

**Severe malnutrition with and without HIV-1 infection in hospitalised children in
Kampala, Uganda: differences in clinical features, haematological findings and CD4⁺
cell count.**

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Abstract

Background: Whereas the interaction between HIV infection and malnutrition has been extensively documented there is paucity of data on haematological indices and lymphocyte subsets of severely malnourished children in relation to HIV infection. The aim of the study was to describe clinical features of severely malnourished children, haematological, CD4⁺ and CD8⁺ cell counts in relation to HIV/AIDS infection. **Methods:** Study setting was Paediatric wards of Mulago hospital, Uganda's national referral and teaching hospital. A repeat cross sectional study design. This paper reports on 315 severely malnourished children (weight-for-height < -3 z-score or presence of oedema) studied. At admission, CD4⁺ and CD8⁺ cells were measured (FACScan) and HIV serology was confirmed (by ELISA) for those > 18 months, and RNA PCR for those ≤ 18 months. Complete blood count, including differential counts was done using a Beckman Coulter counter. **Results:** Of the 315 children, 119 (38%) were female; median age 17 months (IQR 12- 24); with no difference in HIV status by sex or age. The children had a high prevalence of infections: pneumonia (68%), diarrhoea (38%), urinary tract infection (26%) and bacteraemia (18%), with no significant difference by HIV status (HIV infected vs. HIV uninfected). However, HIV infected children were more likely as compared with HIV uninfected severely malnourished children to have persistent diarrhoea, odds ratio (OR 2.0, 95% CI 1.2-3.6). The median white blood cell count (8700 vs 10700); and lymphocyte count (2687 vs. 4033), were significantly lower in HIV positive than HIV negative children. Severely wasted (marasmus) children were more likely to have CD4⁺ percentages <15% (RR 4.2, 95% CI 1.4-13) and <20% (RR 3.1, 95% CI 1.5-6.5) when compared with children with oedematous malnutrition (kwashiorkor and marasmic-kwashiorkor) even after controlling for HIV infection. **Conclusion:** Severe protein energy malnutrition in children is associated with a depletion of haematological and lymphocyte subsets which is exacerbated by the presence of HIV-1 infection.

Background

Severe malnutrition has been associated with acquired immunodeficiency among children worldwide referred to as Nutritionally Acquired Immunodeficiency Syndrome or NAIDS [1, 2]. With the advent of the human immunodeficiency virus (HIV) pandemic, the role of malnutrition in immunodeficiency has often been overlooked, and indeed only a handful of studies have investigated CD4⁺ and CD8⁺ lymphocyte subsets in severely malnourished children [3, 4].

There is very little information on the effect of the added burden of HIV/AIDS infection on the clinical features [5-7] and cellular immunity of severely malnourished children. The objective of this study was to describe the clinical features, haematological, CD4⁺ and CD8⁺ lymphocyte subsets of severely malnourished children by HIV status.

Methods

Subjects

This was a repeat cross sectional study. All severely malnourished children consecutively admitted to the paediatric wards of Mulago, Uganda's national referral and teaching hospital during two periods, September –November 2003 and September – December 2004 were followed up from admission to outcome (death or discharge). Four hundred and fifty severely malnourished (weight-for-height of < -3 z- score and/or presence of oedema) below 60 months of age, whose parents or caregivers gave informed consent, were included in the study.

This paper reports on the 315 children with complete HIV test results, CD4, CD8 count and percentage results. The laboratory work-up was incomplete for 135 children due to lack of reagents (89), inadequate blood volume (38), haemolysis (6) and not bled (2).

We recorded demographic characteristics (age, sex) and clinical features (presence of oedema, weight, height/length, diarrhoea), haematological tests (HB, WBC and differentials, presence of malaria parasites), HIV tests (ELISA and PCR), microbial tests (blood and urine culture and sensitivity), immunologic tests (CD4⁺ and CD8⁺ counts and percentages) and chest x-ray reports of the children. CD4⁺ and CD8⁺ cells were measured (FACScan) and HIV serology was confirmed (by ELISA) for those > 18 months, and RNA PCR for those ≤ 18 months. Complete blood count, including differential counts was done using a Beckman Coulter counter [8].

HIV status was defined as positive when the standard HIV algorithm of two enzyme-linked immunoassays (EIA) gave positive result for the HIV-1 virus for children above 18 months and when the Western blot, Realtime polymerase chain reaction (RT-PCR) test result was positive for HIV-1 virus for children below 18 months. HIV status negative was defined as negative results of the EIA and Western blot tests. We used CD4 percentage to categorize HIV/AIDS but not clinical definition as all of them were severely malnourished.

Severe malnutrition was defined according to the WHO classification, oedematous malnutrition (presence of symmetrical oedema involving at least the feet) and severe wasting (weight for height < 3 SD of the NCHS/WHO reference values with no oedema) [9].

The children were divided in 2 groups according their HIV test results into HIV positive and HIV negative

The study protocol was approved by the Regional Committee for Medical Ethics, Bergen, Norway (REK Vest), Makerere University Faculty of Medicine Ethics and Research Committee, Mulago Hospital Ethics Committee and the Uganda National Council for Science and Technology.

Statistical analysis was done using SPSS version 11.5. Medians were used to measure central tendency and inter quartile range (IQR) for the spread of haemoglobin concentration, leucocytes (WBC), total lymphocytes, CD4⁺ and CD8⁺ cell counts. Children were grouped by their gender (male, female), age groups in months (≤ 24 months and > 24 months), presence of oedematous malnutrition and HIV infection, CD4⁺ levels (CD4⁺-cell % $< 20\%$, and CD4⁺-cell % $< 15\%$). Chi square and Wilcoxon-Mann-Whitney tests and multivariate analysis were used to test for differences by HIV status and gender and type of severe malnutrition (oedematous vs. severe wasting) A 2- tailed p-value of < 0.05 was considered significant.

Results

Of the 315 children, 119 (38%) were female and the median age was 17.0 months (IQR 12 – 24). One hundred and seventy (45%) had oedematous malnutrition (kwashiorkor and marasmic-kwashiorkor), and 123 (39%) were HIV infected. The HIV uninfected children were more likely to present with oedema (OR 2.2 (95% CI 1.4 – 3.4)). Only 27 (9%) of the severely malnourished children had no identifiable infection on admission, 51 (16%) had one type of infection and the majority, 227 (72 %) had more than one type of infection on admission. The infections included pneumonia (68%), diarrhoea (38%), urinary tract infection (26%), bacteraemia (18%), malaria (9%) and oral thrush (11%), (Table 1). Overall, there was no significant difference in the prevalence of infection by HIV status. However, HIV-1 infected children were more likely to have persistent diarrhoea and oral thrush (Table 1).

The median haemoglobin concentration of the severely malnourished children was below 9 g/dL, which is, by WHO criteria, moderate anaemia they also define severe anaemia as < 5 g/dL and very severe anaemia as < 4mg/dL. There was no significant difference in levels of haemoglobin concentration by type of severe malnutrition or HIV status, (Table 2).

The total white blood cell count was significantly lower in HIV positive than HIV negative children 8.9×10^6 (IQR 5.4 –11.3) versus 9.1×10^6 (7.2 –13.5), ($p=0.028$). The HIV positive children with severe wasting had lower total white blood cell count compared to those with oedema, (Table 2). This difference was not observed in the HIV negative children.

The total lymphocyte counts were 2.9×10^9 (IQR 2.0 – 4.9) for HIV positive and 4.5×10^9 (IQR 2.9 – 6.3) for HIV negative children, ($p=0.008$). Absolute lymphocyte counts were 2.7×10^9 (IQR 1.8 – 4.9) for HIV infected and 4.0×10^9 (2.8 – 5.6) for HIV uninfected children,

($p < 0.001$). HIV infected children with severe wasting (marasmus) had lower total lymphocyte, monocyte, and neutrophil counts than those with oedema. This was not observed amongst the HIV negative children.

The median (IQR) CD4⁺ and CD8⁺ cell counts for HIV positive children were 497 (280 – 1379) and 880 (490 – 1750), and for HIV negative children, 1265 (829 – 1758) and 588 (331 – 913), respectively. Similarly, the median (IQR) CD4⁺ and CD8⁺ cell percentages for HIV infected children were 18% (12% – 34%) and 31% (23% – 50%) and for HIV uninfected children, 33% (26% – 40%) and 15% (13% – 21%), respectively. The differences by HIV status were both significant, $p < 0.001$. Regardless of their HIV status, children with severe wasting (marasmus) had significantly lower CD4⁺ count, CD4⁺ and CD8⁺ percentages and CD4⁺ / CD8⁺ ratios than those with oedematous malnutrition (kwashiorkor and marasmic-kwashiorkor), (Table 2, Figure 1)

One third of the severely malnourished children had low CD4⁺ percentages of less than 20%. Moreover, 17% of them had very low CD4⁺ cell percentage of less than 15%. Fifty five out of the 315 children (17%) had CD4⁺ percentage below 15% while a similar number had CD4⁺ percentage between 15 – 24 %, table 3. Of these, 22% of children who had CD4⁺ percentage < 15% and 35% of children with CD4⁺ cell percentage between 15 – 24 % were not infected with the HIV-1 virus. Children with marasmus were more likely to have CD4⁺ percentages below 15%, (OR 4.2, CI 1.4-12.6); and between 15 – 25 %, (OR 2.0, CI 0.6 – 6.8) than children with oedema. This difference persisted even after controlling for HIV status, (table 2).

Discussion

In this paper we describe the clinical features/diagnoses, haematological, CD4⁺ and CD8⁺ cell counts and percentages of severely malnourished children by HIV status. All children had a high prevalence of multiple infections including pneumonia, diarrhoea, bacteraemia, malaria, urinary tract infection and oral thrush. The proportion and types of infections did not differ significantly by HIV status in this study. Apart from persistent diarrhoea and oral thrush, no significant difference was observed in the proportions of severely malnourished children with respiratory infections, blood stream infections or urinary tract infections by HIV status.

The relationship between infection, malnutrition, and HIV/AIDS has been previously described [10-13]. Severe wasting in the absence of oedema and persistent diarrhoea have been reported as features commonly observed in severe malnutrition with concurrent HIV infection [6, 11, 13-15]. In this study, only severe wasting was significantly associated with HIV infection after a multivariate analysis (OR 2.3, 95% CI 2.2- 4.3, p= 0.01). The similarity in clinical features of severe malnutrition and HIV/AIDS affects accurate clinical diagnosis of HIV infection in developing countries where there is co-existence of the two diseases with inadequate HIV testing facilities [16].

Depletion of haematological indices other than the haemoglobin concentration was more marked in children who were infected with the HIV 1 virus. These significant differences were not observed in the HIV negative group. The reason for this is not clear but a recent study reported 5 malnourished children with mixed infection who had a higher monocyte count than the 4 with only respiratory infection although their HIV status was not reported [3]. Alterations in haematological functions in malnutrition been documented [17]. Both

granulocyte and lymphocyte suppression is an indication of reduced haemopoietic function and as observed in this study, the additional burden of HIV-1 infection seems to further reduce this function.

The median CD4⁺ cell counts and percentages were lower than the recently published median CD4⁺ count and percentage of healthy Ugandan children younger than five years [18].

Surprisingly, this included children who had tested negative for HIV-1. Extremely low levels of CD4⁺ cell percentages consistent with a laboratory diagnosis of AIDS have rarely been described in HIV uninfected children with or without mixed infections. Reports on proportions of T cells % and CD4⁺ cell percentage in severely malnourished children are inconsistent [3, 4, 19, 20]. The difference observed may be influenced by difference in study designs and sample size.

CD4⁺ cells percentages in this study were lower in children who presented with severe wasting (marasmus) and remained consistent in both samples and in the HIV infected and non infected groups. Earlier studies reported that oedematous malnutrition had lower T cells [19], [21], while others found no difference by type of malnutrition [4]. The reason for these controversies is not clear. All we know is that severe malnutrition alters immunological competence through a number of mechanisms including apoptosis of the thymus gland [22, 23] and micronutrient deficiencies[24].

Conclusion

Severe protein energy malnutrition is associated with depletion of haematological and lymphocyte subsets exacerbated by the presence of HIV 1 infection. Cell mediated immunosuppression is more marked in non-oedematous severe malnutrition regardless of HIV status.

Authors' contributions

All authors participated in the design of the study, interpretation of the results, statistical analysis and writing the manuscript. HB supervised patient recruitment, follow-up and data collection. All authors read and approved the final manuscript.

Competing interests

We declare that we have no competing interests.

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Legend

Figure 1. Box and whisker plot showing the median, interquartile and range of CD4⁺ percentage of lymphocytes of severely malnourished children by HIV status and type of malnutrition

Table 1: Characteristics, medical conditions and diagnosis of children below 60 months of age with severe malnutrition, Mulago hospital, Uganda

	HIV/AIDS		Odds ratio (95% CI)
	Infected	uninfected	
	n (%)	n (%)	
Symptoms and signs	<i>(n=123)</i>	<i>(n=192)</i>	
Diarrhoea (All)	52 (42)	67 (35)	1.4 (0.9 – 2.2)
Persistent diarrhoea (> 2weeks)	32 (26)	28 (15)	2.1 (1.1 – 3.8)
Oral thrush	20 (37)	15 (8)	2.3 (1.1 – 4.7)
Bilateral Oedema (nutritional)	53 (43)	119 (62)	0.5 (0.3 – 0.7)
Severe dehydration	7 (5.7)	11 (5.7)	1.0 (0.4 – 2.7)
Chest x ray findings	<i>(n=109)</i>	<i>(n=158)</i>	
Bronchopneumonia	26 (24)	48 (30)	0.7 (0.4 – 1.3)
Interstitial Pneumonia	40 (37)	48 (30)	1.3 (0.8 – 2.2)
Suspected tuberculosis	14 (13)	18 (11)	1.2 (0.5 – 2.4)
Blood tests	<i>(n=122)</i>	<i>(n=191)</i>	
Malaria parasites	10 (9)	19 (11)	0.9 (0.4 – 1.9)
Severe anaemia, (Hb<5 g/dL)	10 (8)	2 (6)	1.3 (0.6 – 3.3)
Bacteraemia	24 (20)	32 (17)	1.2 (0.7 – 2.2)
Urine tests	<i>(n=10)</i>	<i>(n=160)</i>	
Bacteruria	33 (30)	36 (23)	1.5 (0.9 – 2.6)

Table 2. The medians and interquartile ranges of haemoglobin levels, white blood counts and differentials, CD4⁺ and CD8⁺ cell counts , percentages and ratios of all the 315 severely malnourished children below 60 months of age, by their HIV status and type of malnutrition

	HIV/AIDS			
	Infected		Uninfected	
	Oedema n = 53 median (IQR)	No oedema n = 70 median (IQR)	Oedema n =119 median (IQR)	No oedema n =73 median (IQR)
Haemoglobin g/dL	8.2 (6.4–9.6)	7.3 (6–9.1)	8.0 (6.1–9.3)	8.4 (6.7–9.8)
Total WBC (10 ⁹ /L)	11.0 (8.3–17)	7.2 (4.2–12)	10.0 (7.7–17)	11.0 (8.8–15)
Neutrophils (10 ⁹ /L)	6.2 (3.1–8.5) *	2.9 (2.3–7.7)	5.4 (3.5–8.8)	6.1 (3.2–9.0)
Neutrophils (%)	59.0 (34–70)	61.0 (37–73)	55.0 (48–66)	53.0 (38–63)
Monocytes (10 ⁹ /L)	667.0 (182–1246) *	153.0 (83–263)	217.0 (107–540)	412.0 (176–534)
Monocytes (%)	5.7 (2–10)*	2.0 (1.0–2.8)	2.0 (1–5)	3.5 (2–6.5)
Total Lymphocyte (10 ⁹ /L)	3.3 (2.4–6.3)	2.5 (1.7–4.1)*	4.5 (2.6–7.2)	4.4 (3.6–5.7)
Lymphocytes (%)	39.0 (23–57)	36.0 (26–50)	39.0 (31–49)	42.0 (32–55)
CD4 ⁺ cell count	630.0 (305–1759)***	379.0 (123–713)	1354 (894–1914)***	1169 (682–1600)
CD4 ⁺ cell %	20.0 (14–42)***	14.0 (5–25)	35.0 (29–44)***	27.0 (22–37)
CD8 ⁺ cell count	1046 (521–1896)	811.0(462–1363)	822.0 (492–1367)*	595 (328–1054)
CD8 ⁺ cell %	23.0 (20–39)*	41.0 (27–56)	15.0 (12 –21)	16.0 (13–21)
CD4 ⁺ / CD8 ⁺ ratio	0.9 (0.4 –1.6)***	0.4 (0.1–0.9)	2.2 (1.6–3.0)	1.9 (1.2 –2.8)

* p- value < 0.05 ** p-value <0.005 *** p-value < 0.001

Table3. Distribution of the 314 severely malnourished children by type of severe malnutrition their cellular Immunological category and HIV status.

	HIV/AIDS	
	Infected n=53	Uninfected n=119
Oedema	n (%)	n (%)
CD4 ⁺ ≥ 25%*	28 (53)	110 (93)
CD4 ⁺ 15 – 24%**	15 (28)	5 (4)
CD4 ⁺ < 15%***	10 (19)	4 (3)
No oedema	n=71	n=71
CD4 ⁺ ≥ 25%	16 (22)	49 (69)
CD4 ⁺ 15 – 24%	22 (31)	15 (21)
CD4 ⁺ < 15%	33 (47)	7 (10)

*No evidence of suppression, ** Evidence of moderate suppression, *** Severe suppression

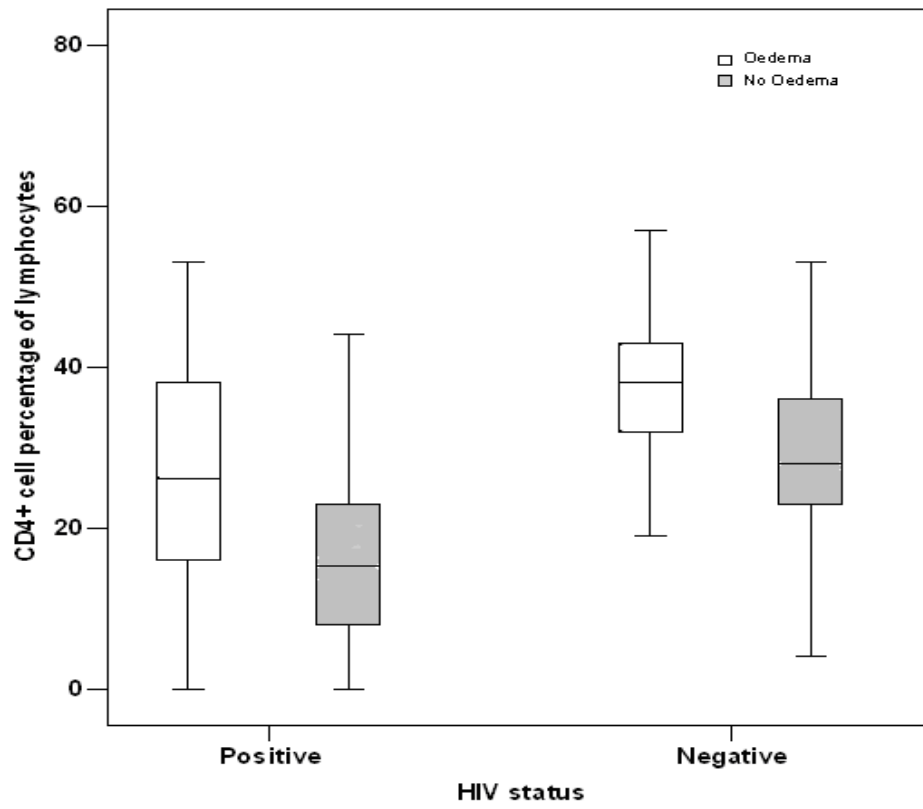


Figure 1