Aetiology, Pathogenesis & Consequences of Severe Anaemia in Malawian Children : HIV and other factors

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Chapter 4

Long term outcome of severe anaemia in Malawian children

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Submitted

ABSTRACT

Background

Severe anaemia is a common, frequently fatal, syndrome in African children admitted to hospital, but its long term outcome is unknown. Early reports that survivors may be at risk of additional late morbidity and mortality may have significant implications for child survival in Africa. We assessed the short and long term outcome of severe anaemia in Malawian children and identified potential risk factors.

Methods and Findings

We conducted a prospective case-control study of children presenting to hospital with severe anaemia (haemoglobin $\leq$5g/dl) and their (hospital and community) controls. All children were aged between 6 and 60 months and were followed up for 18 months to assess the incidence of (in-hospital and post-discharge) mortality and further severe anaemia.

A total of 377 *cases*, 377 hospital controls and 380 community controls were recruited. The in-hospital mortality was 6.4% among the cases. Mortality in the 18-month follow-up period among cases was 12.6%, significantly greater than in hospital controls (2.9%) or community controls (1.4%)($p<0.001$). HIV was the most important risk factor for mortality (HR 10.5, 95% CI 4.0-27.2). The incidence of severe anaemia during the follow-up period among the cases was 80 per 100 person-years (95% CI 57-113), significantly higher than the 5 per 100 person-years (95% CI 2-11) in the combined controls ($p<0.001$).

Conclusions

Severe anaemia carries a high 'hidden' morbidity and mortality occurring in the months after initial diagnosis and treatment. Because severe anaemia is very common, this is likely to contribute importantly to overall under-five mortality. If not adequately addressed, severe anaemia may be an obstacle to achievement of the fourth millennium development goal on child survival. Strategies to diagnose and properly treat HIV-infected children early may reduce the high post-discharge mortality in severe anaemia.

INTRODUCTION

Of children admitted to hospital in Africa, 12-29% have severe anaemia requiring a blood transfusion[1,2,3]. In-hospital mortality in this group is commonly between 4% and 10%[1,2,3,4].

Investigators in Kenya reported unexpectedly high post-discharge mortality and recurrent severe anaemia rates in children within two months of a severe anaemia episode[3]. There has been no subsequent attempt to confirm or investigate these findings. An excess risk of death in this large group could contribute an important, and potentially correctable, component to the high mortality rate among young children in Africa.

In this study our aims were, firstly, to document mortality and the incidence of recurrent severe anaemia during a period of 18 months after admission for severe anaemia, and secondly, to identify risk factors for post-discharge mortality, in order to develop more effective management and preventive strategies.

METHODS

This study was carried out as part of a follow-up to a large case-control study investigating the aetiological factors for severe anaemia in children[5]. It was conducted in southern Malawi at Queen Elizabeth Central hospital in Blantyre and Chikwawa District hospital. In Blantyre (urban site) malaria is seasonal, while in Chikwawa (rural site) malaria transmission is intense throughout the year.

Children were recruited from July 2002 for 2 years and followed up until February 2006 from the hospital's Paediatric Accident and Emergency Unit (Blantyre) or Under-fives Clinic (Chikwawa). Each of these is a facility available to the public without charge, functioning in daylight hours for six days each week. Severely anaemic children (*cases*) were recruited if they had a blood haemoglobin concentration ([Hb]) of less than 5.0 g/dl, were aged 6-60 months and had not received a blood transfusion during the preceding four weeks. For each *case* a Hospital Control (*HC*) was recruited on presentation to the same out-patient department for a condition other than severe anaemia, and when the index *case* was discharged from hospital a Community Control (*CC*) was recruited from among apparently healthy children residing within 100-1000 metres of the home of the case. *HC* and *CC* had to be aged between 6 and 60 months, and to have a blood [Hb] of 5g/dl or more. Informed consent was obtained from the guardians of the children and the study was approved by the ethics committees of the University of Malawi and the Liverpool School of Tropical Medicine, UK.

Procedures

The process of recruitment included a detailed medical history and physical examination. Samples of venous blood, urine and stool were collected. Children requiring hospitalisation (all cases and a minority of *HC*s) were managed in a research ward and according to standard protocols.

All three study groups (*cases*, *HC* and *CC*) were actively followed up at 1, 3, 6, 12 and 18 months. Guardians were additionally asked to return with the child to a study clinic whenever the child was sick. During each follow-up visit, a standard clinical form was completed and, if necessary, the child was treated by the attending clinician. At the time of this study, anti-retroviral therapy (ART) was not provided to children in Malawi. Deaths and severe anaemia episodes were recorded, and if they occurred outside the study clinics, they were investigated as completely as possible using the patient's 'Health Passport' book, hospital records and home interviews with parents or guardians. Deaths occurring during the initial hospitalisation period were recorded as in-hospital mortality. Deaths occurring after discharge but during the study follow-up period were recorded as post-discharge mortality.

All laboratory assays were done blinded to patient study group. Haemoglobin was measured on site using the HemoCue B-Haemoglobin analyser (HemoCue, Ängelholm, Sweden) and subsequently by Coulter counter analyser (Beckman Coulter, Durban, South Africa). Ferritin was measured using the electrochemiluminescence immunoassay (Modular Analytics E170, Roche Diagnostics, Switzerland. Soluble transferrin receptor (sTfR) levels were measured using ELISA (Ramco Laboratories, TX, USA). Iron deficiency was defined as a sTfR/log ferritin index of 5.6 or more[6,7]. Malaria was defined as the presence of *Plasmodium falciparum* asexual parasites in the blood. Stool samples were examined for helminth infection[8] by Kato-Katz and Polymerase Chain Reaction (PCR). Urine specimens were examined for *Schistosoma haematobium* using a semiquantative concentration method[9]. Bacterial cultures were carried out only on *cases* and *HC* according to a standard method using an automated BacT/Alert system (BioMérieux Industry, MO, USA) and cultured for 7 and 56 days for routine pathogens and mycobacteria respectively. Mixed growth or growth of Micrococci, Bacillus species or coagulase-negative staphylococci were considered contaminants. HIV testing was performed according to WHO guidelines using two rapid tests (Determine, Abbott-Laboratories, Japan; Unigold, Trinity-Biotech, Ireland). Discordant results and reactive results in children less than 18 months were resolved by PCR[10]. For genetic analysis, DNA was extracted using a Nucleon extraction kit (Amersham biosciences, UK) and tested by Cytronomics and arms-PCR for a predefined set of polymorphisms including glucose-6-phosphate dehydrogenase deficiency (G6PD, variant A- 202/376)[11] , sickle cell disease and single nucleotide polymorphism in the promoter region of the IL-10 and TNF-α genes[12].

Statistical analysis

Data were analyzed using SPSS for Windows© version 12 and STATA© version 9. Discrete data were analyzed by the chi-squared or Fisher's exact test. Continuous data were analysed by independent samples Student's t-tests. Survival times were recorded as the duration of follow-up from the date of recruitment or the date of discharge (if hospitalised at time of recruitment) until the date of a severe anaemia episode or post-discharge mortality. Severe anaemia was defined as [Hb] less than 5g/dl during the follow-up period and at least four weeks after a previous severe anaemia episode. Survival times were compared using Kaplan-Meier plots. Wasting was defined as a Z-score <-2 weight-for-height and stunting as a Z-score <-2 height-for-age.

Hazard ratios (HR) of predictors for post-discharge mortality and their 95% confidence intervals were estimated by using Cox Regression. Factors associated with post-discharge mortality in the univariate model were then included in a multivariate model if either $p<0.1$ or if they were important confounders and effect modifiers.

A survey in Chikwawa of pre-school children found an annual incidence of severe anaemia of 7%. Samples of 380 *cases* and 380 controls provided power to detect factors increasing the incidence of severe anaemia from 7% in the controls to 13.5% in the *cases* (i.e. to detect a HR of 2.0) at an alpha level of 5%.

RESULTS

A total of 1138 children were recruited, of whom 381 (33.5%) were *cases*, 377 (33.1%) were *HC* and 380 (33.4%) were *CC*. Four children among the *cases* were excluded from the final analysis because of undeterminable discharge dates. Of all children included in the analysis, 53.4% (606/1134) were from the urban site (Blantyre). Over the 18 months study period 17.6% (195/1110) were lost to follow-up, the commonest reasons being emigration out of the study area and withdrawal of consent (Table 1). There were no significant differences in baseline characteristics of the children lost to follow-up compared to those that completed the study follow-up period.

There were a few differences in the baseline characteristics of the three groups. *Cases* had a higher prevalence of stunting, wasting and malaria compared to controls. Bacteraemia was more common among *cases* (15.2%, 54/355) than *HC* (4.0%, 14/353) with 72.1% (49/68) caused by non-typhoidal Salmonella. Blood cultures were not done on *CC*. HIV prevalence was significantly higher among the *cases* (12.7%, 45/553) than *HC* (8.1%, 27/335) or *CC* (4.0%, 14/347).

Table 1: Baseline characteristics and outcome by study group

	All cases	Hospital Controls	Community Controls
Sample size			
Recruited	381	377	380
Excluded from analysis[1]	4	0	0
Included in analyses	*377*	*377*	*380*
Baseline characteristics			
Urban site	53.6% (202/377)	53.3% (201/377)	53.4% (203/380)
Age (in months)[2]	20.4 (12.8)	22.5 (12.1)	25.3 (13.1)
Male	46.4% (175/377)	52.3% (197/377)	49.7% (189/380)
History of previous transfusion	15.2% (57/376)	5.9% (22/376)	4.2% (16/380)
Educated mother	88.2% (321/364)	68.4% (258/377)	78.7% (296/376)
Wasting (z-score ≤ 2)	15.9% (52/328)	8.3% (28/339)	4.2% (15/356)
Stunting (z-score ≤ 2)	53.2% (175/329)	36.8% (125/340)	45.4% (162/357)
[Hb] (g/dl)[2]	3.6 (0.8)	9.6 (2.2)	9.9 (1.9)
Iron deficiency	46.4% (6/207)	65.7% (136/207)	73.1% (152/208)
Malaria	59.0% (222/376)	41.0% (154/376)	44.7% (167/374)
HIV	12.7% (45/353)	8.1% (27/335)	4.0% (14/347)
Bacteraemia	15.2% (54/355)	4.0% (14/353)	–
Mortality			
Total deaths	65	10	5
All mortality incidence[3]	148	20	9
In-hospital mortality	6.4% (24/377)	0% (0/377)	–
Post-discharge follow-up (days)[4]	537 (0 - 715)	540 (0 - 700)	540 (0 - 694)
Losses to follow-up at: 6 months	6.8% (24/353)	6.1% (23/377)	3.7% (14/380)
12 months	10.2% (36/353)	10.6% (40/377)	6.8% (26/380)
18 months	17.8% (63/353)	19.6% (74/377)	15.3% (58/380)
Total deaths post-discharge	41	10	5
Post-discharge mortality[5] at: 6 months	8.6%	1.7%	0%
12 months	10.8%	2.3%	0.6%
18 months	12.6%	2.9%	1.4%
Severe anaemia post-discharge			
Number of children (episodes)	32 (42)	4 (5)	2 (2)
One or more episodes[5] at: 6 months	6.7%	0.8%	0.3%
12 months	9.1%	1.1%	0.6%
18 months	10.2%	1.1%	0.6%

[1] no hospital discharge date, outcome unknown; [2] mean (sd); [3] per 100 person-years; [4] median (range); [5] adjusted for losses to follow-up.

The overall mortality rate (in-hospital and post-discharge) was significantly higher among the *cases*, 17.2% (65/377), than the combined control groups 2.0% (15/757, p<0.001, Table 1). The overall incidence of death among *cases* was 148 per 100 person-years (95% CI

116-189), and they were about ten times more likely to die than controls (HR 9.5, 95% CI 5.4-16.7). In-hospital mortality among the *cases* was 6.4% (24/377) while the post-discharge mortality after 18 months of follow-up was 12.6% (Kaplan-Meier estimates, adjusted for losses to follow-up). The post-discharge mortality was significantly higher (Log rank test, p<0.001) than that observed among the *HC* (2.9%) or *CC* (1.4%). Post-discharge deaths among *cases* occurred after a median time of 4.1 months (IQR 1.8-8.1), 70.7% (29) occurring within the first six months (Figure 1).

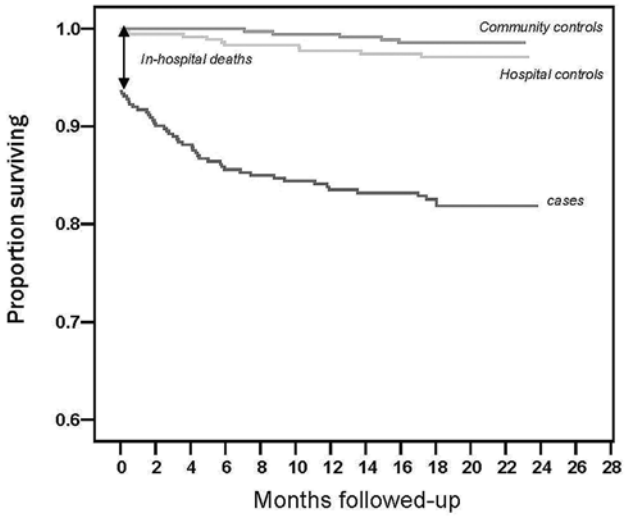

Figure 1: Survival curve showing the time to post-discharge death during the follow-up period of severely anaemic children (*cases*) and hospital and community controls (see colour section). Log rank test, p<0.001.

For *cases*, risk factors for post-discharge mortality are presented in Table 2 and Figure 2. Children who died were more commonly HIV-infected than survivors and after adjusting for confounders and effect modifiers, HIV was strongly associated with death in the follow-up period (HR 10.5, 95% CI 4.0-27.2). Overall mortality was 60.0% (27/45) among HIV infected as compared to 10.7% (33/308) among HIV uninfected severely anaemic children (p<0.001). Bacteraemia was associated with a non-significantly increased risk of post-discharge mortality (HR 2.2, 95% CI 0.8-5.6). Malaria, study site, sickle cell disease, G6PD or hookworm infection did not significantly predict post-discharge death.

Recurrence of severe anaemia was observed in 10.2% (Kaplan-Meier estimates, adjusted for losses to follow-up) of *cases* during the 18-month follow-up period, with a median time to event of 2.9 months (IQR 1.4-6.6) (Figure 3). *Cases* had a significantly higher rate of severe anaemic events (Log rank test, p<0.001) during the follow-up period than *HC* (1.1%) or *CC* (0.6%). Of these events 65.6% (21/32) occurred within the first six months after discharge. The incidence of severe anaemia in the follow-up period among the *cases* was 80 per 100 person-years (95% CI 57-113) compared to 5 per 100 person-years (95% CI 2-11) for the combined controls (p<0.001). The prevalence of HIV infection was not

Table 2: Baseline characteristics of severe anaemia cases by outcome

	All deaths (n= 65)	Post-discharge mortality (n= 41)	Survivors (n= 312)
Urban site[1]	63.1%	61.0 %	51.6%
	(41/65)	(25/41)	(161/312)
Age (in months)[2]	19.6	17.8	20.5
	(12.5)	(10.8)	(12.8)
Male[1]	38.5%	46.3%	48.1%
	(25/65)	(19/41)	(150/312)
History of previous transfusion[1]	14.1%	12.2%	15.4%
	(9/64)	(5/41)	(48/312)
Educated mother[1]	86.2%	81.6%	88.6%
	(50/58)	(31/38)	(271/306)
Wasting[1]	29.4%	20.0%	13.4%
	(15/51)	(7/35)	(37/277)
Stunting[1]	62.7%	65.7%	51.4%
	(32/51)	(23/35)	(143/278)
Splenomegaly	58.7 %	50.0%	64.5%
	(37/63)	(20/40)	(198/307)
Hb (g/dl)[2]	3.4	3.5	3.6
	(0.8)	(0.6)	(0.9)
Iron deficiency[1]	50.0%	52.0%	45.7%
	(16/32)	(13/25)	(80/175)
Malaria[1]	46.9%	53.7%	61.5%
	(30/64)	(22/41)	(192/312)
HIV[1]	45.0%	48.8%	5.8%
	-2760	(20/41)	(18/293)[3]
Bacteraemia[1]	31.6%	29.3%	12.1%
	(18/57)	(12/41)	(36/298)

[1] %, (n); [2] mean (SD); [3]p<0.0001 between Survivors and All deaths or post-discharge mortality

significantly greater among *cases* that had recurrent severe anaemia than those that did not (16.7%, 5/30 versus 12.4%, 40/323 respectively, p= 0.5).

DISCUSSION

The in-hospital case-fatality rate of 6.4% in the present study is within the range of previously reported studies in Sub-Saharan Africa[1,2,3,13]. In-hospital deaths usually occur shortly after admission and respiratory distress has been found to be an important predictor of a fatal outcome[2]. Earlier identification of these children in the community and more timely transfusion may decrease these early deaths.

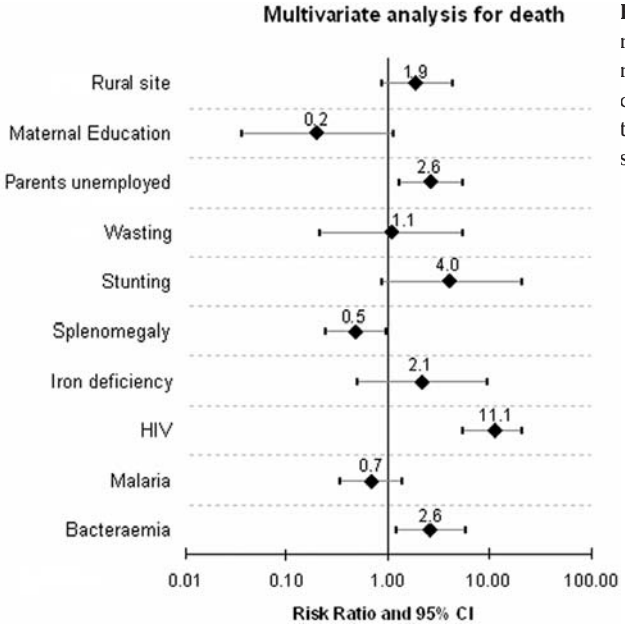

Figure 2: Adjusted Hazard Ratios of main risk factors for post-discharge mortality among severely anaemic children. Other factors included in the univariate analysis included: sickle cell, G6PD, hookworm

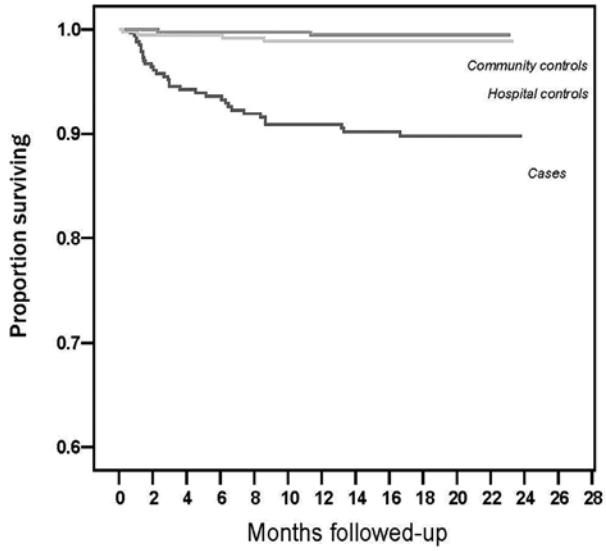

Figure 3: Survival curve showing the time to severe anaemia during the follow-up period of severely anaemic children (*cases*) and hospital and community controls (see colour section). Log rank test, p<0.001

Of serious concern is the high post-discharge mortality observed in this study, with most deaths occurring within six months after admission for severe anaemia. Outside of the context of a research study, such deaths may remain undetected by the health services; even if recorded, the link with a previous episode of severe anaemia may be unrecognized.

Before this study, the longest follow-up of children after an episode of severe anaemia was eight weeks, in an investigation in rural Kenya[3]. Even in that relatively short period, the post-discharge mortality rate was found to be 14%, which the investigators attributed to suboptimal medical care during admission to hospital (poor disease recognition and inadequate treatment). The present study has been the first attempt to validate these Kenyan findings in a different setting and with an extended follow-up period. We provided a good level of supervised medical care during the initial admission, and were therefore surprised to find that both post-discharge mortality and the rate of recurrence of severe anaemia, although better than in Kenya, remained high.

Several possible explanations for the high incidence of post-discharge mortality and recurrent severe anaemia should be considered. Firstly, it is possible that the underlying cause of the initial episode of severe anaemia was neither diagnosed nor treated, and therefore haemoglobin levels may have deteriorated again after discharge. Considering the extensive diagnostic investigations[5] and frequent follow-up visits that were carried out by dedicated medical staff, this explanation appears unlikely.

Secondly, the aetiology of the severe anaemia episode may have been identified correctly, but treatment failure occurred as result of multi-drug resistant malaria or bacterial infections. It has been shown that recrudescent and new malaria infections during the first six months can negate the initial haematological improvement attained from receiving blood transfusion[3]. Children with malaria in this study were treated with a combination of quinine for at least three days and sulfadoxine-pyrimethamine according to national policy. Although generally efficacious, this regimen is known to have an increasing failure rate in formal studies in Malawi, so that recrudescent malaria may have contributed to recurrent anaemia in some children. Similarly although sepsis or positive blood culture was treated with broad spectrum antibiotics, guided by drug sensitivity findings, un-eradicated or new bacterial infections may have developed and contributed to morbidity and mortality.

Thirdly, *cases* may have been seen in the end-stage of a chronic underlying disease, with severe anaemia being merely a marker of disease severity. This hypothesis is partly supported by the fact that HIV infection was found to be the most important independent risk factor for post-discharge mortality. Anaemia is not only a common paediatric manifestation of HIV, but has been shown to be strongly and consistently associated with the progression of HIV disease and death[14]. At the time of this study, ART was not available to children in Malawi. Currently 'unexplained moderate anaemia' is a stage III criterion for commencing ART, but in practice this is often not used in deciding which children should be started on ART. If our finding of a very high HIV-related case fatality rate in severely anaemic children is confirmed in other studies, it may be appropriate to

consider adding severe anaemia to the criteria for stage IV disease in the WHO paediatric HIV staging system.

Finally, inadequate blood transfusion may explain the high rates of post-discharge mortality and recurrent severe anaemia, an explanation also suggested in the Kenyan study[3]. We have tried to prevent this happening by evaluating haemoglobin levels after transfusion and before discharge, and repeating transfusions if necessary. Nevertheless, in *cases* haemoglobin concentrations were still well below the normal range following transfusion – a situation that is likely to prevail in many health facilities in Africa – and a relatively minor additional haematological insult could have major consequences.

The importance of bacteraemia has been shown in a previous study in Malawi, where it was associated with severe anaemia[15]. Non-typhoidal salmonellae, principally *S. typhimurium* and *S. enteritidis*, are widely prevalent in Africa and are associated with malaria and HIV infection[16]. In our study 15% of children admitted with severe anaemia were bacteraemic, but bacteraemia was not an independent risk factor for post discharge mortality or recurrence of severe anaemia. Whether routine treatment with antibiotics should be given to all children with severe anaemia remains a matter of debate. Our data suggest that this would be a reasonable policy where blood cultures cannot be carried out.

Our analysis included only risk factors that could be ascertained at the time of admission. Causes of a post-discharge death, if it occurred in the community, could only be investigated retrospectively by using the verbal autopsy method. Verbal autopsies are known to be neither very sensitive nor specific; therefore the true causes of post-discharge mortality in the study population could not be clearly determined[17].

This study provides disturbing evidence of consequences of severe anaemia on child health and survival. It is commonly thought that most deaths due to severe anaemia occur in-hospital[2]. This study shows that there is an even greater mortality post-discharge. This 'hidden' post-discharge mortality, calculated at 12.6% during 18 months (compared to 2% in the combined controls), is likely to be an underestimate, as it is based on the assumption that all children lost to follow-up survived. As severe anaemia is very common[1], the impact on overall under-five mortality is likely to be considerable. Increased attention to the prevention and management of severe anaemia in African children is urgently needed if the fourth millennium development goal (MDG4) – a significant reduction in child mortality – is to be achieved.

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