Observed full blood count and lymphocyte subset values in a cohort of clinically healthy South African children from a semi-informal settlement in Cape Town

D Lawrie,[1] MSc (Med), PhD; **H Payne**,[2,3] BSc Hons, MB ChB Hons, MRCPCH;
M Nieuwoudt,[4] PhD (Bioengineering), Dip Nucl Med Tech, BSc (Physiology, Psychology); **D K Glencross**,[1] MB BCh, MMed

[1] *Department of Molecular Medicine and Haematology, National Health Laboratory Service and Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa*
[2] *Institute of Child Health, University College London, UK*
[3] *Children's Infectious Diseases Clinical Research Unit, Department of Paediatrics, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, Cape Town, South Africa*
[4] *South African Department of Science and Technology/National Research Foundation Centre of Excellence in Epidemiological Modelling and Analysis, Faculty of Science, Stellenbosch University, Stellenbosch, Western Cape, South Africa*

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

Background. The paediatric full blood count and lymphocyte subset reference intervals used by the National Health Laboratory Service (NHLS), South Africa (SA), are taken from two international reference interval publications. Differences in reference intervals suggest that international data sets may not be appropriate for use in SA.
Objective. To study immunohaematological values of a group of clinically healthy children from an informal settlement in Cape Town, SA, to assess whether international paediatric reference intervals used by the NHLS are appropriate.
Methods. A cross-sectional study of 207 female and 174 male HIV-uninfected children living in an informal settlement in Cape Town was performed. Full blood counts, automated differential counts and lymphocyte subset analysis were done using internationally accepted technologies. Data were categorised by age and reference intervals compiled using medians and 95% confidence intervals (CIs). Gender comparisons were calculated by non-parametric tests.
Results. Although median and 95% CI values differed slightly, physiological trends for red cell, platelet, white blood cell differential and lymphocyte subsets were similar to international reference intervals currently in use at the NHLS. Benign ethnic neutropenia was not a significant finding, and gender-specific intervals were not necessary until 12 years of age. Lower overall median values for haemoglobin and haematocrit, and higher median values for mean cell volume and red cell distribution width, were noted. Assessment of haemoglobin, red cell distribution width and calculated Mentzer ratios suggested underlying iron deficiency in 14.2% of participants.
Conclusion. Paediatric immunohaematological reference intervals observed in this study are similar to, and support continued use of, international paediatric reference intervals. Underlying iron and related nutritional deficiencies may be contributing to lower haemoglobin levels noted in local children. A larger nationwide study, including all ethnic groups, is recommended.

S Afr Med J 2015;105(7):589-595. DOI:10.7196/SAMJnew.7914

Integrated productive haemopoeisis is fundamental to a successful immune response. Neutrophils, monocytes, eosinophils and basophils are essential components of the primary innate immune response and, further, initiate the secondary immune response by processing and presenting pathogenic antigens to the adaptive immune cells in the lymph nodes. After antigenic stimulation, the lymphocytes respond via T-cell-dependent cellular and B-cell-dependent humoral immune reactions.[1] A laboratory-derived immunohaematological profile usually includes full blood count parameters, a white blood cell (WBC) differential count and lymphocyte subsets (T, B and natural killer cells). Lymphocyte subsets are further defined to provide important physiological and functional information such as the proportions of naïve, memory and activated T and B cells.

Comprehensive immunohaematological analysis is therefore used to screen paediatric patients for underlying immune suppression associated with primary immunodeficiency such as X-linked agammaglobulinaemia and severe combined immunodeficiency. It can also be used to exclude secondary immunodeficiency caused by overwhelming infections such as HIV, parvovirus, hepatitis B and tuberculosis,[2] and primary T-cell and B-cell as well as post-transplantation[3,4] lymphoproliferative disorders.

Although the optimal approach to assessing and monitoring immunohaematological status is to record individual patient baseline values prior to infection or clinical illness, this is not generally feasible. Standard clinical and laboratory practice is to compare the patient's results with established reference intervals (that reveal the expected immunohaematological intervals of 90 - 95% of a 'normal healthy population'[5]). Currently, the paediatric full blood count and lymphocyte subset reference intervals used by the National Health Laboratory Service (NHLS) in South Africa (SA) are taken from two international reference interval publications.[2,6] Differences in reference intervals attributed to ethnicity, diet, endemic infections, altitude, laboratory testing protocols and statistical methodologies used[7,8] suggest that international data sets may not necessarily be appropriate for local use in SA.

Objective

An opportunity to establish whether the reference interval sets[2,6] used are representative of local children's full blood count and lymphocyte subset reference intervals presented itself during a recent clinical paediatric study at a child wellness clinic in Cape Town (H Payne *et al.* – unpublished data). The aim of this report was to establish reference intervals of full blood count and lymphocyte subset values in a cohort of apparently healthy SA children of diverse ethnicity, residing in a semi-informal settlement in Cape Town, SA, to assess the appropriateness of the full blood count and lymphocyte subset paediatric reference intervals currently used in the NHLS.

Methods

This was a cross-sectional study performed on 381 clinically healthy, HIV-uninfected children from the Wesbank semi-informal settlement in Cape Town. In Wesbank,[9] 27% of adults are unemployed, 62% have not completed their complete education to grade 12 level, and 39% of households earn <R19 200 per year. Sixteen per cent of the population lives in informal dwellings, and 5% of the population is >65 years of age. The infant mortality rate is 23.2/1 000 live births, 7% of babies are born to mothers aged <18 years, and 20% of babies weigh <2 500 g at birth.

There were 207 female and 174 male participants, with an age range of 2 weeks - 12.6 years. Each age group had an approximately 50:50 male-to-female ratio. The children were black Africans (n=85) or of mixed ethnic ancestry (n=296). An attending paediatrician confirmed that the children were clinically healthy and well nourished on the day of phlebotomy. Wellness criteria included: (*i*) previous registration at the child wellness clinic; (*ii*) attendance with the biological mother and Road-to-Health card; (*iii*) a documented clinical history without current infection (defined as within the last week) or chronic medical conditions; and (*iv*) no prescribed medications. The Stellenbosch University Human Ethics Committee approved the study (M12/01/005).

Venous blood samples were collected into dipotassium EDTA, transported and stored at 20°C (standard deviation 4°C) prior to preparation and analysis. All testing was completed within 24 hours of collection. Rapid HIV antibody analysis (Alere Determine, USA) confirmed that the participants were HIV-seronegative on the day of phlebotomy. An automated full blood count and white cell differential analysis was performed using a Beckman Coulter LH-750 Haematology Analyser (Beckman Coulter, USA). Samples were examined for the following parameters: total white blood cell (WBC) count and five-part automated differential count, red blood cell (RBC) count, haemoglobin (Hb), haematocrit (Hct), mean cell volume (MCV), red cell distribution width (RDW) and platelet (Plt) count. Blood films were reviewed manually to visually confirm automated differential counts and exclude gross morphological abnormalities. A Mentzer index (MCV/RBC)[10-12] was calculated for each participant. Dual-platform absolute lymphocyte subset counts and percentages were prepared using the Becton Dickinson FACSLyse/wash method (BDIS, USA). Samples were stained using the following directly labelled antibodies: CD3 APC, CD3 FITC, CD16 PE, CD19 FITC, CD45 PerCP, CD45RO PE, CD45RA FITC, HLA DR APC (BDIS) and CD4 FITC, CD8 PE and CD56 PE (Beckman Coulter). All samples were analysed on a FACSCalibur flow cytometer (Becton Dickinson, USA) using CellQuest analysis software (Becton Dickinson). The accuracy of the full blood count and lymphocyte subset values was subject to strict manufacturer and internationally recommended internal and external quality assurance procedures. Laboratories performing testing are accredited under the South African National Accreditation System (certificate numbers M0025A and M0106B) and subscribe to the NHLS National Quality Assessment programmes and the UK National External Quality Assessment Schemes to confirm longitudinal accuracy of all laboratory parameters.

Statistical analysis

The Clinical and Laboratory Standards Institute *Approved Guideline* (3rd edition) for defining and establishing reference intervals was used to determine and calculate the reference intervals.[5] No data points were removed from statistical analysis, as sample integrity and testing quality were closely monitored (and ensured) throughout the study. The data were categorised into the following age groups: 0 - 3 months, 3 - 6 months, 6 - 12 months, 1 - 2 years, 2 - 6 years, 6 - 12 years and >12 years, in keeping with similar international publications. Differences between genders were calculated with the non-parametric Mann-Whitney U-test and the reference intervals were reported as medians with 95% confidence intervals (CIs). Data analysis was performed using Microsoft Excel (Frontline Systems Inc., USA), Stata version 12 (StataCorp LP, USA) and GraphPad Prism 5 Software (GraphPad Software, USA).

Results

Table 1 summarises the values obtained for the full blood count and white cell differential parameters. Table 2 summarises the immune monitoring values, i.e. lymphocyte subsets, activated T cells and naive and memory subsets. No gender-specific statistically significant differences (Mann-Whitney p-values <0.05) were noted across any of the age groups for any of the full blood count parameters or lymphocyte subsets analysed. The Mentzer index, RDW and Hb values for participants identified with possible iron deficiency are summarised in Table 3.

Discussion

RBC parameters

In this study, the overall median RBC, Hb and Hct values increased from infancy to 12 years of age, with a cumulative increase of ±24% for RBCs (3.50 v. 4.60 × 10^{12}/L), ±17% for Hb (10.6 v. 12.8 g/dL) and ±18% for HCT (0.31 v. 0.38 L/L between 0 - 3 months and 12 years of age. The median MCV (92.2 fL) was highest at 0 - 3 months, decreasing steadily until approximately 2 years of age (72.5 fL); subsequently values increased, reaching previously reported adult values. Although the median RDW values were variable, no specific trends were noted across any of the paediatric age groups, falling within an interval of 13.5 - 16.3 fL. Analysis of the median values for RBC parameters and comparison with published literature confirms that the RBC parameters noted (Fig. 1) are in keeping with 'normal' physiological anaemia of childhood.[1] Increasing physiological demands for RBCs result in an overall decreased median Hb concentration and production of smaller RBCs (decreased median MCV),[6] with greater variation in size (increased median RDW). The MCV begins to decrease in healthy neonates after ±1 week, and RBC production increases when the Hb drops to a level of about 11 g/dL (at ±2 months of age), attributable to reduced red cell lifespan (60 - 80 days v. 120 days for an adult), influence of erythropoietin,[1] etc.

No statistically significant gender-related differences in RBC parameters were noted until 12 years of age (p>0.05). Although gender-specific discrepancies have been noted previously in the literature, they are more evident from puberty and are attributed to the hormonal influences of oestrogen and androgens (Hb in males is reported to increase by as much as 2 g/dL in response to testosterone[13]). Although not shown in this study, ethnicity and altitude have been noted to contribute to RBC parameter variation[6] in other studies.

Table 1. Full blood count calculated medians and 95% CIs for the study group

	Age ranges						
	0 - 3 months (*n*=45)	**3 - 6 months (*n*=40)**	**6 - 12 months (*n*=54)**	**1 - 2 years (*n*=79)**	**2 - 6 years (*n*=118)**	**6 - 12 years (*n*=37)**	**>12 years (*n*=8)**
RBCs (× 10^{12}/L)	3.5 (2.7 - 4.4)	4.2 (3.1 - 4.9)	4.4 (3.9 - 5.2)	4.4 (3.9 - 5.1)	4.3 (3.9 - 4.8)	4.2 (3.8 - 4.7)	4.6 (4.0 - 5.2)
Hb (g/dL)	10.6 (8.7 - 14.7)	11.4 (8.5 - 12.7)	10.8 (9.5 - 13.1)	10.8 (8.7 - 12.5)	11.2 (9.4 - 12.5)	11.8 (10.0 - 13.1)	12.8 (11.1 - 13.3)
Hct (L/L)	0.31 (0.25 - 0.41)	0.34 (0.25 - 0.38)	0.32 (0.28 - 0.39)	0.32 (0.27 - 0.37)	0.34 (0.29 - 0.38)	0.35 (0.31 - 0.38)	0.38 (0.32 - 4.00)
MCV (fL)	92.2 (82.2 - 99.4)	79.2 (68.7 - 88.6)	73.5 (66.8 - 81.2)	72.5 (64.5 - 82.2)	78.9 (64.5 - 86.4)	83.2 (74.3 - 87.7)	81.1 (75.4 - 86.8)
RDW (%)	14.7 (12.8 - 18.2)	13.5 (11.7 - 16.8)	15.9 (13.4 - 21.3)	16.3 (14.1 - 20.8)	14.8 (12.8 - 19.4)	13.9 (12.7 - 15.9)	13.9 (13.3 - 16.3)
Plt (× 10^9/L)	420 (243 - 581)	390 (228 - 687)	367 (253 - 610)	349 (192 - 551)	326 (197 - 471)	319 (206 - 454)	280 (180 - 371)
WBCs (× 10^9/L)	9.6 (5.7 - 14.9)	10.4 (6.9 - 19.7)	12.0 (5.3 - 20.0)	10.7 (6.9 - 18.5)	8.7 (5.8 - 13.6)	7.6 (4.0 - 10.0)	8.6 (4.9 - 13.0)
Neutrophils (%)	24.1 (15.6 - 39.0)	29.6 (11.6 - 48.8)	31.7 (13.0 - 52.3)	31.5 (20.0 - 51.1)	42.0 (25.0 - 61.2)	48.2 (28.4 - 65.3)	54.4 (45.5 - 57.2)
Neutrophils (× 10^9/L)	2.36 (1.26 - 5.20)	2.88 (1.22 - 8.11)	3.99 (1.13 - 8.08)	3.55 (1.79 - 7.87)	3.63 (1.59 - 6.64)	3.69 (1.85 - 6.33)	4.19 (2.60 - 7.30)
Lymphocytes (%)	60.0 (49.0 - 72.8)	58.8 (42.3 - 77.5)	58.0 (36.8 - 73.9)	57.0 (37.7 - 69.0)	43.4 (26.1 - 62.1)	37.5 (21.5 - 60.9)	35.1 (27.7 - 45.8)
Lymphocytes (× 10^9/L)	5.56 (3.23 - 11.07)	6.12 (3.79 - 12.43)	6.20 (3.10 - 11.45)	5.75 (3.58 - 10.58)	3.81 (1.93 - 6.11)	2.71 (1.50 - 3.89)	2.85 (1.78 - 3.95)
Monocytes (%)	11.0 (5.0 - 18.3)	7.9 (3.2 - 16.6)	8.0 (2.8 - 13.4)	7.0 (3.0 - 12.0)	6.0 (2.5 - 11.0)	5.3 (1.6 - 11.2)	6.1 (4.2 - 8.0)
Monocytes (× 10^9/L)	1.03 (0.52 - 2.40)	0.97 (0.32 - 2.37)	0.89 (0.30 - 1.93)	0.73 (0.29 - 1.61)	0.53 (0.20 - 1.06)	0.38 (0.12 - 0.97)	0.46 (0.22 - 0.75)
Eosinophils (%)	3.0 (1.0 - 6.3)	3.0 (0 - 10.0)	3.1 (1.0 - 8.0)	3.1 (0 - 10.0)	5.9 (1.5 - 18.1)	4.8 (0.9 - 12.9)	6.8 (1.1 - 16.0)
Eosinophils (× 10^9/L)	0.25 (0.07 - 0.89)	0.33 (0 - 1.24)	0.29 (0.05 - 1.34)	0.32 (0 - 1.37)	0.51 (0.11 - 1.20)	0.27 (0.10 - 1.16)	0.50 (0.06 - 1.05)
Basophils (%)	0.2 (0 - 1.1)	0.2 (0.1.1)	0.3 (0 - 1.3)	0.2 (0 - 1.0)	0.3 (0 - 1.0)	0.3 (0 - 0.8)	0.3 (0 - 0.4)
Basophils (× 10^9/L)	0.02 (0 - 0.11)	0.03 (0 - 0.12)	0.03 (0 - 0.17)	0.02 (0 - 010)	0.03 (0 - 0.08)	0.02 (0 - 0.04)	0.03 (0 - 0.10)

The RBC parameter variation, particularly in median Hb (~1 - 2 g/dL lower) and Hct (~4 - 7% lower), across all age groups may be attributed to a combination of ethnicity and altitude. However, subclinical iron deficiency and/or mild anaemia could not be ruled out. Although iron deficiency and other metabolic disorders, including vitamin B_{12} and folate deficiencies, were not formally excluded in this cohort, haematological indices and the Mentzer index calculation were assessed.[10] Iron deficiency was suspected if a concurrent Hb <10 g/dL, RDW >15%[11] and Mentzer index >13[10,12] was found. As noted in another local adult reference interval study,[14] the findings suggested that 14.2% (54/381) of the children tested in this study had possible iron deficiency (Table 3). Further studies are required to confirm this finding.

Platelets

The overall median Plt counts were highest at 0 - 3 months (420 × 10^9/L) and were comparable to adult values described previously.[1,6] However, although the Plts remained within adult reference limits, they showed a steady decrease of approximately 33% until 12 years of age (280 × 10^9/L) (Fig. 2). The median Plt count of the cohort appeared to stabilise at ±12 years of age, similar to reference intervals currently in use and a previously published local adult study.[15] Individual gender-specific differences become apparent at puberty as a result of hormonal influences. In girls, Plt counts increase by approximately 25 × 10^9/L owing to the effect of the hormone oestradiol.[13] Additional increases are linked to the onset of menstruation and prevalence of iron deficiency anaemia.[1,6]

WBC parameters

Total white cell and differential count

The median total WBC count increased from 9.6 × 10^9/L at 0 - 3 months, reaching a peak of 12 × 10^9/L at 12 months. Thereafter, median WBC counts declined to reach adult values[6] at 12 years

Table 2. Measured immune monitoring data for the study group, medians and 95% CIs

	Age ranges						
	0 - 3 months (*n*=45)	**3 - 6 months (*n*=40)**	**6 - 12 months (*n*=54)**	**1 - 2 years (*n*=79)**	**2 - 6 years (*n*=118)**	**6 - 12 years (*n*=37)**	**>12 years (*n*=8)**
Cell marker (cells/μl), median (95% CI)							
$CD3^+$	3 650 (2 028 - 7 625)	3 999 (2 341 - 8 451)	4 212 (1 795 - 7 334)	3 787 (2 392 - 6 616)	2 652 (1 360 - 4 202)	1 853 (1 017 - 2 913)	1 944 (1 146 - 2 544)
$CD4^+$	2 656 (1 520 - 5 160)	2 927 (1 573 - 5 441)	2 474 (1 085 - 4 562)	2 190 (1 374 - 3 928)	1 579 (816 - 2 705)	1 092 (568 - 2 013)	1 205 (724 - 1 785)
$CD8^+$	949 (428 - 2 478)	1 135 (593 - 3 176)	1 355 (622 - 3 244)	1 280 (669 - 3 247)	970 (414 - 1 599)	648 (340 - 1 210)	704 (318 - 980)
Ratio $CD4^+/CD8^+$	2.58 (1.29 - 5.49)	2.33 (1.19 - 4.96)	1.70 (0.90 - 3.92)	1.69 (0.78 - 3.88)	1.80 (0.91 - 3.13)	1.67 (0.95 - 2.47)	1.74 (1.58 - 2.63)
$CD19^+$	835 (351 - 1823)	1 179 (666 - 3 310)	1 037 (485 - 2 348)	1 156 (610 - 2 699)	584 (274 - 1 468)	322 (163 - 563)	303 (105 - 623)
$CD3^+HLA^-DR^+$	172 (94 - 709)	222 (90 - 1 361)	387 (157 - 916)	365 (142 - 1 069)	217 (92 - 507)	124 (56 - 284)	157 (54 - 205)
$CD4^+HLA^-DR^+$	117 (45 - 228)	129 (45 - 380)	107 (44 - 229)	125 (56 - 282)	84 (34 - 153)	49 (20 - 137)	60 (23 - 77)
$CD8^+HLA^-DR^+$	46 (10 - 487)	99 (21 - 950)	213 (58 - 699)	199 (44 - 849)	112 (29 - 414)	65 (29 - 168)	84 (27 - 131)
$CD4^+CD45^+RA^+$	1 828 (954 - 4 064)	2 383 (1 200 - 4 049)	1 749 (793 - 3 700)	1 687 (930 - 2 655)	944 (388 - 2 018)	603 (92 - 1 278)	551 (344 - 1 086)
$CD4^+CD45^+RO^+$	868 (261 - 1 801)	634 (314 - 1 384)	576 (359 - 1 293)	634 (343 - 1 218)	562 (343 - 1 218)	523 (305 - 845)	660 (311 - 708)
Ratio $CD4^+$ naive/ memory	2.6 (0.74 - 7.00)	3.72 (1.55 - 6.45)	2.83 (1.19 - 5.51)	2.57 (1.35 - 5.64)	1.76 (0.71 - 3.69)	1.15 (0.10 - 2.48)	1.21 (0.76 - 1.60)
$CD8^+CD45^+RA^+$	812 (370 - 2 044)	873 (402 - 2 323)	974 (443 - 1 939)	964 (459 - 2 688)	719 (295 - 1 247)	466 (258 - 800)	425 (200 - 731)
$CD8^+CD45^+RO^+$	146 (50 - 683)	259 (81 - 1174)	337 (83 - 1 583)	292 (72 - 1 162)	188 (69 - 532)	159 (48 - 455)	213 (68 - 339)
Ratio $CD8^+$ naive/ memory	4.93 (1.48 - 14.00)	3.40 (0.94 - 10.60)	3.36 (0.81 - 14.30)	3.43 (1.16 - 12.90)	3.56 (1.25 - 9.98)	2.86 (1.24 - 6.92)	2.52 (1.06 - 3.66)
$CD16^+CD56^+$	489 (179 - 1 310)	399 (137 - 1 699)	367 (117 - 1 860)	435 (111 - 1 372)	284 (120 - 782)	214 (52 - 697)	277 (128 - 642)
$CD3^-CD56^+$	448 (189 - 1 450)	396 (126 - 1 647)	368 (123 - 1 840)	385 (125 - 1 253)	283 (119 - 744)	219 (61 - 680)	294 (136 - 624)
Cell marker, % (95% CI)							
$CD3^+$	69.3 (58.0 - 79.2)	67.8 (47.0 - 79.7)	68.3 (47.3 - 76.2)	67.0 (52.4 - 76.2)	70.5 (54.6 - 79.3)	71.8 (50.7 - 80.5)	64.5 (56.2 - 74.5)
$CD4^+$	49.5 (37.6 - 63.0)	45.9 (37.6 - 63.0)	41.2 (26.7 - 57.8)	39.7 (26.5 - 56.3)	41.6 (30.2 - 53.8)	41.9 (29.2 - 54.2)	42.0 (37.7 - 55.3)
$CD8^+$	18.2 (10.7 - 28.1)	19.6 (11.4 - 30.2)	24.1 (12.7 - 33.9)	24.4 (13.7 - 35.3)	24.5 (15.1 - 35.7)	25.9 (15.2 - 32.3)	23.3 (17.2 - 26.8)
Ratio $CD4^+/CD8^+$	2.58 (1.29 - 5.49)	2.33 (1.19 - 4.96)	1.70 (0.90 - 3.92)	1.69 (0.78 - 3.88)	1.80 (0.91 - 3.13)	1.67 (0.95 - 2.47)	1.74 (1.58 - 2.63)
$CD19^+$	15.5 (8.0 - 29.5)	20.1 (10.5 - 38.7)	18.6 (9.3 - 33.5)	20.0 (10.7 - 33.0)	15.7 (9.4 - 30.5)	11.5 (8.4 - 18.0)	12.1 (5.9 - 17.1)
$CD3^+HLA^-DR^+$	5.1 (2.6 - 14.9)	5.6 (2.4 - 20.7)	9.7 (3.6 - 19.3)	9.0 (3.0 - 19.6)	8.0 (4.0 - 19.9)	6.9 (3.1 - 16.0)	7.3 (4.0 - 10.4)

Continued ...

Table 2. (Continued) Measured immune monitoring data for the study group, medians and 95% CIs

	Age ranges						
	0 - 3 months (*n*=45)	3 - 6 months (*n*=40)	6 - 12 months (*n*=54)	1 - 2 years (*n*=79)	2 - 6 years (*n*=118)	6 - 12 years (*n*=37)	>12 years (*n*=8)
$CD4^+HLA^-DR^+$	4.0 (2.0 - 8.1)	4.2 (1.8 - 8.7)	4.3 (2.3 - 8.9)	5.6 (2.3 - 12.0)	5.7 (2.2 - 10.0)	4.3 (1.9 - 15.0)	4.2 (2.5 - 6.5)
$CD8^+HLA^-DR^+$	4.5 (1.6 - 28.7)	10.0 (1.5 - 38.9)	16.9 (5.4 - 35.1)	14.4 (4.5 - 46.2)	12.5 (4.6 - 30.1)	9.3 (5.1 - 23.9)	11.4 (6.4 - 17.8)
$CD4^+CD45^+RA^+$	72.2 (42.6 - 87.5)	78.8 (60.8 - 86.6)	73.9 (54.4 - 84.6)	72.0 (57.4 - 84.9)	63.8 (41.6 - 78.7)	53.5 (8.9 - 71.3)	54.6 (43.1 - 61.5)
$CD4^+CD45^+RO^+$	27.8 (12.5 - 57.4)	21.2 (13.4 - 39.2)	26.1 (15.4 - 45.6)	28.0 (15.1 - 42.6)	36.2 (21.3 - 58.4)	46.5 (28.7 - 91.1)	45.4 (38.5 - 56.9)
Ratio $CD4^+$ naive/ memory	2.6 (0.7 - 7.0)	3.7 (1.6 - 6.5)	2.8 (1.2 - 5.5)	2.6 (1.4 - 5.6)	1.8 (0.7 - 3.7)	1.2 (0.1 - 2.5)	1.2 (0.8 - 1.6)
$CD8^+CD45^+RA^+$	83.1 (59.6 - 93.3)	77.3 (48.3 - 91.4)	77.1 (44.8 - 93.5)	77.4 (53.6 - 92.8)	78.1 (55.6 - 90.9)	74.9 (55.4 - 87.9)	71.6 (51.4 - 78.6)
$CD8^+CD45^+RO^+$	16.9 (6.7 - 40.4)	22.7 (8.6 - 51.7)	22.9 (6.5 - 55.2)	22.6 (7.2 - 46.4)	21.9 (9.1 - 44.4)	25.9 (12.6 - 47.0)	28.4 (21.4 - 48.6)
Ratio $CD8^+$ naive/ memory	4.9 (1.5 - 14.0)	3.4 (0.9 - 10.6)	3.4 (0.8 - 14.3)	3.4 (1.2 - 12.9)	3.6 (1.3 - 10.0)	2.9 (1.2 - 6.9)	2.5 (1.1 - 3.7)
$CD16^+CD56^+$	9.3 (3.6 - 22.5)	6.5 (3.0 - 17.7)	7.0 (3.2 - 18.4)	7.1 (2.4 - 20.0)	7.9 (3.4 - 18.1)	7.9 (2.9 - 26.9)	9.9 (6.0 - 19.2)
$CD3^-CD56^+$	9.2 (4.0 - 20.7)	6.5 (3.0 - 18.5)	7.0 (3.2 - 17.8)	6.4 (2.5 - 19.1)	7.9 (3.6 - 17.5)	8.2 (3.1 - 26.3)	10.6 (5.9 - 19.8)

of age, with an overall 10% decrease in median total WBC counts between 0 - 3 months and 12 years of age (9.6 v. 8.6 × 10^9/L). Between 0 - 3 months and 12 years of age, the overall median absolute neutrophils increased by 44% (2.36 × 10^9L v. 4.19 × 10^9L) and lymphocytes decreased by 49% (5.56 × 10^9L v. 2.85 × 10^9L). Monocyte counts decreased by 55% (1.03 × 10^9L v. 0.46 × 10^9L), while eosinophils and basophils increased by 50% (0.25 × 10^9L v. 0.50 × 10^9L) and 33% (0.02 × 10^9L v. 0.03 × 10^9L), respectively. These trends were also noted in the corresponding relative neutrophil, lymphocyte, monocyte, eosinophil and basophil percentage values. Lymphocytes are the predominant WBC until ±4 years of age and then decline, with neutrophils becoming increasingly predominant as the child grows.[6] This study confirmed this observation, showing a median lymphocyte count of 5.75 × 10^9/L v. 3.55 × 10^9/L for neutrophils at 1 - 2 years, with lymphocyte numbers decreasing to 2.71 × 10^9/L and neutrophils increasing to 3.69 × 10^9/L by 6 - 12 years of age (Fig. 3). Neutrophil reference intervals calculated from this local paediatric population were in keeping with those reported from westernised countries.[6] These are higher than intervals reported from other African countries,[7,8] where benign genetic neutropenia, which may be linked to the Duffy-null trait, is thought to confer protection against *Plasmodium vivax* malaria.[16] The similarity of this locally derived neutrophil reference interval to westernised countries could also be attributed to the large number of participants of mixed ethnic ancestry in this study (*n*=296).

Baseline eosinophil counts are reported to be increased in some African countries as a result of endemic parasitic infections.[8] Endemic parasitic infections are reported to be prevalent in children from the neighbouring Eastern Cape Province and, with the reported rapid influx of migrants from the Eastern Cape into Cape Town, increased eosinophil counts may have been expected in this study. However, the results from this and a previous SA study[15,17] reveal that eosinophil counts are low and in keeping with westernised countries. This is possibly the result of successful local implementation of deworming programmes in children that has ensured minimal helminthiasis, despite reports that >90% of children from the Eastern Cape region are infected by *Ascaris* and/or *Trichuris*.[18] Further, although a schistosomiasis prevalence of 73.2% was reported in a study of schoolchildren in the rural Eastern Cape, the eosinophil counts of this cohort of children suggest that schistosomiasis is also not prevalent in this population group.[19]

Table 3. Summary of Mentzer index, RDW and Hb values for boys and girls identified as having possible iron deficiency

Mentzer index	RDW (%)	Hb (g/dL)
Boys		
27.0	15.5	7.3
15.8	16.9	7.7
17.5	18.4	7.9
27.1	15.5	8.2
14.5	19.4	8.3
15.6	16.8	8.7
15.4	17.1	8.8
12.4	19.8	8.8
13.0	19.4	8.8
35.6	15.6	8.9
16.0	24	8.9
14.0	18	9.2
18.8	20.5	9.2
16.4	20	9.2
19.5	21.5	9.3
15.5	18.4	9.4
32.8	15.3	9.4
14.5	20.8	9.5

Continued ...

Table 3. (Continued) Summary of Mentzer index, RDW and Hb values for boys and girls identified as having possible iron deficiency

Mentzer index	RDW (%)	Hb (g/dL)
17.4	16.2	9.5
16.9	17.8	9.6
15.6	20.9	9.6
17.2	18.9	9.6
11.1	23.6	9.7
16.6	17.9	9.7
17.6	16.4	9.7
16.1	18.1	9.7
14.7	16.8	9.7
17.9	20.5	9.8
16.8	17.7	9.8
20.6	16.6	9.8
16.8	18	9.9
15.3	17.3	9.9
Girls		
15.4	18.9	8.2
32.9	17.9	8.5
12.5	20.0	8.5
27.2	16.1	8.5
37.3	16.6	8.7
26.8	15.4	9.0
26.6	19.0	9.2
13.7	16.8	9.4
16.3	18.6	9.4
20.9	15.3	9.4
18.9	21.3	9.5
16.2	18.4	9.5
15.5	17.1	9.5
16.2	17.7	9.6
17.2	17.3	9.7
35.0	17.1	9.5
17.5	17.5	9.8
15.9	19.5	9.9
15.7	18.7	9.9
14.7	21.0	9.9
13.8	20.3	9.9
16.6	17.5	9.9

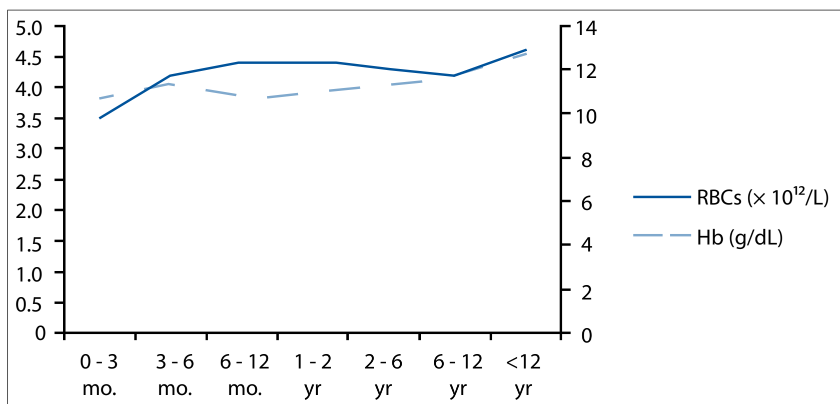

Fig. 1. Graphical representation of childhood physiological anaemia in this study group, similar to that previously described.[1] *From ~3 - 6 months of age until 6 - 12 years of age, there is an inverse relationship between the median Hb and median RBC cell count. Between 6 and 12 years of age, the parameters converge and increase proportionally until adulthood.*

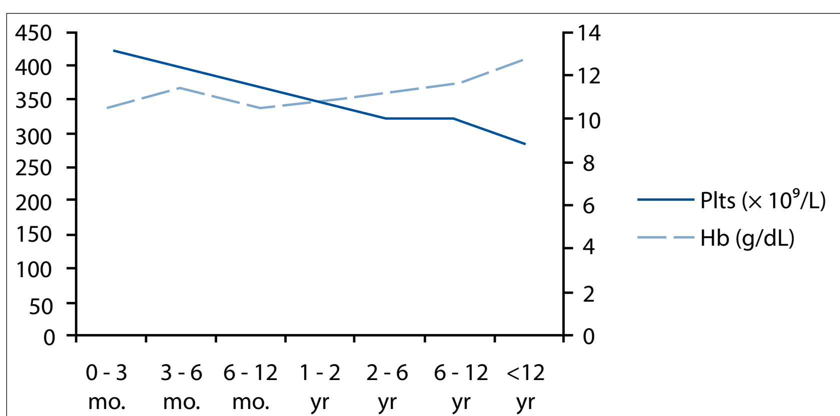

Fig. 2. Comparison of the median Hb trend v. the median Plt count trend. As illustrated, while Hb increases steadily with age, Plts show a decreasing trend with age and only stabilise at puberty.

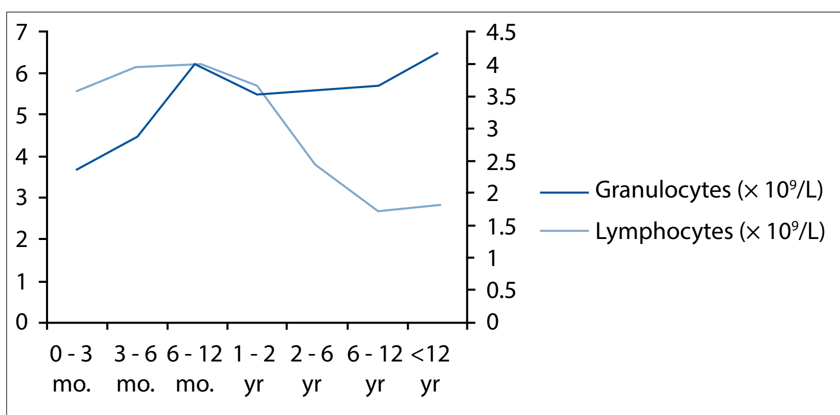

Fig. 3. Comparison of median granulocyte numbers v. lymphocyte numbers. As illustrated, from about 2 years of age lymphocyte numbers decrease markedly while granulocyte numbers stabilise and slowly begin to increase, in keeping with normal childhood physiological development.[1]

Lymphocyte subsets (T, B and natural killer cells)

Analysis of the absolute T, B and natural killer lymphocyte subset numbers showed a decreasing trend with age, in keeping with previously described normal physiological total WBC and lymphocyte count intervals.[6] The median T-cell numbers decreased by ±47%, CD4-positive cells by 55% and CD8-positive cells by 26%. As reported elsewhere, developmental changes in the composition of the peripheral B-cell pool are most noticeable up to 5 years of age, and the total number of B cells decreases with age.[1] In this cohort, the median number of CD19-positive B cells decreased by 36%, in keeping with international reports.[2] The median number of $CD3^{-}/CD16^{+}/CD56^{+}$ natural killer cells decreased by 43% between 0 - 3 months and 12 years of age, in keeping with international trends and the current reference intervals used for reporting.[2]

T-cell activation

To assess T-cell activation, HLA-DR expression was measured on the total $CD3^+$ T-cell population, on $CD3^+/CD8^+$ cytotoxic T lymphocytes and on $CD3^+/CD4^+$ helper T lymphocytes. HLA-DR expression is typically increased on T cells during activation and on $CD8^+$ lymphocytes, specifically with increasing age.[20] In this cohort of children, an overall decrease of 9% in the total number of activated T cells between 0 - 3 months and 12 years of age (172×10^6/L v. 157×10^6/L) was noted. However, further investigation of the T-cell subsets showed a 51% decrease in activated $CD3^+/CD4^+$ lymphocytes (117×10^6/L v. 60×10^6/L) and a 55% increase in activated $CD3^+/CD8^+$ cytotoxic T cells (46×10^6/L v. 84×10^6/L), as reported elsewhere.[2]

Naive and memory subsets

Analysis of CD45RA and CD45RO expression on $CD3^+/CD4^+$ T-helper lymphocytes in this study group was in keeping with international reports and showed consistent conversion of naive T-helper cells to memory T-helper cells from 0 - 3 months (ratio 2.6) across the range of paediatric age groups, to achieve a ratio of 1.21 by 12 years of age.[2] Expression of CD45 isoforms, CD45RA and CD45RO, on CD4- and CD8-positive T-cells varies depending on the stage of maturation and the T-cell receptor response to antigenic stimulation.[21] CD45RA has been shown to be associated with CD4 and be more efficient at promoting T-cell activation after T-cell receptor stimulation. Analysis of the CD45RA and CD45RO isoforms therefore remains important for monitoring immune suppression associated with infections such as HIV.[21]

Conclusion

Paediatric full blood count and lymphocyte subset reference intervals often differ between laboratories and across centres, and vary between populations and regions. Locally, a lack of comprehensive local paediatric full blood count and lymphocyte subset reference intervals has led to the use of internationally published reference intervals, which may not be appropriate for our local populations. Reference intervals reported here, derived from a cohort of children from an informal settlement in the Western Cape Province, SA, confirm expected active immunohaemopoeisis of childhood.[1,6]

Locally established reference intervals,[17,22] using limited participants from a single area (where a multitude of unknown factors may affect outcome), may not necessarily be appropriate for defining whether a particular individual's blood counts are 'normal' (or not) if the individual is of different ethnic origin or socioeconomic background or lives in a different region. International Clinical Laboratory Standards Institute study guidelines are also not specific enough to exclude all variables that could affect each parameter studied.[5] The reference intervals reported here, although limited, do provide important insight into local paediatric reference intervals. In the absence of a local fully representative population study, including paediatric participants from across SA (with known ethnic, social and economic demographics), this study serves to validate the use of the published international reference intervals provided with NHLS paediatric immunohaematology laboratory reports. Ethical considerations will preclude studies on healthy, normal children. However, conducting similar studies during paediatric clinical trials in different regions across SA could provide valuable opportunities to extend this work. In the meantime, this article supports the continued use of the international paediatric reference intervals currently used by the NHLS for full blood count, white cell differential and lymphocyte subsets.

References

1. Kaushansky K, Lichtman MA, Beutler E, Kipps TJ, Seligsohn U, Prchal JT. Williams Hematology. 8th ed. McGraw-Hill, 2010.
2. Shearer WT, Rosenblatt HM, Gelman RS, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: The Pediatric AIDS Clinical Trials Group P1009 study. J Allergy Clin Immunol 2003;112(5):973-980. [http://dx.doi.org/10.1016/j.jaci.2003.07.003]
3. Yang F, Li Y, Braylan R, Hunger SP, Yang LJ. Pediatric T-cell post-transplant lymphoproliferative disorder after solid organ transplantation. Pediatr Blood Cancer 2008;50(2):415-418. [http://dx.doi.org/10.1002/pbc.21072]
4. Kaleem Z, Hassan A, Pathan MH, White G. Flow cytometric evaluation of posttransplant B-cell lymphoproliferative disorders. Arch Pathol Lab Med 2004;128(2):181-186. [http://dx.doi.org/10.1043/1543-2165(2004)128<181:FCEOPB>2.0.CO;2]
5. Clinical Laboratory Standards Institute. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory: Approved Guideline. 3rd ed. Vol. 28, No. 20. Wayne, PA: CLSI, 2010:61.
6. Bates I, Lewis SM. Reference ranges and normal values. In: Bain BJ, Bates I, Laffan MA, Lewis M, eds. Dacie and Lewis Practical Haematology. Philadelphia, PA: Elsevier Churchill Livingstone, 2012:11-22. [http://dx.doi.org/10.1016/B978-0-7020-3408-4.00002-3]
7. Buchanan AM, Muro FJ, Gratz J, et al. Establishment of haematological and immunological reference values for healthy Tanzanian children in Kilimanjaro Region. Trop Med Int Health 2010;15(9):1011-1021. [http://dx.doi.org/10.1111/j.1365-3156.2010.02585.x]
8. Lugada ES, Mermin J, Kaharuza F, et al. Population-based hematologic and immunologic reference values for a healthy Ugandan population. Clin Diagn Lab Immunol 2004;11(1):29-34. [http://dx.doi.org/10.1128/CDLI.11.1.29-34.2004]
9. Gie J, Small K, Haskins C. Planning District Profiles. Cape Town: City of Cape Town, 2007:1-16.
10. Mentzer WC, Jr. Differentiation of iron deficiency from thalassaemia trait. Lancet 1973;1(7808):882. [http://dx.doi.org/10.1016/S0140-6736(73)91446-3]
11. Sazawal S, Dhingra U, Dhingra P, et al. Efficiency of red cell distribution width in identification of children aged 1-3 years with iron deficiency anemia against traditional hematological markers. BMC Pediatr 2014;14(8):1-6. [http://dx.doi.org/10.1186/1471-2431-14-8]
12. Vehapoglu A, Ozgurhan G, Demir AD, et al., Hematological indices for differential diagnosis of beta thalassemia trait and iron deficiency anemia. Anemia 2014(2014), article ID 576738. [http://dx.doi.org/10.1155/2014/576738]
13. Sikaris KA. Physiology and its importance for reference intervals. Clin Biochem Rev 2014;35(1):3-14. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=24659833 (accessed 9 June 2015).
14. Lawrie D, Coetzee LM, Glencross DK. Iron deficiency anaemia in healthy South African women despite iron fortification. S Afr Med J 2008;98(8):606-607.
15. Lawrie D, Coetzee LM, Becker P, Mahlangu J, Stevens W, Glencross DK. Local reference ranges for full blood count and CD4 lymphocyte count testing. S Afr Med J 2009;99(4):243-248.
16. Reich D, Nalls MA, Kao WH, et al. Reduced neutrophil count in people of African descent is due to a regulatory variant in the Duffy antigen receptor for chemokines gene. PLoS Genet 2009;5(1):e1000360. [http://dx.doi.org/10.1371/journal.pgen.1000360]
17. Badenhorst CJ, Fourie J, Steyn K, et al. The haematological profile of urban black Africans aged 15-64 years in the Cape Peninsula. East Afr Med J 1995;72(1):19-24. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7781549 (accessed 9 June 2015).
18. Adams VJ, Markus MB, Adams JF. Paradoxical helminthiasis and giardiasis in Cape Town, South Africa: Epidemiology and control. Afr Health Sci 2005;5(3):276-280. [http://dx.doi.org/10.5555/afhs.2005.5.3.276]
19. Meents EF, Boyles TH. *Schistosoma haematobium* prevalence in school children in the rural Eastern Cape Province, South Africa. S Afr J Epidemiol Infect 2010;25(4):28-29.
20. O'Gorman MR, Millard DD, Lowder JN, Yogev R. Lymphocyte subpopulations in healthy 1-3-day-old infants. Cytometry 1998;34(5):235-241. [http://dx.doi.org/10.1002/(SICI)1097-0320(19981015)34:5<235::AID-CYTO5>3.0.CO;2-0 [pii]
21. Altin JG, Sloan EK. The role of CD45 and CD45-associated molecules in T cell activation. Immunol Cell Biol 1997;75(5):430-445. [http://dx.doi.org/10.1038/icb.1997.68]
22. Kiepiela P, Coovadia HM, Coward P, Woodhead R, Abdool-Karim SS, Becker P. Age-related lymphocyte sub-population changes among healthy Africans from birth to adulthood. Ann Trop Paediatr 1989;9(4):199-205. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2482000 (accessed 9 June 2015).

Accepted 19 January 2015.