Published in final edited form as:

Paediatr Int Child Health. 2012 ; 32(4): 213–227. doi:10.1179/2046905512Y.00000000034.

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

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Abstract

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 <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 ~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 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 log₁₀-transformed to account for possible skewing of the data and geometric means (antilog of the mean of the log₁₀-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.

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 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 ($0.74 \text{ cells} \times 10^9/\text{L}$) than in Uganda ($0.10 \text{ cells} \times 10^9/\text{L}$) ($P < 0.05$). 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 two decades (e.g. HIVNET 012⁷ and PEPI⁸). 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 WHO¹⁴ 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.

Acknowledgments

Funding source

The study was supported by the HIV Prevention Trials Network (HPTN) and sponsored by the National Institute of Allergy and Infectious Diseases, National Institute of Child Health and Human Development, National Institute on Drug Abuse, National Institute of Mental Health, and the Office of AIDS Research of the National Institutes of Health, US Department of Health and Human Services under Cooperative Agreement #U01 AI46749 with Family Health International and U01 AI48054. The International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) Network succeeded HPTN and continued funding the studies in Malawi and Uganda. Overall support for IMPAACT was provided by the National Institute of Allergy and Infectious Diseases (NIAID) [U01 AI068632], the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), and the National Institute of Mental Health (NIMH) [AI068632]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. This work was supported by the Statistical and Data Analysis Center at Harvard School of Public Health, under the National Institute of Allergy and Infectious Diseases cooperative agreement #5 U01 AI41110 with the Pediatric AIDS Clinical Trials Group (PACTG) and #1 U01 AI068616 with the IMPAACT Group. Support of the sites was provided by the National Institute of Allergy and Infectious Diseases (NIAID) and the NICHD International and Domestic Pediatric and Maternal HIV Clinical Trials Network funded by NICHD (contract number N01-DK-9-001/HHSN267200800001C).

The authors would like to thank all the mothers and infants who participated in the study, the staff at Mulago National Referral Hospital, Makerere University–Johns Hopkins University Research Collaboration in Uganda, the Chilomoni, Limbe and Ndirande Health Clinics and the Queen Elizabeth Central Hospital in Blantyre Malawi, the National Institute of Allergy and Infectious Diseases, the National Institute of Child Health and Human Development, Family Health International, and the IMPAACT Network.

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Table 1

Characteristics of infants enrolled in Malawi and Uganda

Characteristics	Malawi	Uganda	Total
Age-group, days	<i>n</i>	<i>n</i>	<i>n</i> (% 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.

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: 150-420
	22-30	50	323.9	306.5	237.0-398.0	
	31-35	15	376.4	379.0	304.0-412.0	
	36-56	87	368.5	30.0	277.0-428.0	
ALT (U/L)	57-98	62	351.0	328.5	266.0-403.0	
	99-190	87	314.0	274.0	243.0-373.0	
	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: 13-45
	22-30	54	17.3	14.5	11.0-21.0	
AST (U/L)	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	
	0-1	107	86.0	70.1	54.2-103.0	
	2-7	25	48.5	45.0	32.0-60.0	Newborn: 25-75
	8-14	36	38.5	36.0	29.0-45.5	
AST (U/L)	15-21	27	39.7	35.0	28.0-45.0	
	22-30	54	37.9	32.0	28.0-44.0	Infant: 15-60
	31-35	16	34.1	33.0	29.5-37.5	
	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

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: 13–22
	2–7	18	19.7	18.8	18.0–22.0	
	8–14	34	21.1	21.0	19.3–23.0	
	15–21	25	20.1	21.0	18.0–22.0	Infant/child: 20–28
	22–30	50	20.1	20.1	18.2–22.0	
	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	
Creatinine (μmol/L)	99–190	89	19.0	19.0	17.0–21.3	
	0–1	98	68.6	68.6	61.0–76.3	Newborn: 27–88
	2–7	28	30.5	30.5	30.5–38.1	
	8–14	34	38.1	30.5	30.5–38.1	
	15–21	25	30.5	30.5	22.9–30.5	Infant: 18–35
	22–30	51	22.9	22.9	22.9–30.5	
	31–35	15	30.5	22.9	22.9–30.5	
	36–56	89	30.5	30.5	22.9–30.5	
GGT (U/L)	57–98	63	22.9	22.9	15.3–30.5	
	99–190	91	22.9	22.9	15.3–30.5	
	0–1	108	99.8	92.5	53.6–123.9	0–3 w: 0–130
	2–7	23	130.2	66.0	47.0–137.0	
	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: 4–120
	22–30	53	92.5	71.0	45.0–112.0	
	31–35	16	82.0	76.5	43.0–119.0	
	36–56	91	57.9	47.0	33.0–71.0	>3 m: 5–65
	57–98	63	30.4	27.0	22.0–34.7	
	99–190	90	18.1	16.9	11.1–22.0

Alk phos, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IQR, interquartile range; Ref, reference; BUN, blood urea nitrogen; Uganda: CO₂, carbon dioxide; GGT, gamma-glutamyl transferase;

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	
Eosinophil absolute ($\times 10^9/L$)	57–98	59	0.1	0.1	0.1–0.2	
	99–190	95	0.2	0.1	0.1–0.2	
	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	
Monocyte absolute ($\times 10^9/L$)	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	
	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

Parameter	Age, d	n	Mean	Median	IQR	Ref mean 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)
Haemoglobin (g/L)	99-190	95	0.33	0.33	0.31-0.35	190 d: 0.36 (0.31)
	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	
Lymphocyte absolute ($\times 10^9/L$)	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)
	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

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	
Eosinophil (proportion of 1.0)	99-190	95	0.66	0.66	0.60-0.73	190 d: 0.61
	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; SD, standard deviation

* IQR for reference mean not given

Table 4

Infant cytometry values overall and by site

Parameter	Age, m	n	Mean	Median	IQR
CD4 (cells/ μ l)	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/ μ l)	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