instrument; this is illustrated by the absence of platelet reference ranges reported for Lesotho in 1983[4] and the Cape Peninsula in 1995 (Table I).[5] Automated methods have replaced manual methods, e.g. white cell differential and reticulocyte counts. New parameters, such as red cell distribution width (RDW) and mean platelet volume (MPV) are routinely available on automated full blood count analysers, giving supplementary clinical and technical information at no additional cost. Because these are new parameters, local reference intervals were not available at the time and published intervals were therefore used.[6] Original impedance counting and sizing methods have been enhanced or replaced by optical methods, yielding new information, e.g. granulocytes are

Table I. Summary of local South African haematological reference ranges published after 1980

Parameter	Maseru, Lesotho[4]	Witwatersrand[3]	Cape Peninsula[5]
RBC (10^{12}/l)			
Male	4.49 - 5.90	4.43 - 6.03	3.2 - 5.8
Female	3.85 - 5.25	4.07 - 5.13	3.0 - 5.3
HGB (g/dl)			
Male	13.7 - 17.8	13.8 - 17.9	10.3 - 16.7
Female	11.7 - 16.0	12.4 - 15.5	9.0 - 15.2
HCT (l/l)			
Male	0.41 - 0.52	0.39 - 0.51	0.31 - 0.525
Female	0.35 - 0.47	0.36 - 0.45	0.273 - 0.472
MCV (fl)			
Male	81 - 99	77 - 94	82 - 110.4
Female			76.2 - 106.7
MCH (pg)			
Male	27.2 - 33.6	27.5 - 33.3	25.3 - 34.9
Female			24.8 - 33.8
MCHC (g/dl)			
Male	32.1 - 35.5	33.4 - 37.0	29.9 - 36.0
Female			30.3 - 35.4
Platelets (10^9/l)			
Male		164 - 396	
Female		191 - 442	
WBC (10^9/l)			
Male	2.53 - 8.43	3.4 - 9.3	3.7 - 12.6
Female	2.90 - 9.10		
Neutrophils (10^9/l)	0.958 - 6.403	1.96 - 6.52	2.9 - 7.1
Lymphocytes (10^9/l)			
Male	1.012 - 2.972	0.95 - 3.38	2.1 - 6.0
Female	0.840 - 3.256		
Monocytes (10^9/l)	0.082 - 0.607	0.05 - 0.08	0.17 - 0.75
Eosinophils (10^9/l)	0 - 0.276	0 - 0.36	0.14 - 1.56
Basophils (10^9/l)		0 - 0.05	

counted using their peroxidase content. Red blood cell and white blood cell maturation is automatically categorised. Such improvements in full blood count analyser technology have resulted in better overall linearity and precision of results.

Absolute CD4 count reference intervals were established in Gauteng in 1992 using a dual platform (DP) full blood count/flow cytometry method.[7] Subsequently, DP methods were replaced by single platform (SP) methods combining white cell count analyses with absolute CD4 counting by flow cytometry alone. The SP PanLeucoGating CD4 method was introduced into the NHLS in 2002 as the routine CD4 testing method; it has improved accuracy, precision and reproducibility of CD4 testing.[8,9] Although a recent multi-centre study[10] showed no significant differences between DP and SP analyses, it is still necessary to verify the current absolute CD4 count reference range to exclude any DP bias.

The aim of our study was to validate current NHLS reference ranges for the Gauteng region for full blood count and absolute CD4 lymphocyte counts in the face of technological advances and shifting population demographics. In addition, a reference interval for the CD4 percentage of lymphocytes needed to be established.

Materials and methods

Ours was a cross-sectional study performed on 631 female and 88 male HIV-negative, clinically healthy volunteers from the Gauteng region;[11] 63% of the female group, and 56% of the male group, were black, with an average age of 41 for all participants. Inclusion criteria were HIV negativity and general well-being. No exclusions were made on the basis of a history of smoking or contraceptive use, adverse clinical findings, or presence of co-morbid diseases such as TB and hepatitis.[12]

The University of the Witwatersrand Human Ethics Committee approved the study. The laboratory where the study was conducted is certified in terms of the South African National Accreditation System.[13]

After giving informed consent, each volunteer donated a single venous blood sample collected into dipotassium EDTA

(BD Vacutainer Systems, Plymouth, UK). The samples were stored at 20±2ºC prior to preparation and analysis. All testing was completed within 12 hours of collection.

Full blood counts were done on the Beckman Coulter LH 750 Hematology Analyzer (Beckman Coulter, Fullerton, CA, USA). Samples were examined for the following parameters: total white blood cell count (WBC) and 5-part automated differential count, red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), RDW, platelet count (PLT) and MPV.

SP absolute CD4 counts and the percentage CD4 of lymphocytes were obtained using a lyse-no-wash procedure. Samples were stained using the Beckman Coulter Flowcare PLG CD4 reagent kit with Flow Count fluorospheres and analysed on a Beckman Coulter XL-MCL Flow Cytometer.

The accuracy of the full blood count and CD4 values was subject to strict internal and external quality assurance procedures. The laboratory participates in the Beckman Coulter Interlaboratory Quality Assessment Program (IQAP), and the NHLS National Quality Assessment programmes for full blood count and CD4 monitoring; it also takes part in the United Kingdom National External Quality Assessment Scheme (UKNEQAS), African Regional External Quality Assessment Scheme (AFREQAS) for CD4 monitoring, and Royal College of Pathologists of Australia Quality Assessment Programme for the automated full blood count and white differential counts.

Statistical analysis

The guidelines for reference interval determination of the Clinical Laboratory Standards Institute (CLSI, Wayne, PA, USA) were followed.[14] Although these guidelines are meant for establishing new reference intervals, the basic principles also apply to re-assessment of reference ranges.

Of the 631 female participants, 41 females were removed from statistical analyses because their full blood count results were suggestive of anaemia (HGB <12 g/dl and MCV <80 fl);[15] this may have skewed the data. All data sets were tested for normal distribution using the Kolmogorov-Smirnov (KS) and Shapiro-Wilk tests of normality. The 2.5th and 97.5th percentiles were used as reference intervals as the data were normally distributed. Differences between genders were calculated with the non-parametric Mann-Whitney *U*-test, and differences between all ethnic groups within the male and female populations analysed using the Kruskal-Wallis test. A combined gender-specific Asian-coloured-Caucasian group was compared with the corresponding gender-specific black population. Data analysis was performed using StatCorp 2003 (Stata Statistical Software: Release 8.0. Stata Corporation, College Station, Tex., USA) and GraphPad Prism 5 Software (GraphPad Software, La Jolla, CA, USA).

Results

Tables II and III summarise the calculated reference intervals for full blood count and CD4 lymphocyte parameters obtained from this study, alongside the reference ranges currently in use in Gauteng NHLS laboratories. There were significant differences between male and female participants ($p<0.001$) for some parameters (Tables II and III, column 2). Ethnic-specific differences were noted for some parameters between the black participants and the combined Asian/Caucasian/coloured participants (Table IV, column 3).

Discussion

The opportunity to perform this validation presented itself when a large-scale Human Sciences Research Council (HSRC) study was undertaken to assess HIV prevalence among health care workers.[11] This study allowed data collection from a more representative demographic population with a broader age distribution than is currently encompassed by the full blood count reference ranges used in the NHLS Gauteng region.

Red blood cell parameters

An interesting finding of our current study was a decreased upper limit of HGB for males of 17.5 g/dl v. 18.3 g/dl in the 1985 study. A possible explanation for this finding is that there were fewer participants who smoked than in the 1985 study. Smoking increases HGB values. The upper limit for the female participant group did not increase.

Gender-specific differences

Significant differences were noted between males and females for RBC, HGB, HCT, MCV, MCH, MCHC and RDW (Table II). These findings are in keeping with those of previous South African studies.[3-5,16] The gender differences in red blood cell indices and HGB are historically attributed to biological and physiological fluctuations, including menstrual blood loss, hormonal influences of oestrogens and testosterone on erythropoiesis, and the prevalence of iron deficiency anaemia in women.[3,17]

Ethnic differences

Ethnic differences were noted in red blood cell parameters in the female subjects, in keeping with previous southern African studies.[3,16] In the current study, black females had lower red cell counts, HGB and HCT values in general (Table IV). Although it may not be necessary to establish separate reference intervals for these parameters, the ranges for RBC, HGB, and HCT documented for black females fell at the lower end of the current reference intervals. Automated 'canned comment' reporting may suffice to highlight such findings to attending clinicians. No ethnic differences were noted for male participants regarding red blood cell parameters.

Table II. Comparison of the reference ranges from this study with the current reference ranges used in NHLS laboratories for haematology parameters

Parameter	New ranges from this study	Mann-Whitney *p*-value (male v. female)	Currently in use
RBC (10^{12}/l)			
Male	4.19 - 5.85	<0.0001***	4.89 - 6.11
Female	3.93 - 5.40		4.13 - 5.67
HGb (g/dl)			
Male	13.4 - 17.5	<0.0001***	14.3 - 18.3
Female	11.6 - 16.4		12.1 - 16.3
HCT (l/l)			
Male	0.39 - 0.51	<0.0001***	0.43 - 0.55
Female	0.34 - 0.48		0.37 - 0.49
MCV (fl)			
Male	83.1 - 101.6	<0.05*	79.1 - 98.9
Female	78.9 - 98.5		79.1 - 98.9
MCH (pg)			
Male	27.8 - 34.8	<0.001**	27.0 - 32.0
Female	26.1 - 33.5		27.0 - 32.0
MCHC (g/dl)			
Male	33.0 - 35.0	<0.0001***	32.0 - 36.0
Female	32.7 - 34.9		32.0 - 36.0
RDW (%)			
Male	12.1 - 16.3	<0.001**	11.6 - 14.0
Female	12.4 - 17.3		11.6 - 14.0
Platelets (10^9/l)			
Male	171 - 388	<0.0001***	137 - 373
Female	186 - 454		178 - 400
MPV (fl)			
Male	7.1 - 11.0	NS	7.0 - 11.4
Female	7.3 - 11.3		7.0 - 11.4
WBC (10^9/l)			
Male	3.92 - 10.40	NS	3.92 - 9.88
Female	3.90 - 12.60		3.92 - 9.88
Neutrophils (10^9/l)			
Male	1.6 - 6.98	NS	2.0 - 7.5
Female	1.6 - 8.3		
Lymphocytes (10^9/l)			
Male	1.4 - 4.2	<0.05*	1.0 - 4.0
Female	1.4 - 4.5		
Monocytes (10^9/l)			
Male	0.3 - 0.8	<0.05*	0.18 - 0.8
Female	0.2 - 0.8		
Eosinophils (10^9/l)			
Male	0 - 0.95	NS	0 - 0.45
Female	0 - 0.4		
Basophils (10^9/l)			
Male	0 - 0.1	NS	0 - 0.2
Female	0 - 0.1		
% neutrophils			
Male	32 - 76	NS	51 - 76
Female	34 - 72		
% lymphocytes			
Male	18 - 56	NS	26 - 40
Female	21 - 56		
% monocytes			
Male	4 - 12	<0.0001***	5 - 8
Female	3 - 10		
% eosinophils			
Male	0 - 8	<0.05*	0 - 5
Female	0 - 6		
% basophils			
Male	0 - 2	NS	0 - 2
Female	0 - 1		

p-values indicate significant differences between male and female participants for each parameter tested; NS indicates no significant differences.
* p=0.01 - 0.05.
** p=0.001 - 0.01.
*** p<0.001.

Table III. Comparison of the reference ranges from this study compared with the current reference ranges used in the NHLS laboratories for CD4 parameters

Parameter	This study	Mann-Whitney *p*-value (males v. females)	In use
CD4 absolute count (10^6/l)			500 - 2 010
Male	503 - 1 807		
Female	561 - 2 051	<0.0001***	
CD4 % of lymphocytes			Not currently reported
Male	27.7 - 56.7		
Female	31.6 - 58.0	<0.0001***	

p-values indicate significant differences between male and female participants; NS indicates no significant difference.
* p=0.01 - 0.05.
** p=0.001 - 0.01.
*** p<0.001.

Table IV. Summary of the significant differences noted in reference intervals between the black population group and the remaining combined ethnic groups (Asian, coloured and Caucasian)

Parameter	Black ethnic group	Combined Asian, coloured and Caucasian ethnic groups	Significance
RBC (female)	3.89 - 5.32	4.02 - 5.59	p<0.0001***
HGB (female)	11.6 - 16.1	11.6 - 16.8	p<0.0001***
HCT (female)	0.34 - 0.47	0.35 - 0.50	p<0.0001***
RDW (female)	12.5 - 17.3	12.3 - 17.2	p<0.0001***
Eosinophils (female)	0 - 0.4	0 - 0.5	p<0.05*
WBC			
Male	3.93 - 10.04	4.49 - 10.74	p<0.001**
Female	3.8 - 11.40	4.68 - 13.84	p<0.0001***
Neutrophils			
Male	1.46 - 6.40	2.14 - 7.49	p<0.0001***
Female	1.53 - 7.27	2.04 - 8.96	p<0.0001***
% neutrophils			
Male	31 - 72	42 - 81	p<0.0001***
Female	32 - 72	35 - 74	p<0.0001***
Monocytes			
Male	0.2 - 0.7	0.3 - 0.9	p<0.01**
Female	0.2 - 0.8	0.2 - 0.9	p<0.0001***
% lymphocytes			
Male	21 - 58.0	14 - 47	p<0.0001***
Female	23 - 57	19 - 54	p<0.0001***

NS indicates no significant difference.
* p=0.01 - 0.05.
** p=0.001 - 0.01.
*** p<0.001.

Platelet parameters

Studies of differences in platelet counts between gender groups consistently report higher values in females, regardless of ethnicity.[3,18] Gender differences in platelet counts have historically been attributed to hormonal influences.[3] In the current study, similar differences were observed (Table II). No ethnic differences were demonstrated. No gender- or ethnic- specific differences were found in the MPV volume. No significant difference in the MPV volume was found between the reference interval determined in this study and the global reference interval (7 - 11.4 fl).[6]

White blood cell parameters

Previous southern African studies have shown gender- and ethnic-specific differences in total WBCs.[3,16] The present study did not show any significant gender differences, but confirmed ethnic differences in total WBCs between the black participants and other ethnic groups (Tables II and IV).

Significant ethnic differences were found for some types of white blood cells. The absolute and percentage neutrophil counts were generally lower, and the percentage lymphocytes generally higher, in black participants (Table IV), resulting in wider reference intervals.

The current study confirmed ethnic differences in absolute monocyte counts reported elsewhere.[18] This finding in black participants is not clinically significant though interesting, as it was noted previously and may reflect the impact of improved sensitivity in automated monocyte counting.[19]

The differences in white blood cell differential reference intervals found between ethnic groups in the southern African population correlate with published data from other parts of Africa, the UK and the USA.[3,16,18,20,21] When comparing the full blood count and absolute differential counts with reference interval data from the rest of Africa that used similar methodology, our reported reference intervals for the Gauteng region showed the closest similarity to intervals reported for Uganda.[21]

CD4 values

Our gender-specific differences for the CD4 percentage of lymphocytes and absolute CD4 lymphocyte counts (Table III) concur with those in other African, Asian and European countries.[22] However, we found no significant ethnic differences in these figures.

Conclusion

Our study confirmed that, in general, the NHLS's full blood count reference ranges for Gauteng (Mendelow *et al*., unpublished data, 1985 and Tikly *et al*.,[3] 1987) and the CD4 ranges established in 1992[7] compare well with the current study and other recently reported ranges from Europe and Africa. Current reference intervals therefore remain valid for use in the Gauteng region, with some modifications as suggested below for optimisation.

The reference intervals currently in use for red cell parameters accommodate the differences found between males and females for RBC, HGB, HCT, MCV, MCH and MCHC. However, we recommend that the current reference intervals for RDW (11.6 - 14%) be amended to reflect our findings (12.1 - 16.3% for males; 12.4 - 17.3% for females). Amending the RDW reference ranges would result in changes in commenting on red cell morphology. It is anticipated that if our recommendations are adopted, fewer 'red cell anisocytosis' comments would be noted on otherwise normal full blood count reports.

The current NHLS gender-specific platelet reference ranges for males and females remain applicable. A single reference range for MPV is still valid, and the intervals currently in use are adequate.

With ethnic differences noted in some types of white blood cells, it is recommended that a separate reference range is introduced for percentage and absolute neutrophil counts and percentage lymphocytes for black patients. This modification would reduce the number of patients 'misdiagnosed' with neutropenia, as well as erroneous reporting of reversed neutrophil/lymphocyte ratios.

The current absolute CD4 reference range is robust enough to accommodate the differences described in this paper, and the CD4 percentage reference interval established in this study should be implemented. With minor changes and additions to current reference intervals for full blood counts and absolute CD4 testing, these ranges will be more representative of the current patient demographics in Gauteng province and the updated technology in use at NHLS laboratories.

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Accepted 9 December 2008.