Seasonal and sex-specific variations in haematological parameters in 4 to 5.5-month-old infants in Guinea-Bissau, West Africa

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Objective: This study investigated seasonal and sex-specific variations in the haematological parameters and established reference ranges for these parameters in healthy 4 to 5.5-month-old infants in Guinea-Bissau.

Methods: Within a randomised trial of early measles vaccination, over a period of 13 months blood samples were collected from infants aged 4 to 5.5 months. Haematological parameters were determined by an automated cell counter and compared in linear regression models providing geometric mean ratios (GMR).

Results: Blood samples from 501 infants (n=248 boys, 49.5%) were obtained, and 285 (56.9%) were collected in the rainy season. Median age was 4.7 months (range 3.7 to 7.2 months). Eosinophil and platelet counts were lower in the dry season (December to May) than in the rainy season (GMR 0.79 [95% CI 0.68–0.92]) and 0.93 [0.87–1.00], respectively). The calculated reference ranges were wider and generally higher than those from a US population of comparable age, but neutrophil levels were notably lower in Guinea-Bissau.

Conclusions: The study indicated that eosinophil and platelet counts of infants were subject to seasonal variations. The reference ranges for haematological values were comparable to other African populations and corroborated that neutropenia regularly occurs in African infants.

Keywords: Africa, Differential count, Haematology, Infant, Season, Sex difference

Introduction

Studies from The Gambia have shown that season of birth is associated with higher mortality risk up to 15 years of age,[1] that season of vaccine administration may influence the response to certain vaccines[2] and that there are significant increases in leukocyte counts during the rainy season.[3] A study in Guinea-Bissau has previously found that T-lymphocyte subsets were significantly associated with season, with a positive shift in the CD4+:CD8+ T cell ratio from rainy to dry season, and that CD8+ T cells percentages were lower in girls than boys in 0 to 5-month-old infants.[4] Haematological values of infants have been shown to differ between Europe and Africa[5] and may also differ between the genetically diverse African populations; moreover, haematological values and normal ranges change with age, particularly in early life.[4,6,7] Hence, haematological parameters may be specific to location, season, sex and age. As haematology and biochemistry values are used as diagnostic tools by clinicians and as safety and toxicity indicators in clinical trials, population-specific reference ranges are important.

The objectives of the present study were to investigate seasonal and sex-specific variation in haematological parameters in infants in Guinea-Bissau, West Africa, and to establish reference ranges for the haematological parameters for this population. Furthermore, we compared the calculated reference ranges to those from other African countries and North America.

Methods

Setting and population

The study was carried out at the Bandim Health Project (BHP) in Guinea-Bissau. The BHP maintains a Health and Demographic Surveillance System (HDSS) site covering six suburban districts of the capital Bissau. The area has a population of around 103 000 inhabitants with a mixed composition of the major ethnic groups in Guinea-Bissau. The dry season is commonly defined as December–May and the rainy season as June–November, concurring with observed precipitation during the study period (Supplementary Figure 1). No sample size calculation was done; however, covering a full calendar year we aimed to include as many infants as allowed by the main trial enrolment rates and by that we would obtain a study population of around 600 children, making it one of the bigger studies of its kind.

Inclusion

The present study took place from January 2012 to January 2013 as a sub-group study to a randomised controlled trial of the effects of providing an early measles vaccine on child mortality. Infants between 4 and 7 months of age were eligible for inclusion into the trial if they had received their third Pentavalent vaccine (against Diphtheria, Tetanus, Pertussis, *Haemophilus influenzae* type b and hepatitis B) at least 28 days before enrolment. Mothers or guardians of eligible infants living in the HDSS area were visited at home and invited to participate in the trial. Enrolment took place at the local health centre. Oral and written informed consent to participate in the main trial and the present sub-group study was obtained. Information regarding economic status of the household and health history of the infant was obtained by interview.

A paediatrician examined all infants before enrolment. Infants were excluded if they were overtly sick, had a middle upper arm circumference below 115 mm or had congenital malformations. Mild illness, such as diarrhoea and coughing, were not exclusion criteria but were noted by the doctor.

Before randomisation to early measles vaccine or no early measles vaccine, all infants were bled by a trained paediatric nurse in order to assess maternal measles antibody levels. Additional blood was obtained from infants included in this sub-study for haematological analysis. All blood samples were tested for malaria with a blood-smear and microscopy or rapid antigen-based diagnostic test (First Response Malaria antigen Combo diagnostic test, Premier Medical Corporation, Nani Daman, India). No infants included in the study had malaria parasitaemia.

Analytical procedure

Approximately 0.5 to 1 ml of blood was collected in a 1.2 mL EDTA-coated tube by venepuncture of the dorsal hand or the elbow. If no blood sample could be obtained after three attempts, the child would be excluded from the study. The blood samples were kept cold until analysis at the National Public Health Laboratory in Bissau with an ABX Pentra60 differential counter (Horiba ABX Diagnostics, Montpellier, France) within 4 hours of collection.

The ABX provides a total of 26 parameters. The following outcomes were analysed and presented as they represent a broad haematological picture, are of clinical relevance and commonly used in health monitoring: total white cell, lymphocyte, monocyte, neutrophil, eosinophil, basophil, red blood cell and platelet counts as well as haemoglobin concentration.

The data output was visually inspected for artefacts right after analysis. If a parameter was equal to zero or immeasurable, the blood sample was re-analysed to a maximum of three times. If this did not improve the data output, the child would be censored from the analysis.

Statistical methods

The haematological results were double entered, inconsistencies resolved, and the data merged with the trial database. The baseline characteristics were compared using Student's T-test for normally distributed continuous data, and alternatively Kruskal-Wallis' test for non-normally distributed data, or χ^2 test for categorical data. The haematological data was log-transformed to obtain normal distribution. We used linear regression to obtain estimates for the effect of season (overall and stratified by sex) and the effect of sex. The estimates of the effect of season or sex were presented as geometric means ratios (GMR) with 95% CI, where a GMR>1 can be interpreted as a positive effect of dry season or female sex, respectively. Adjustment for age, middle upper arm circumference and weight at inclusion and number of previous hospitalisations did not change the GMRs in any significant way (data not shown); therefore, all results are presented unadjusted.

The reference 95% ranges were calculated according to guidelines of the Clinical and Laboratory Standards Institute using a non-parametric method. The reference range was defined as the range from the 2.5th to the 97.5th percentile.[8] To determine if mild illness (diarrhoea, coughing or an axillary temperature above 37.5°C) had any effect on the reference ranges we did an additional analysis excluding these infants. This did not change the reference ranges in any significant way. For instance, censoring children with mild illness only changed the range for neutrophil counts from 0.5–4.7 cells/mm^3 to 0.5–4.4 cells/mm^3. Therefore, we calculated the reference ranges on the basis of all infants included.

Ethical considerations

Written informed consent was obtained from parent or guardian of all children included and presented data has been anonymised.

The early measles vaccine trial including the present sub-group study (collection of blood samples and the haematological analyses) was approved by the Guinean Ethical Committee (ref. no. 027/CNES/2011) and the Danish Central Ethical Committee gave its consultative approval (case no. 1106261). The main trial was registered at clinicaltrials.gov (NCT01644721).

Results

A total of 658 infants were invited to participate in this sub-study and haematological data were obtained from 522 infants. In total, test results from 501 infants were available for analysis,

285 in the rainy season and 216 in the dry season; 248 from boys and 253 from girls (Figure 1). Infants included in the dry and rainy season were comparable for all background parameters, except the incidence of diarrhoea; infants included in the rainy season were twice as likely to have diarrhoea compared with their peers in the dry season. Boys were significantly heavier, longer and with a larger middle upper arm circumference compared with girls (Table 1).

The effect of season

In the dry season eosinophil counts (GMR 0.79 [0.68–0.92], p<0.01) and platelet counts (GMR 0.93 [0.87–1.00], p=0.04) were significantly lower than the rainy season (Table 2). Haemoglobin concentration (GMR 1.02 [1.00–1.04], p=0.05) and red blood cell counts (GMR 1.02 [1.00–1.04], p=0.04) were slightly, but significantly elevated in the dry season. The other parameters did not show seasonal variation. Adjusting for diarrhoea did not change the conclusions (data not shown). Censoring children with diarrhoea from the analysis, the power was reduced but the estimates for eosinophils and platelets remained significant (GMR 0.76 [0.65–0.89] and GMR 0.93 [0.87–0.99], respectively), whereas the differences for red blood cell counts and haemoglobin concentration were no longer significant (data not shown).

The effect of sex

The haemoglobin concentration was slightly, but significantly, higher in girls than in boys (GMR 1.02 [1.00–1.04], p=0.01). In contrast, red blood cell counts (GMR 0.98 [0.96–1.00], p=0.04) were slightly, but significantly, lower in girls than in boys. Adjustment for middle upper arm circumference or weight of the children did not have an effect on the differences between the sexes (data not shown).

The effect of season, stratified by sex

The association with season tended to differ by sex. Dry season was associated with a tendency towards lower total leukocytes, lymphocytes, monocytes, neutrophils and basophils in boys, with the opposite tendency in girls. This interaction between season and sex was significant for total leukocyte, monocyte and basophil counts. The eosinophil counts were lower in the dry season than the rainy season in both sexes. There were no sex-differences in the seasonal variation of red blood cells, haemoglobin concentration and platelets.

Reference ranges

Based on our data, reference 95% ranges of haematological parameters for infants from Guinea-Bissau aged 4 to 5.5 months were produced, overall and specific for sex and season, respectively, and compared by sex to those of US children and by season to those of Gambian children (Table 3). In Table 4 the overall reference ranges were compared to the available data from children in other Sub-Saharan African countries.
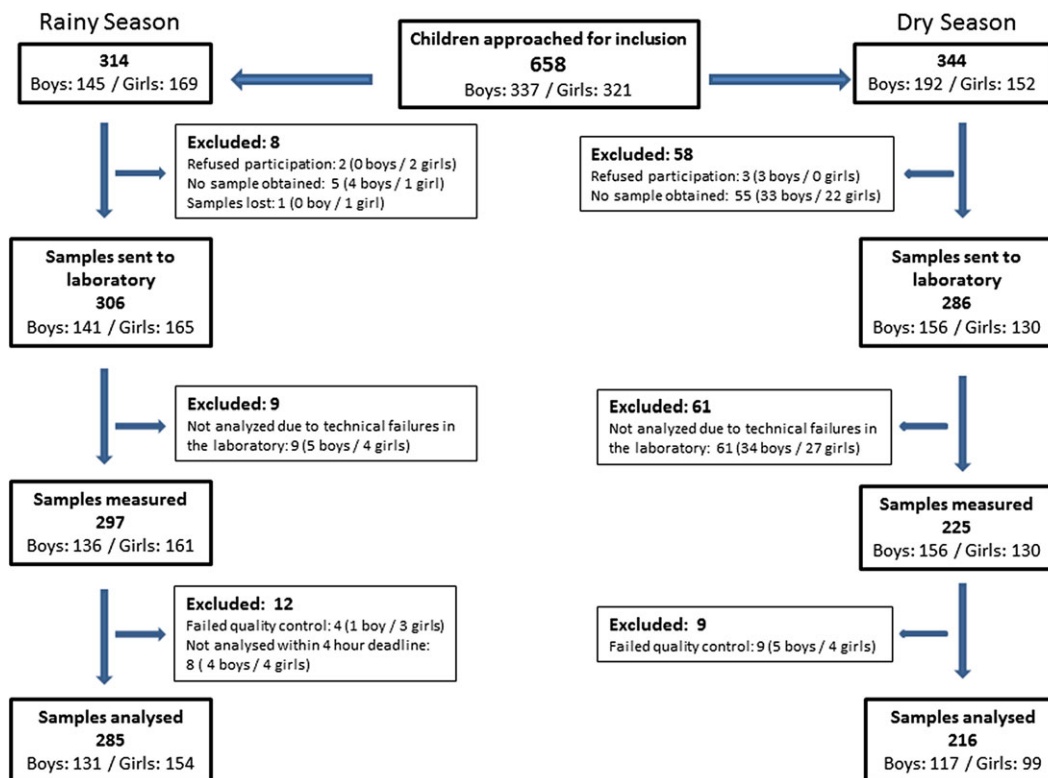

Figure 1. Flow chart of study participants.

Table 1. Characteristics of the study population, overall and stratified by season and sex

	Dry season	Rainy season	p for season	Girls	Boys	p for sex	Total
Total inclusions, n (%):	216 (43.1%)	285 (56.9%)	NA	253 (50.5%)	248 (49.5%)	NA	501 (100%)
Age at inclusion:							
Median age in months (range)	4.7 (3.7–7.2)	4.7 (3.7–7.2)	NS[a]	4.7 (3.7–7.1)	4.7 (8.8–7.2)	NS[a]	4.7 (3.7–7.2)
Anthropometry:							
Mean weight in kg (SD)	7.2 (1.1)	7.1 (0.9)	NS	6.8 (0.9)	7.4 (1.0)	<0.001	7.1 (1)
Mean height in cm (SD)	64 (2.7)	63 (2.4)	NS	63 (2.5)	64 (2.3)	0.006	63 (1)
Median MUAC in mm (range)	142 (116–178)	138 (116–176)	NS[a]	138 (116–176)	142 (116–178)	<0.001[a]	140 (116–178)
Median maternal MUAC in mm (range)	274 (208–396)	274 (196–432)	NS[a]	276 (200–432)	272 (196–398)	NS[a]	274 (196–432)
Morbidity on day of enrolment:							
% with diarrhoea at enrolment (n/N)	6.5% (14/216)	12.3% (35/285)	0.03	8.3% (21/253)	11.3% (28/248)	NS	9.8% (49/501)
% with coughing at enrolment (n/N)	37.0% (80/216)	40.4% (115/285)	NS	34.8% (88/253)	43.2% (107/248)	NS	38.9% (195/501)
Previous health events:							
% with one or more hospital admissions before enrolment (n/N)	3.2% (7/216)	3.9% (11/285)	NS	4.4% (11/253)	2.8% (7/248)	NS	3.8% (19/501)
% received oral polio vaccine at birth (n/N)	86.6% (187/216)	88.1% (251/285)	NS	89.3% (226/253)	85.5% (212/248)	NS	87.4% (438/501)
% received BCG vaccine at birth (n/N)	100% (216/216)	99.3% (283/285)	NS	99.2% (251/253)	100% (248/248)	NS	99.6% (499/501)
Socio economic indicators:							
% with electricity installed (n/N)	51.9% (112/216)	47.7% (136/285)	NS	48.2% (122/253)	50.8% (126/248)	NS	49.5% (248/501)
% with functioning electricity, if electricity installed (n/N)	65.2% (73/112)	72.1% (98/136)	NS	72.1% (88/122)	65.9% (83/126)	NS	68.9% (171/248)

MUAC: mid-upper arm circumference; NA: not applicable; NS: not significant.

[a] Truncated continuous data were analysed by Kruskall Wallis' test, normally distributed continuous data by Students T-test or categorical data by χ^2 test.

Discussion

Main findings

The eosinophil counts showed the most significant seasonal variation, being lower in the dry season than in the rainy season. This association was separately significant in girls, but not in boys. Moreover, the study indicated a sex-differential association with season for white blood cells, most pronounced for total leukocyte, monocyte and basophil counts. The levels of these cell types were slightly lower in the dry season than the rainy season in boys, but tended to be higher in the dry season in girls. Finally, independent of season, the study indicated significant sex-differences for haemoglobin concentration and red blood cell counts, although of a lesser magnitude. Compared with US infants of comparable age, the reference ranges from Guinea-Bissau generally showed wider intervals, with higher upper limits for most cell types, though not for neutrophils which were much lower in Guinea-Bissau, a tendency also seen in other African children.

Strengths and weaknesses

The ABX Pentra60 technology used for our haematological analysis has previously been shown to deliver precise measurements.[9] The automated cell counter was calibrated using a control blood sample (Horiba ABX Diagnostics) to ensure correct measurements.

Due to temporary technical difficulties in January to March 2012, we were not able to analyse as many samples as anticipated during the dry season, resulting in a higher number of samples from the rainy season. This impaired the power of our statistical analyses, and may have attenuated potential signals of seasonal variation in this particular period. However, the

Table 2. Haematological parameters by season and sex

	All–season	All–sex	Boys only	Girls only	
	Dry season vs rainy season GMR (95% CI)	Girls vs boys GMR (95% CI)	Dry season vs rainy season GMR (95% CI)	Dry season vs rainy season GMR (95% CI)	p for interaction between season and sex
Total leukocytes	0.99 (0.94–1.04)	0.99 (0.94–1.04)	0.93 (0.87–1.01)	1.04 (0.97–1.12)	0.04
Lymphocytes	1.00 (0.94–1.06)	0.98 (0.93–1.05)	0.96 (0.88–1.05)	1.03 (0.95–1.13)	NS
Monocytes	1.00 (0.94–1.06)	0.95 (0.89–1.01)	0.92 (0.84–1.01)	1.07 (0.98–1.17)	0.02
Neutrophils	0.98 (0.88–1.09)	1.03 (0.93–1.15)	0.89 (0.76–1.03)	1.09 (0.93–1.27)	NS
Eosinophils	0.79 (0.68–0.92)[a]	0.99 (0.85–1.15)	0.88 (0.71–1.09)	0.70 (0.56–0.87)[a]	NS
Basophils	1.03 (0.94–1.12)	1.04 (0.95–1.13)	0.94 (0.82–1.07)	1.13 (1.00–1.28)[a]	0.05
Red blood cells	1.02 (1.00–1.04)[a]	0.98 (0.96–1.00)[a]	1.01 (0.99–1.04)	1.02 (1.00–1.05)	NS
Haemoglobin concentration	1.02 (1.00–1.04)[a]	1.02 (1.00–1.04)[a]	1.02 (1.00–1.05)	1.02 (0.99–1.05)	NS
Platelets	0.93 (0.87–1.00)[a]	0.98 (0.91–1.04)	0.94 (0.85–1.03)	0.92 (0.84–1.01)	NS

GMR: geometric mean ratios; NS: not significant.
Estimates for the differences by season or sex presented as GMR with 95% CI; a GMR>1 indicates a higher level in dry season than in rainy season or a higher level in girls than in boys.
[a] Significant estimates (p<0.05).

Table 3. Reference 95% ranges of haematological parameters in Guinean infants, overall and stratified by season and sex, compared with United States and Gambian infants

	Guinea-Bissau					United States		Gambia	
	Aged 110–220 days					Aged 61–180 days[18]		Aged 12–23 months[7]	
	All	Dry season	Rainy season	Boys	Girls	Boys	Girls	Dry season	Rainy season
Total leukocytes (10^3/mm^3)	5.4–18.7	5.3–19.2	5.3–17.3	5.2–19.5	5.4–18.1	7.9–13.4	6.9–12.8	boys: 6.0–15.7 girls: 6.2–16.9	boys: 6.4–20.8 girls: 6.5–19.2
Lymphocytes (10^3/mm^3)	2.6–12.0	2.3–12.1	2.9–11.9	2.6–12.0	2.5–11.9	2.3–5.5	1.9–5.4	3.0–10.5	3.5–13.5
Monocytes (10^3/mm^3)	0.7–3.0	0.7–3.1	0.7–2.8	0.7–3.2	0.7–2.9	0.3–1.1	0.2–1.2	0.10–1.37	0.09–1.46
Neutrophils (10^3/mm^3)	0.5–4.7	0.5–4.8	0.4–4.6	0.4–4.8	0.5–4.3	2.6–7.5	2.2–7.1	boys: 1.4–6.2 girls: 1.3–6.8	boys: 1.3–8.5 girls: 1.4–7.9
Eosinophils (10^3/mm^3)	0.06–1.44	0.06–1.48	0.07–1.42	0.08–1.38	0.06–1.47	0.05–0.36	0.01–0.28	0.05–1.31	0.05–1.31
Basophils (10^3/mm^3)	0.06–0.44	0.06–0.57	0.05–0.39	0.05–0.54	0.06–0.46	0.01–0.06	0.01–0.07	NA	NA
Red blood cells (10^6/mm^3)	3.7–5.6	3.7–5.7	3.6–5.6	3.6–5.6	3.7–5.7	3.7–4.6	3.6–4.6	NA	NA
Hemoglobin (g/dL)	9.0–13.4	9.0–13.7	8.8–13.4	8.8–13.4	9.2–13.4	9.6–12.4	9.9–12.4	6.8–12.7	6.9–12.0
Platelets (10^3/mm^3)	176–788	159–782	189–853	185–783	158–800	215–448	147–423	146–886	163–922

NA: not available.
Reference ranges based on number of participants: Guinea-Bissau: 501 (dry season: 216; rainy season: 285); United States: Boys ranging 511 to 1197; girls ranging from 511 to 1113; Gambia: 346.

reduced enrolment was non-selective in respect to the infants, hence, it should not bias the results. To establish whether the observed seasonal association was indeed a true phenomenon, enrolments should have lasted more than a single year. The strength of the study is the homogeneity of the cohort in respect to age, geographical location, vaccinations and socioeconomic background. However, this may also constrain the relevance of the reference ranges for use in other populations.

The analysis of nine outcome parameters stratified by sex and season entails a risk of mass significance. The estimates were not adjusted for multiple testing and should, therefore, be interpreted with appropriate caution.

Table 4. Reference 95% ranges of haematological parameters from different African sites

	Guinea-Bissau (2012) (n=501) Age: 4–5.5 months Environment: urban Analysis: venous blood, automated cell counter	Gabon (2006–2008)[5] (n=267) Age: 1–2 months Environment: not described Analysis: venous blood, automated cell counter	Mozambique (2003)[6] (n=NA) Age: 12–24 months Environment: mostly rural Analysis: capillary blood, automated cell counter	The Gambia (2006–2010)[19] (n=458) Age: 2–5 months Environment: peri-urban Analysis: venous blood, automated cell counter
Total leukocytes ($\times10^3$/mm^3)	5.4–18.7	5.8–13.5	6.6–16.6	5.6–15.4
Lymphocytes ($\times10^3$/mm^3)	2.6–12.0	3.4–8.7	NA	NA
Monocytes ($\times10^3$/mm^3)	0.7–3.0	0.7–2.5	NA	NA
Neutrophils ($\times10^3$/mm^3)	0.5–4.7	0.5–3.5	NA	NA
Eosinophils ($\times10^3$/mm^3)	0.06–1.44	0.07–0.58	NA	NA
Basophils ($\times10^3$/mm^3)	0.06–0.44	0.07–0.37	NA	NA
Red blood cells ($\times10^6$/mm^3)	3.7–5.6	2.9–4.8	NA	NA
Hemoglobin (g/dL)	9.0–13.4	8.4–12.8	6.8–12.4	Boys: 9.0–12.7 Girls: 9.3–12.9
Platelets ($\times10^3$/mm^3)	176–788	143–638	133–750	136–709

NA: not available.

The effect of season

Our findings of higher platelet counts and lower haemoglobin concentration in the rainy season corroborate a recent study in 12 to 23-month-old Gambian children, whereas contrastingly the Gambian study also found higher total leukocyte, monocyte and neutrophil counts in the rainy season.[7] Higher total leukocyte counts were also found in another Gambian study of infants below one year of age.[3] Of note, the latter Gambian study found that besides rainy season sampling, rainy season births were positively associated with total leukocyte and lymphocyte counts, which remained significant at later ages in infancy.[3]

In Bissau infants, eosinophil counts were higher in the rainy season. Elevated eosinophil counts may be caused by parasitic infections, of which some show seasonal fluctuations in low-income tropical sites.[10] One such parasite is *Cryptosporidium parvum*, which is prevalent in Guinea-Bissau in the early rainy season,[11] and which has been shown to also infect young children.[10] However, much more common causes of diarrhoea in young children in Guinea-Bissau are rotavirus and *Escherichia coli*,[12] which should not cause eosinophilia. Notably, the association between season and eosinophilia remained after excluding children with diarrhoea, indicating that parasite infection or diarrhoea of which both may be more prevalent in the rainy season did not explain the higher eosinophil counts in the rainy season. Since symptomatic illness showed seasonal variation, one could speculate if sub-clinical illness, also varied with the season, and hence may have contributed to the observed seasonal variation sustaining after correcting for symptomatic illness, but this remains speculative. We found significantly higher haemoglobin concentration and red blood cell counts in the dry season. Since the differences are minor, these findings may be statistically but not necessarily biologically significant. Platelet counts were found to be higher in the rainy season. This could be an example of reactive thrombocytosis, most likely caused by previous infections.[13]

Effect of sex

Leukocyte counts and leukocyte subset counts were not different in boys and girls, contrasting findings in Gambian children aged 1–5 years, where total leukocytes and neutrophils were higher in girls than in boys.[7] Haemoglobin concentration was slightly but significantly higher in girls than in boys, whereas red blood cell counts were slightly lower in girls than in boys. Seemingly at odds with each other, these associations may be chance findings. Other studies including a wider age group found no sex differences in haematological parameters before age 10–12 years.[14,15] After that age haemoglobin concentration and red blood cell counts are found to be higher in boys than in girls.[16,17]

Reference ranges

The sex-specific reference ranges from Guinea-Bissau were compared with published reference ranges of US infant boys and girls, aged 61 to 180 days, taken from emergency room patients in Washington DC, and excluding haematological and oncological cases.[18] For boys and girls, total leukocytes and white blood cell differential counts were more variable and tended to be skewed upwards compared with US infants, except for the neutrophil counts, which were lower, as was the haemoglobin concentration. Platelet counts were also more variable and generally higher in Guinea-Bissau compared with the US.

In our review of the literature we identified four other studies reporting haematological reference ranges from sub-Saharan Africa, namely from Gabon,[5] The Gambia[7,19] and Mozambique.[6] These studies were of comparable design, although some heterogeneity in analytical procedure, environment and age were noted (Table 4). Where available, these African studies also reported wider ranges for total leukocyte counts and narrower and lower neutrophil counts compared with the US infants. Except the Gambian study in >1 year old infants, the lower limit of the neutrophil range was so low that it indicated that several children had severe neutropenia. It is well established that adult Africans and Americans of African descent have lower neutrophil counts than Caucasians,[20–22] which has also been reported in African infants,[23] and a reference range similar to the present was also seen in the study from Gabon,[5] but lower than that of The Gambia.[7] A study from Malawi found that 10% of children below 18 weeks had severe neutropenia, defined as less than 0.75×10^3 cells/mm^3. In all cases the neutropenia was resolved by 10 months of age and no children devolved severe infections.[23] These findings were also seen in a study from Zimbabwe.[24] Thus, severe neutropenia seems to occur regularly in African infants.

Compared to Guinea-Bissau, the studies from Gabon and Mozambique reported slightly lower ranges for haemoglobin concentration, whereas the ranges in Gambian 2 to 5-month-old infants were closer to the ranges from Guinea-Bissau. Only from Gabon were red blood cell counts reported, and the range was skewed slightly downwards compared with Guinea-Bissau. For platelet counts, wider reference ranges with higher upper limits were also seen in all the African sites compared with the US infants; the platelet range from Guinea-Bissau was slightly higher than the other African studies, but in Gambian children >1 year old.

It should be noted that total leukocyte counts and haemoglobin concentration may be higher in capillary blood than in venous blood.[25–27] All studies but one (Mozambique) used venous blood; indeed, the leukocyte lower limit was slightly higher in the Mozambique study compared with the other African studies.

Conclusions

In conclusion, the present study suggests seasonal variation of several haematological parameters in infants in Guinea-Bissau, some being sex specific. The obtained reference ranges were similar to those found in other African studies and differed from those obtained in a US population. Neutrophil counts were remarkably low, as also seen in other African infant populations.

Supplementary data

Supplementary data are available at Transactions online (http://trstmh.oxfordjournals.org/).

Authors' contributions: OB oversaw data collection and inclusion, performed laboratory procedures, performed the statistical analysis and wrote first draft of the manuscript. KJJ participated in the design of the study, supervised laboratory procedures, supervised statistical analysis and contributed to writing the final draft. AA supervised statistical analysis and contributed to writing the final draft. CB performed inclusion examination and supervised the inclusion process. CM was principal investigator of the main trial to this sub study and supervised inclusion procedures. SBS supervised data collection and inclusion procedure. AP contributed to writing the final draft. CSB participated in the design of the study, supervised statistical analysis and contributed to writing the final draft. All authors read and approved the final manuscript. CBS is guarantor of the paper.

Competing interests: None declared.

Ethical approval: The early measles vaccine trial including the present sub-group study (collection of blood samples and the haematological analyses) was approved by the Guinean Ethical Committee (ref. no. 027/CNES/2011) and the Danish Central Ethical Committee gave its consultative approval (case no. 1106261). The main trial was registered at clinicaltrials.gov (NCT01644721).

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