Distribution of haematological and chemical pathology values among infants in Malawi and Uganda

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Background: Data on paediatric reference laboratory values are limited for sub-Saharan Africa.
Objective: To describe the distribution of haematological and chemical pathology values among healthy infants from Malawi and Uganda.
Methods: A cross-sectional study was conducted among healthy infants, 0–6 months old, born to HIV-uninfected mothers recruited from two settings in Blantyre, Malawi and Kampala, Uganda. Chemical pathology and haematology parameters were determined using standard methods on blood samples. Descriptive analyses by age-group were performed based on 2004 Division of AIDS Toxicity Table age categories. Mean values and interquartile ranges were compared by site and age-group.
Results: A total of 541 infants were included altogether, 294 from Malawi and 247 from Uganda. Overall, the mean laboratory values were comparable between the two sites. Mean alkaline phosphatase levels were lower among infants aged $\leqslant$21 days while aspartate aminotransferase, creatinine, total bilirubin and gamma-glutamyl transferase were higher in those aged 0–7 days than in older infants. Mean haematocrit, haemoglobin and neutrophil counts were higher in the younger age-groups (<35 days) and overall were lower than US norms. Red and white blood cell counts tended to decrease after birth but increased after $\sim$2 months of age. Mean basophil counts were higher in Malawi than in Uganda in infants aged 0–1 and 2–7 days; mean counts for eosinophils (for age groups 8–21 or older) and platelets (for all age groups) were higher in Ugandan than in Malawian infants. Absolute lymphocyte counts increased with infant age.
Conclusion: The chemical pathology and haematological values in healthy infants born to HIV-uninfected mothers were comparable in Malawi and Uganda and can serve as useful reference values in these settings.

Keywords: Chemical pathology, Haematology, Infant, Malawi, Reference laboratory value, Uganda

Introduction

In developed countries, the normal values of laboratory parameters for the general population are well characterized and established to monitor laboratory testing and assist in clinical management. In resource-limited settings, standard reference laboratory data are limited; most comparisons are based on reference values from populations in developed countries.[1] However, differences in genetic, nutritional and environmental factors may undermine use of reference values from developed country populations and could potentially lead to clinical misclassification and inappropriate management.[2,3] In research studies that monitor safety, the lack of site-specific data is challenging and use of data from other populations can lead to inaccurate reporting and assessment of adverse events and their severity. For example, in HIV intervention research involving clinical trials of efficacy and safety, almost all studies use the Toxicity Table from the Division of AIDS (DAIDS) at the US National Institutes for Health (NIH).[4] In an earlier report, we compared the proportions of Ugandan and Malawian non-HIV-exposed children who would have been classified as normal or abnormal based on the US laboratory toxicity tables and discussed the limitations of this

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approach to monitor adverse events in African children.[5] In this paper, we describe the distribution of haematological and blood chemical pathology parameters in healthy Malawian and Ugandan infants.

Methods

Study design, location and population

A cross-sectional paediatric laboratory study was conducted in Malawi and Uganda between 2006 and 2007. Details of the design and methods used have been described elsewhere.[5] Briefly, the surveys were conducted at Mulago Hospital, Kampala, Uganda and at the Queen Elizabeth Central Hospital and three health centres in Blantyre, Malawi. The study protocol was approved by the institutional review boards in Malawi and Uganda, and at the Johns Hopkins University.

Study procedures

Eligible mother–infant pairs were identified at referral hospitals in Blantyre and Kampala. In both, participants were recruited at birth and before the infant was discharged from hospital following delivery, or at a scheduled postnatal visit. Mothers with an HIV-negative test result within 30 days of screening for the study were asked to participate. Mothers who did not know their HIV status were appropriately counselled and offered testing using the standard rapid test for each respective site. If the HIV test results were negative, and the mother was willing to have her child participate in the study, written informed consent was obtained from her before the infant was enrolled in the study. Infants were enrolled into age-groups similar to those of the 2004 DAIDS Toxicity Tables.[4]

Healthy, full-term newborns weighing >2.5 kg or infants up to 6 months (⩽6 months) of age born following an uncomplicated pregnancy to HIV-negative mothers were eligible. To be eligible, infants had to be without any life-threatening conditions, acute illnesses or major congenital anomalies. Infants from multiple-gestation births were also eligible. Infants who had a history of blood transfusion were excluded. Each infant was assigned to an appropriate age interval within the first 6 months of life. In Malawi, the age-groups included birth (day 0), 1–7, 8–21, 22–35, 36–56, 84–98 and 170–190 days. The groups in Uganda were similar, except for two intervals, 57–123 and 124–159 days, where the older age-groups did not completely overlap with the Malawi age-groups. In both places, infants were enrolled consecutively as they were identified to achieve a target of approximately 30 evaluable infants per age-group from birth to 6 months at each of the sites.

At the time of enrolment, demographic data and a brief medical history were obtained. A study clinician or nurse then performed a physical examination, including vital signs to rule out acute illnesses. A venous blood sample was drawn from healthy infants during the same clinic visit while infants found to be sick were treated and rescheduled for the blood draw and enrolment in an appropriate age-group at a subsequent visit.

Laboratory methods

Venous blood, 2–3 ml, was collected in a 4.0-ml EDTA and plain vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) during working days of the week. The blood sample collected in the EDTA tube was used for complete blood count (CBC) with differential count and flow cytometry while the sample collected in the plain tube was used for chemistries and pathology. Blood samples were collected during morning hours and transported to the respective research laboratories in Blantyre and Kampala within 30–60 minutes. The research laboratories in Malawi and Uganda both participate in the same DAIDS-recommended proficiency tests, including UK NEQAS for CD4+ T-cells. They are approved by DAIDS to provide laboratory support for various research networks. Both laboratories have well established quality-control measures based on written, standard operating procedures, and are monitored by an independent agency. The quality-control measures cover all steps including collection of samples in the clinic, transport and receipt of samples at the laboratory, processing and testing as appropriate, and reporting of results. Laboratory personnel are certified in Good Clinical and Laboratory Practice.

Haematological measurements for CBC in Malawi and Uganda were analysed using a Beckman–Coulter AcT5 Part Diff (California, USA). The CBC report included the following parameters: white blood cell count (WBC), red blood cell count, haemoglobin, haematocrit, platelets, mean corpuscular volume, mean corpuscular haemoglobin concentration, and five-part WBC differential, absolute count and percentages.

In Malawi, blood chemical pathology parameters were analysed using a Beckman–Coulter CX5 Chemistry analyser (Brea, California, USA) while in Uganda a COBAS Integra 400+ (Roche Diagnostics, Indianapolis, IN, USA) was used. Chemistry analytes included alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, creatinine, blood urea nitrogen, sodium, potassium, chloride and bicarbonate. Other chemistry analytes tested were albumin, amylase, creatinine kinase, glucose and uric acid.

Cytometric measurements (CD4+ and CD8+ cell counts) were analysed using a Becton Dickinson (San Jose, California, USA) FACS Count in Malawi and

the Becton Dickinson FACS-Calibur instrument in Uganda. Laboratory tests were completed on the days on which samples were collected.

Statistical analysis

Enrolled infants aged between 0 and 6 months of age were considered in seven non-overlapping age categories. Descriptive and parametric statistics were used to determine and describe the distributions of laboratory values. Mean, median and interquartile range (IQR) values were calculated for each age-group. Appropriate statistical tests of significance were performed using *t*-tests for mean values and the χ^2 test for categorical values. Data are presented in tables for the two sites combined (overall) and comparisons between the two sites are graphically shown as line-graphs of mean values by age category. For comparison, reference values from other sources, whenever available, are shown in a separate column in these tables.[6] The data were also $\log_{10}$-transformed to account for possible skewing of the data and geometric means (antilog of the mean of the $\log_{10}$-transformed data) were calculated for each laboratory parameter. The overall results of the geometric means and the graphical presentation of these means by site were comparable with the parametric estimates and therefore the transformed data are not shown.

Results

A total of 569 healthy, full-term infants, 0–6 months old, born to HIV-negative mothers were enrolled from both sites in Malawi and Uganda. Of these, 541 infants had evaluable laboratory data, 247 from Uganda and 294 from Malawi. Table 1 shows the general characteristics of these infants by site. There were no gender differences by age category, and mean birthweights were similar in both places.

Table 1 Characteristics of infants enrolled in Malawi and Uganda

Characteristics	Malawi	Uganda	Total
Age-group, days	*n*	*n*	*n* (% male)
Birth*	76	61	137 (50.4)
2–7	10	21	32 (48.4)
8–14	33	9	42 (45.2)
15–21	10	17	27 (51.9)
22–30	32	24	56 (44.6)
31–35	9	9	18 (33.3)
36–56	62	32	94 (57.5)
57–98	37	29	66 (56.1)
99–190	41	57	98 (44.9)
Birthweight, kg, mean (SD)	3.1 (0.48)	3.2 (0.44)	
Male	158	128	286
Female	152	131	283
Total (%)	310 (54.5)	259 (45.5)	569

* 0–1 day.

Chemical pathology

Table 2 shows the overall chemistry results in the Malawian and Ugandan infants. The results have also been stratified by site, plotting mean values by age-group. The chemistry results from Malawi and Uganda were generally comparable by age-group in both cohorts of infants. Mean alkaline phosphatase (200 U/L) was lower in infants aged $\leqslant$21 days than in older infants (>300 U/L). Mean alanine aminotransferase, chloride, carbon dioxide, potassium and sodium levels were approximately similar in all age-groups. Aspartate aminotransferase, creatinine, total bilirubin and gamma-glutamyl transferase were higher in infants 0–7 days old compared with older infants. In addition, mean values of gamma-glutamyl transferase were slightly higher in all age-groups in the Ugandan infants than in the Malawian infants while creatinine was slightly higher in Malawian infants than in Ugandan infants. However, these differences were not statistically significant.

Haematology

Table 3 shows the overall haematology values in Malawian and Ugandan infants. Mean values and ranges for the majority of the parameters were similar by age-group in the two populations. However, overall mean basophil counts were higher in infants in age groups 0–1 and 2–7 days in Malawi (0.74 cells $\times 10^9$/L) than in Uganda (0.10 cells $\times 10^9$/L) ($P<0.05$). Mean eosinophil counts were higher in Ugandan infants aged 8–21 days or older than in Malawian infants ($P<0.05$). Mean platelet counts were also higher in Ugandan infants in all age-groups than in Malawian infants ($P<0.05$). In both populations, the mean haematocrit, haemoglobin, neutrophil, white blood cell count and red blood cell counts were higher in the younger age-group, <35 days, than in the older age-groups. There was a decline in red and white blood cell counts until approximately 2 months of age and an increasing trend thereafter. Absolute lymphocyte counts increased with infants' increasing age.

Cytometry

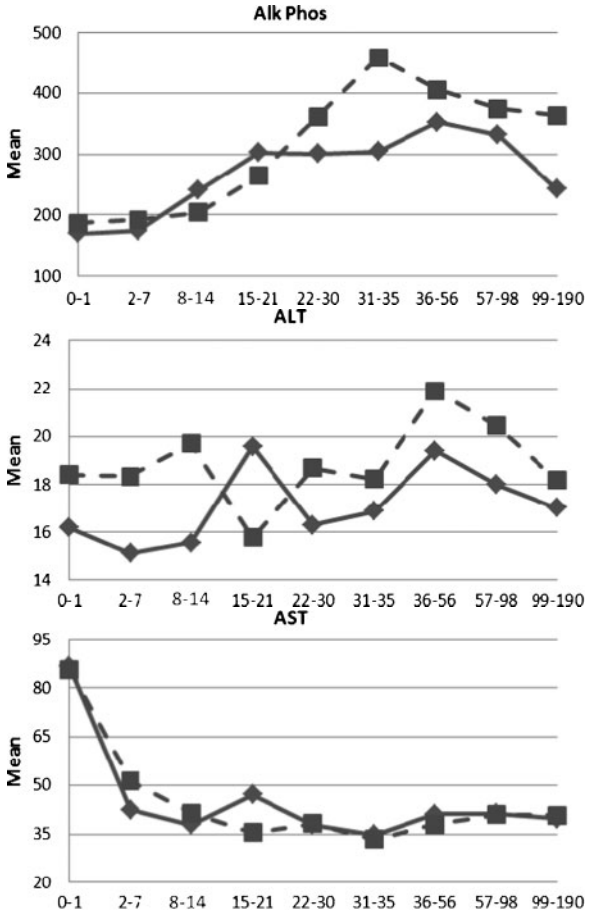
Table 4 shows the results of cytometry measurements. Although the data were not obtained from both sites for each age-group, mean CD4+ and CD8+ cell counts were approximately similar. At both sites, there was a comparable early increase in trends of both CD4+ and CD8+ cell counts, followed by a decline.

Discussion

This study describes the distribution of chemical pathology and haematological indices in two healthy, HIV-unexposed, -uninfected African cohorts of infants. The Malawian and Ugandan research sites have been actively enrolling children in prevention-of-mother-to-child transmission studies for nearly

Table 2 Infant blood chemistry values overall and by site

Parameter	Age, d	*n*	Mean	Median	IQR	Ref. mean[6]
Alk phos (U/L)	0–1	94	176.3	179.7	144.0–206.0	
	2–7	20	184.1	187.5	162.0–217.0	
	8–14	34	235.1	240.0	187.0–278.0	
	15–21	25	279.9	286.0	214.0–321.0	Infant:
	22–30	50	323.9	306.5	237.0–398.0	150–420
	31–35	15	376.4	379.0	304.0–412.0	
	36–56	87	368.5	30.0	277.0–428.0	
	57–98	62	351.0	328.5	266.0–403.0	
	99–190	87	314.0	274.0	243.0–373.0	
ALT (U/L)	0–1	102	17.5	15.0	12.0–20.0	
	2–7	26	17.2	14.0	12.0–22.0	
	8–14	35	16.4	15.0	11.0–20.0	
	15–21	27	17.2	14.0	10.0–24.0	Infant:
	22–30	54	17.3	14.5	11.0–21.0	13–45
	31–35	16	17.6	17.0	12.0–20.0	
	36–56	91	20.3	17.0	12.0–24.0	
	57–98	63	19.1	18.0	13.0–24.0	
	99–190	91	17.7	15.0	12.0–20.0	
AST (U/L)	0–1	107	86.0	70.1	54.2–103.0	
	2–7	25	48.5	45.0	32.0–60.0	Newborn:
	8–14	36	38.5	36.0	29.0–45.5	25–75
	15–21	27	39.7	35.0	28.0–45.0	
	22–30	54	37.9	32.0	28.0–44.0	Infant:
	31–35	16	34.1	33.0	29.5–37.5	15–60
	36–56	90	40.1	36.0	31.0–43.0	
	57–98	63	41.0	41.0	34.0–46.0	
	99–190	90	40.4	38.0	33.0–46.0	



Alk phos, alkaline phosphatase; ALT, alanine aminotransferase;
AST, aspartate aminotransferase; IQR, interquartile range; Ref, reference

Table 2 continued

Parameter	Age, d	*n*	Mean	Median	IQR	Ref mean[6]
Bilirubin total (μmol/L)	0–1	97	87.2	77.0	54.7–95.8	<137
	2–7	28	141.9	141.9	73.5–194.9	<205
	8–14	33	88.9	71.8	32.5–128.3	
	15–21	26	63.3	53.0	22.2–80.4	Infants:
	22–30	51	39.3	25.7	13.7–49.6	<21
	31–35	15	23.9	20.5	13.7–29.1	
	36–56	86	17.1	12.0	8.6–20.5	
	57–98	61	10.3	8.6	6.8–12	
	99–190	91	10.3	8.6	5.1–13.7	
BUN (mmol/L)	0–1	82	3.8	3.6	2.6–4.6	Newborn:
	2–7	23	3.5	2.5	1.4–3.6	0.7–6.7
	8–14	32	3.2	3.0	2.3–3.9	
	15–21	26	2.4	2.1	1.8–3.2	
	22–30	48	2.3	2.1	1.8–2.9	Infant/child 1.8–6.4
	31–35	15	2.1	2.1	1.4–2.5	
	36–56	85	2.0	1.8	1.4–2.5	
	57–98	63	1.7	1.8	1.1–2.1	
	99–190	88	1.5	1.4	1.1–1.8	
Chloride (mmol/L)	0–1	98	108.3	108.2	106.0–110.9	Newborn:
	2–7	18	108.1	108.1	105.0–110.0	98–113
	8–14	34	107.9	107.7	105.8–110.6	
	15–21	25	105.0	104.0	102.9–107.0	Child/adult:
	22–30	50	105.8	105.9	104.0–108.1	98–107
	31–35	15	106.3	106.0	104.0–108.6	
	36–56	86	106.8	107.2	104.6–109.0	
	57–98	62	106.8	106.8	104.2–108.9	
	99–190	88	106.3	105.0	104.0–109.1	

BUN, blood urea nitrogen; IQR, interquartile range; Ref, reference

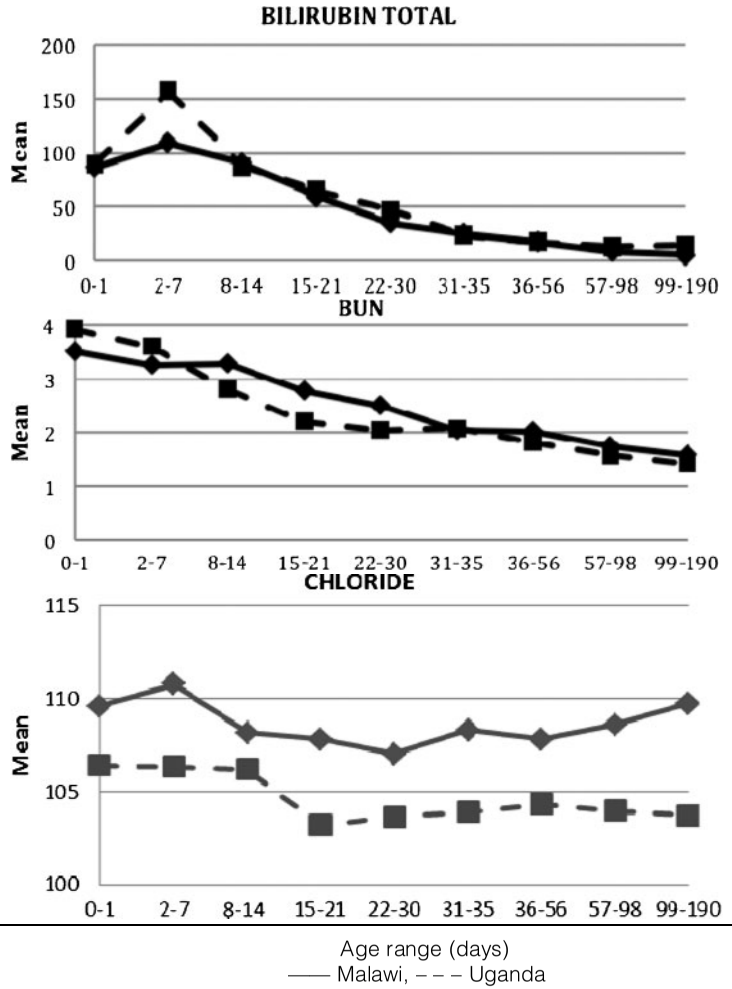
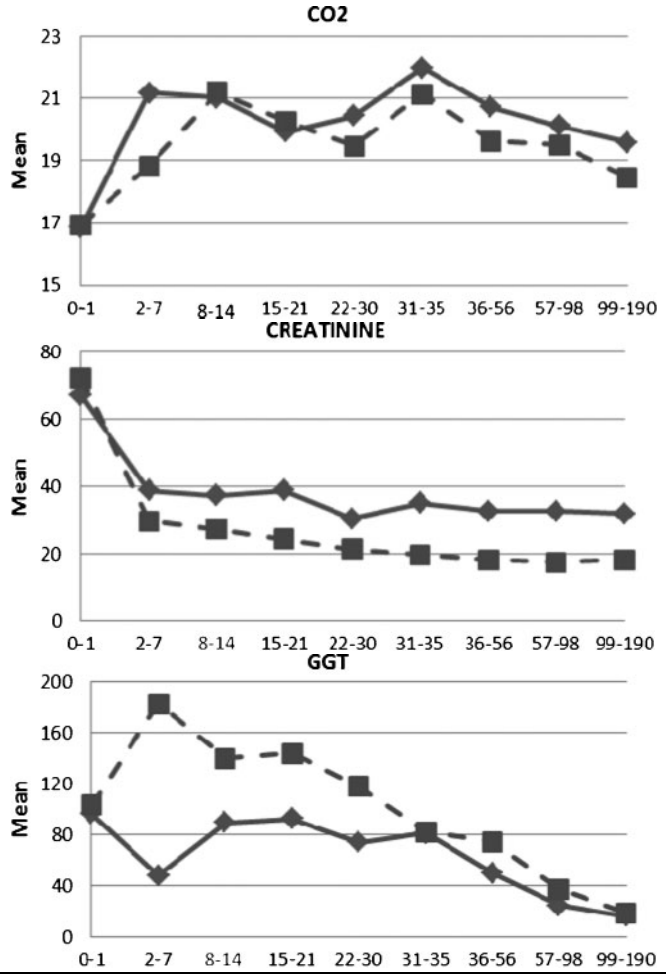


Table 2 continued

Parameter	Age, d	*n*	Mean	Median	IQR	Ref mean[6]
CO_2 (mmol/L)	0–1	95	16.9	17.0	15.0–18.8	Newborn:
	2–7	18	19.7	18.8	18.0–22.0	13–22
	8–14	34	21.1	21.0	19.3–23.0	
	15–21	25	20.1	21.0	18.0–22.0	Infant/child:
	22–30	50	20.1	20.1	18.2–22.0	20–28
	31–35	15	21.6	22.0	19.0–25.1	
	36–56	85	20.4	20.6	18.9–22.0	
	57–98	62	19.9	20.0	18.0–21.6	
	99–190	89	19.0	19.0	17.0–21.3	
Creatinine (μmol/L)	0–1	98	68.6	68.6	61.0–76.3	Newborn:
	2–7	28	30.5	30.5	30.5–38.1	27–88
	8–14	34	38.1	30.5	30.5–38.1	
	15–21	25	30.5	30.5	22.9–30.5	Infant:
	22–30	51	22.9	22.9	22.9–30.5	18–35
	31–35	15	30.5	22.9	22.9–30.5	
	36–56	89	30.5	30.5	22.9–30.5	
	57–98	63	22.9	22.9	15.3–30.5	
	99–190	91	22.9	22.9	15.3–30.5	
GGT (U/L)	0–1	108	99.8	92.5	53.6–123.9	0–3 w:
	2–7	23	130.2	66.0	47.0–137.0	0–130
	8–14	35	98.5	84.0	64.0–317.0	
	15–21	27	124.9	85.0	65.0–116.0	3 w–3 m:
	22–30	53	92.5	71.0	45.0–112.0	4–120
	31–35	16	82.0	76.5	43.0–119.0	
	36–56	91	57.9	47.0	33.0–71.0	>3 m:
	57–98	63	30.4	27.0	22.0–34.7	5–65
	99–190	90	18.1	16.9	11.1–22.0	



CO_2, carbon dioxide; GGT, gamma-glutamyl transferase; IQR, interquartile range; Ref, reference

Table 2 continued

Parameter	Age, d	*n*	Mean	Median	IQR	Ref mean[6]
Potassium (mmol/L)	0–1	91	6.1	5.6	5.0–6.3	Newborn:
	2–7	19	5.8	5.5	5.2–6.0	3.7–5.9
	8–14	34	5.4	5.4	5.1–5.8	
	15–21	24	5.6	5.6	5.0–6.0	Infant:
	22–30	49	5.6	5.5	5.3–5.8	4.1–5.3
	31–35	15	5.5	5.5	5.2–5.9	
	36–56	85	5.8	5.7	5.3–6.0	
	57–98	58	5.7	5.2	5.1–5.7	
	99–190	87	5.1	5.0	4.7–5.3	
Sodium (mmol/L)	0–1	99	139.5	139.7	136.8–141.9	<1 y:
	2–7	18	138.2	137.9	136.0–141.0	130–145
	8–14	34	138.7	139.0	137.0–140.0	
	15–21	25	136.7	136.0	134.8–137.6	
	22–30	50	136.4	136.2	134.9–138.2	
	31–35	15	137.2	137.4	135.0–139.5	
	36–56	86	136.8	137.0	135.9–137.9	
	57–98	62	137.3	137.0	136.0–138.6	
	99–190	88	137.2	137.0	136.0–138.8	

IQR, interquartile range; Ref, reference

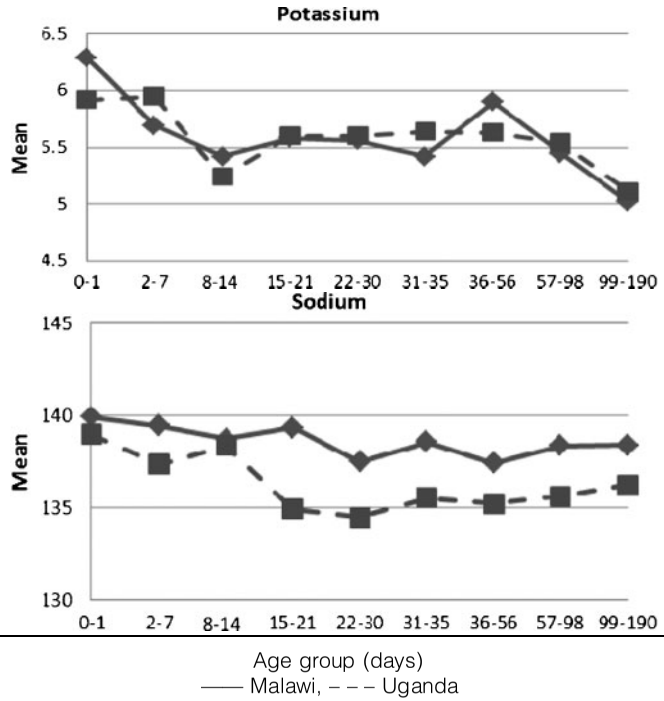
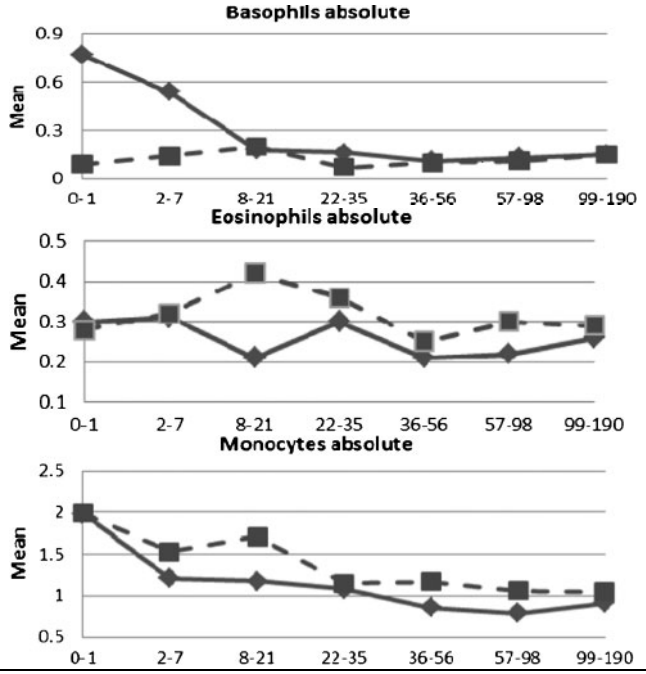


Table 3 Infant haematology values overall and by site

Parameter	Age, d	*n*	Mean	Median	IQR	Ref mean[6]
Basophil absolute ($\times 10^9$/L)	0–1	127	0.5	0.2	0.1–0.5	
	2–7	30	0.3	0.1	0.0–0.3	
	8–21	65	0.2	0.2	0.1–0.2	
	22–35	70	0.1	0.1	0.1–0.2	
	36–56	91	0.1	0.1	0.07–0.14	
	57–98	59	0.1	0.1	0.1–0.2	
	99–190	95	0.2	0.1	0.1–0.2	
Eosinophil absolute ($\times 10^9$/L)	0–1	126	0.3	0.2	0.2–0.3	
	2–7	30	0.3	0.2	0.2–0.3	
	8–21	65	0.3	0.3	0.1–0.4	
	22–35	71	0.3	0.3	0.2–0.4	
	36–56	91	0.2	0.2	0.1–0.3	
	57–98	58	0.3	0.2	0.2–0.4	
	99–190	95	0.3	0.8	0.6–1.2	
Monocyte absolute ($\times 10^9$/L)	0–1	126	2.0	1.8	1.4–2.5	1.1
	2–7	30	1.4	1.3	1.0–1.7	1.1
	8–21	65	1.4	1.3	1.0–1.7	14 d: 1.0
	22–35	71	1.1	1.0	0.7–1.5	28 d: 0.7
	36–56	92	1.0	0.9	0.7–1.2	
	57–98	60	0.9	0.8	0.6–1.2	
	99–190	95	1.0	0.9	0.7–1.1	190 d: 0.6



IQR, interquartile, Ref., reference

Table 3 continued

Parameter	Age, d	*n*	Mean	Median	IQR	Ref mean (−2 SD)[6]
Haematocrit (proportion of 1.0)	0–1	127	0.53	0.53	0.47–0.59	1–3 d: 0.56 (0.45)
	2–7	31	0.51	0.50	0.46–0.57	
	8–21	65	0.44	0.44	0.41–0.47	14 d: 0.53 (0.41)
	22–35	71	0.38	0.38	0.34–0.42	28 d: 0.44 (0.33)
	36–56	92	0.34	0.33	0.31–0.36	
	57–98	60	0.32	0.32	0.30–0.34	60 d: 0.35 (0.28)
	99–190	95	0.33	0.33	0.31–0.35	190 d: 0.36 (0.31)
Haemoglobin (g/L)	0–1	127	175.1	176.0	157–192	1–3 d: 85 (145)
	2–7	31	169.7	168.0	155–185	
	8–21	65	145.7	147.0	136–157	14 d: 166 (134)
	22–35	71	127.5	127.0	114–139	28 d: 139 (107)
	36–56	92	113.7	112.5	104–123	
	57–98	60	105.8	107.0	99–113	60 d: 112 (94)
	99–190	95	109.7	110.0	101–116	190 d:126 (111)
Lymphocyte absolute ($\times 10^9$/L)	0–1	127	4.9	4.6	3.8–5.5	
	2–7	30	4.8	4.5	3.6–5.7	
	8–21	65	5.4	5.2	4.2–6.0	
	22–35	71	6.1	5.7	5.0–6.8	
	36–56	92	5.6	5.7	4.4–6.7	
	57–98	60	6.8	6.5	4.5–9.0	
	99–190	95	6.8	6.3	5.0–7.7	

IQR, interquartile range; Ref, reference; SD, standard deviation

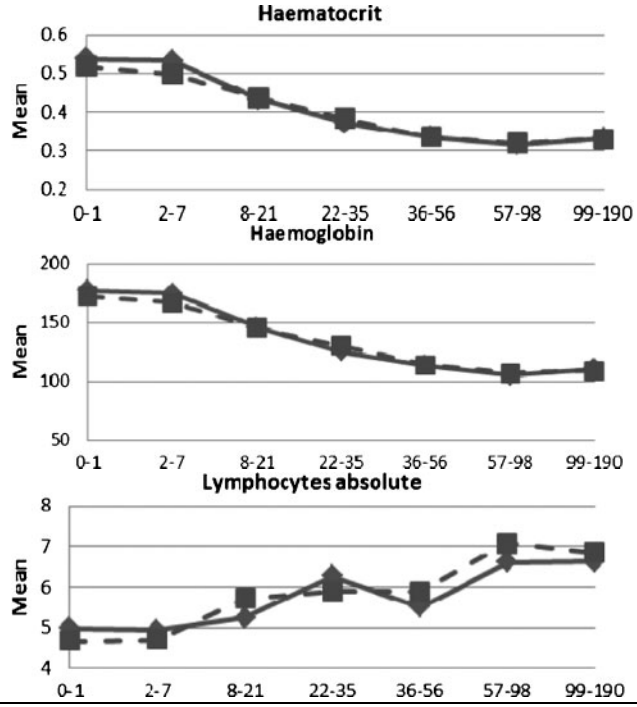
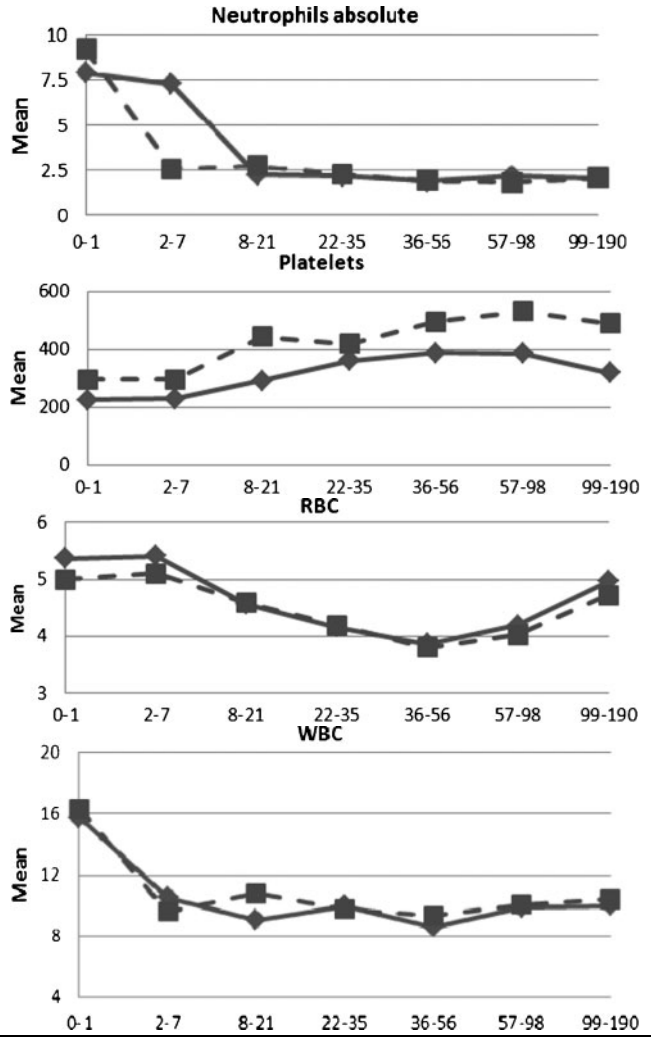


Table 3 continued

Parameter	Age, d	*n*	Mean	Median	IQR	Ref mean (IQR)[6]
Neutrophil absolute ($\times 10^9$/L)	0–1	125	8.5	8.1	5.9–10.9	0–1 d: 11.5 (5–21)
	2–7	30	4.2	2.4	1.7–3.5	7 d: 5.5 (1.5–10)
	8–21	65	2.5	2.2	1.6–2.9	14 d: 4.5 (1–9.5)
	22–35	71	2.2	2.0	1.4–2.9	28 d: 3.8 (1–8.5)
	36–56	91	1.9	1.8	1.3–2.3	
	57–98	58	2.0	1.6	1.2–2.5	
	99–190	95	2.1	2.0	1.4–2.5	190 d: 3.8 (1–8.5)
Platelets ($\times 10^9$/L)*	0–1	127	255.0	250.0	171.0–322.0	1–3 d: 192
	2–7	31	274.0	264.0	219.0–329.0	
	8–21	65	351.8	355.0	252.0–448.0	14 d: 252
	22–35	71	385.1	374.0	285.0–486.0	
	36–56	92	424.6	426.5	294.5–535.5	
	57–98	60	448.1	457.5	347.5–555.0	
	99–190	95	420.3	425.0	307.0–520.0	
Red blood cells ($\times 10^{12}$/L)	0–1	72	5.4	5.2	4.7–5.7	
	2–7	10	5.4	5.1	4.6–5.7	
	8–21	39	4.6	4.5	4.1–5.1	
	22–35	41	4.2	4.2	3.7–4.6	
	36–56	60	3.9	3.8	3.5–4.2	
	57–98	34	4.2	4.1	3.9–4.3	
	99–190	38	5.0	4.7	4.5–5.1	
White blood cells ($\times 10^9$/L)	0–1	127	16.0	15.2	12.4–19.0	1–3 d:
	2–7	31	9.9	8.8	7.6–11.4	18.9 (9.4–34)
	8–21	65	9.8	9.5	7.5–11.3	14 d: 11.4 (5–20)
	22–35	71	9.9	9.5	7.9–11.4	28 d: 10.8 (4–19.5)
	36–56	92	8.8	8.7	7.3–10.2	
	57–98	60	10.1	9.8	6.7–12.0	
	99–190	95	10.3	9.9	7.9–11.9	190 d: 11.9 (6–17.5)



IQR, interquartile range; Ref, reference; *IQR for reference mean not given

Table 3 continued

Parameter	Age, d	*n*	Mean	Median	IQR	Ref mean[6]
Basophil (proportion of 1.0)	0–1	127	0.019	0.013	0.003–0.023	
	2–7	30	0.020	0.014	0.003–0.028	
	8–21	65	0.019	0.016	0.012–0.020	
	22–35	70	0.012	0.012	0.005–0.016	
	36–56	91	0.012	0.011	0.009–0.015	
	57–98	59	0.011	0.011	0.008–0.015	
	99–190	95	0.014	0.013	0.010–0.016	
Neutrophil (proportion of 1.0)	0–1	125	0.52	0.53	0.46–0.59	0–1 d: 0.61
	2–7	30	0.29	0.25	0.22–0.35	7 d: 0.45
	8–21	65	0.25	0.24	0.21–0.27	14 d: 0.40
	22–35	71	0.22	0.21	0.18–0.27	28 d: 0.35
	36–56	91	0.22	0.21	0.17–0.27	
	57–98	58	0.20	0.18	0.14–0.23	
	99–190	95	0.20	0.20	0.14–0.24	190 d: 0.32
Monocyte (proportion of 1.0)	0–1	126	0.12	0.12	0.10–0.14	0–1 d: 0.06
	2–7	30	0.15	0.15	0.13–0.18	7 d: 0.09
	8–21	65	0.14	0.14	0.11–0.16	14 d: 0.09
	22–35	71	0.11	0.11	0.09–0.14	28 d: 0.07
	36–56	92	0.11	0.10	0.08–0.13	
	57–98	60	0.09	0.09	0.07–0.11	
	99–190	95	0.10	0.09	0.08–0.11	190 d: 0.05

IQR, interquartile range; Ref, reference

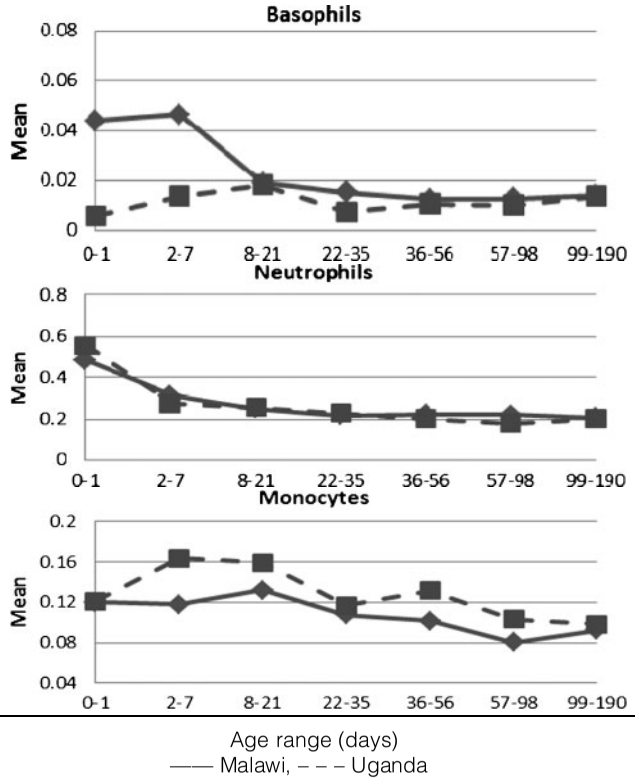


Table 3 continued

Parameter	Age, d	*n*	Mean	Median	IQR	Ref mean[6]
Lymphocyte (proportion of 1.0)	0–1	127	0.32	0.30	0.26–0.36	0–1 d: 0.31
	2–7	30	0.50	0.51	0.44–0.59	7 d: 0.41
	8–21	65	0.55	0.56	0.50–0.60	14 d: 0.48
	22–35	71	0.62	0.63	0.58–0.67	28 d: 0.56
	36–56	92	0.64	0.65	0.58–0.69	
	57–98	60	0.67	0.69	0.63–0.74	
	99–190	95	0.66	0.66	0.60–0.73	190 d: 0.61
Eosinophil (proportion of 1.0)	0–1	126	0.018	0.015	0.011–0.023	0–1 d: 0.02
	2–7	30	0.029	0.026	0.026–0.037	7 d: 0.04
	8–21	65	0.030	0.027	0.016–0.040	
	22–35	71	0.040	0.032	0.020–0.040	
	36–56	91	0.027	0.023	0.016–0.031	14–190 d:
	57–98	58	0.027	0.022	0.015–0.033	0.03
	99–190	95	0.028	0.022	0.014–0.036	

IQR, interquartile range; Ref, reference

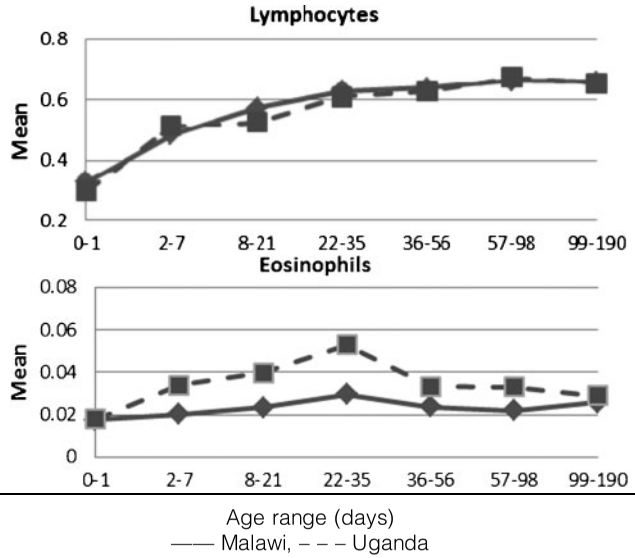
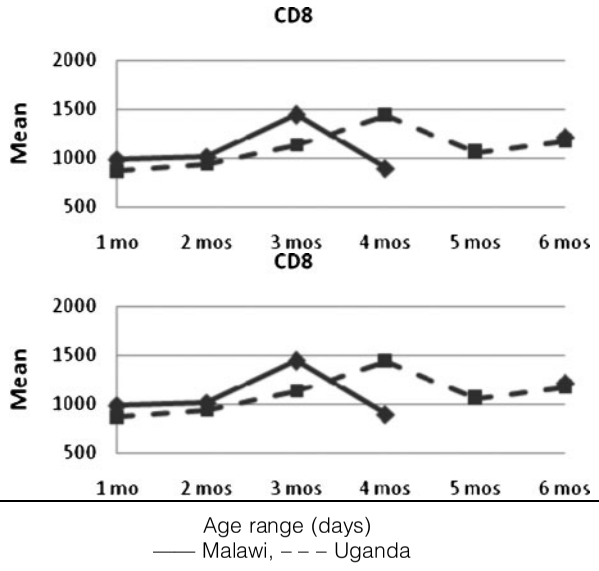


Table 4 Infant cytometry values overall and by site

Parameter	Age, m	*n*	Mean	Median	IQR
CD4 (cells/μl)	1	235	2169.2	2000.0	1626.0–2586.0
	2	103	2294.8	2295.0	1692.0–2883.0
	3	36	2074.1	2010.0	1507.5–2467.5
	4	13	2356.5	2210.0	2016.0–2545.0
	5	29	2373.2	2371.0	1639.0–3006.0
	6	53	2247.7	2000.0	1636.0–2804.0
CD8 (cells/μl)	1	235	926.8	819.0	614.0–1095.0
	2	103	991.1	849.0	625.0–1372.0
	3	36	1286.1	1288.0	743.0–1624.0
	4	13	1202.4	1025.0	755.0–1395.0
	5	29	1096.0	1012.0	757.0–1305.0
	6	53	1178.3	1053.0	803.0–1405.0

IQR, interquartile range



two decades (e.g. HIVNET 012[7] and PEPI[8]). These two sites, as well as others involved in paediatric and prevention-of-mother-to-child research in sub-Saharan Africa, have historically used the US NIH DAIDS Toxicity Tables to monitor safety.[4,5] This approach has provided consistency in reporting across research sites. However, both physiological and environmental factors may limit comparability of populations across countries and continents, as has been shown in prior studies demonstrating differences by region, race and ethnicity.[9,10] For example, a study conducted among HIV-uninfected infants in Zimbabwe showed that haematological and immunological parameters varied significantly from values considered to be normal in Caucasian infants.[3] Another study in a paediatric population in Tanzania showed that their absolute neutrophil counts were lower than those of reference laboratory values used in industrialized countries.[11] Such differences may lead to misinterpretation of normal versus abnormal values in study participants in developing countries.[5]

The findings reported here suggest that in the African context there are no clinically important differences between Malawian and Ugandan healthy, HIV-unexposed infants for most laboratory haematological and blood chemistry pathology mean values measured. It is likely that the underlying genetic, nutritional and other exposures are similar in these two sub-Saharan African settings. However, as previously reported from this same group of infants, the description of abnormal 'toxicity' levels differs when using US-based normal indices. For example, using US standards in the 2004 DAIDS Toxicity Tables,[4] absolute neutrophil count and haemoglobin indices would be classified as abnormally low in a substantial subset of healthy Malawian and Ugandan infants.[5]

Likewise, some differences are noted when comparing the laboratory means and ranges from this study of East and Southern African infants with those of Caucasian infants typically represented in reference tables. For example, mean haemoglobin and haematocrit values of Malawian and Ugandan infants in this study, despite being similar to results from other studies in Africa,[3,9] were consistently lower than those of Caucasian infants after the first week of life.[12] From these data, it is difficult to determine whether such differences are because of physiological or other factors, such as infant feeding practices (e.g. exclusive breastfeeding by women with low nutritional status in resource-limited settings).[13]

Limitations

The sample sizes for some age categories were small and the selection of age categories was mainly based on the US-based DAIDS Toxicity Tables rather than biologically relevant cut-offs. In Malawi and Uganda, the DAIDS tables are commonly used for monitoring adverse events in clinical trials of HIV treatment regimens. Although the research laboratories were certified to perform these assays, there were differences in some of the equipment used at the two sites which may have influenced interpretation of the laboratory values.

Overall, the laboratory findings of this study were similar to reports from other studies in African infants and strengthen the need to establish local or regional reference values to improve clinical management and monitor safety.[1–5] The burden of disease and malnutrition in African children is substantial, irrespective of HIV infection. Knowing the mean values and ranges in healthy paediatric cohorts within the same geographic setting will facilitate management of these diseases. Additionally, knowing baseline values will improve management and monitoring of HIV-infected children as recommended by the WHO[14] and others.[11,15] For researchers, development of locally relevant reference ranges is critical to evaluate safety and efficacy of innovative interventions, including new drugs for prevention and treatment of HIV and other infections.

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References

1 Lugada ES, Mermin J, Kaharuza F, Ulvestad E, Were W, Langeland N, *et al*. Population-based hematologic and immunologic reference values for a healthy Ugandan population. Clin Diagn Lab Immunol. 2004;11:29–34.
2 Quinto L, Aponte JJ, Sacarlal J, Espasa M, Aide P, Mandomando I, *et al*. Haematological and biochemical indices in young African children: in search of reference intervals. Trop Med Int Health. 2006;11:1741–8.
3 Troy SB, Rowhani-Rahbar A, Dyner L, Musingwini G, Shetty AK, Woelk G, *et al*. Hematologic and immunologic parameters in Zimbabwean infants: a case for using local reference intervals to monitor toxicities in clinical trials. J Trop Pediatr. 2012;58:59–62.
4 National Institute for Allergy and Infectious Disease. Division of AIDS toxicity table for grading the severity of adult and pediatric adverse experiences. Bethesda, MD: NIAID, 2004.
5 Lubega IR, Fowler MG, Musoke PM, Elbireer A, Bagenda D, Kafulafula G, *et al*. Considerations in using US-based laboratory toxicity tables to evaluate laboratory toxicities among healthy Malawian and Ugandan infants. J Acquir Immune Defic Syndr. 2010;55:58–64.
6 Gunn V, Nechyba C. Harriet Lane Handbook: A Manual for Pediatric House Officers, 16th edn. Philadelphia, PA: Mosby, 2002; pp. 288–95, 549–57.
7 Guay LA, Musoke P, Fleming T, Bagenda D, Allen M, Nakabiito C, *et al*. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. Lancet. 1999;354:795–802.
8 Kumwenda NI, Hoover DR, Mofenson LM, Thigpen MC, Kafulafula G, Li Q, *et al*. Extended antiretroviral prophylaxis to reduce breast-milk HIV-1 transmission. N Engl J Med. 2008;359:119–29.
9 Scott-Emuakpor AB, Okolo AA, Omene JA, Ukpe SI. Normal hematological values of the African neonate. Blut. 1985;51:11–18.
10 Bain BJ. Ethnic and sex differences in the total and differential white cell count and platelet count. J Clin Pathol. 1996;49:664–6.
11 Buchanan AM, Muro FJ, Gratz J, Crump JA, Musyoka AM, Sichangi MW, *et al*. Establishment of haematological and immunological reference values for healthy Tanzanian children in Kilimanjaro Region. Trop Med Int Health. 2010;15:1011–21.
12 Mwinga K, Vermund SH, Chen YQ, Mwatha A, Read JS, Urassa W, *et al*. Selected hematologic and biochemical measurements in African HIV-infected and uninfected pregnant women and their infants: the HIV Prevention Trials Network 024 protocol. BMC Pediatr. 2009;9:49.
13 World Health Organization. Guidelines on HIV and Infant Feeding: Principles and Recommendations for Infant Feeding in the Context of HIV and a Summary of Evidence. Geneva: WHO, 2010.
14 World Health Organization. Antiretroviral Therapy for HIV Infection in Infants and Children: Towards Universal Access. (Recommendations for a Public Health approach: 2010 revision). Geneva: WHO, 2010.
15 Brugnara C. Reference values in infancy and childhood. In: Nathan DG, Orkin SH, Ginsburg D, Look AT, eds. Nathan and Oski's Hematology of Infancy and Childhood, 6th edn. Philadelphia, PA: WB Saunders, 2003; pp. 1835–64.