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

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Chapter 8

Discussion and Conclusions
Severe anaemia

Severe anaemia is a major public health problem in sub-Saharan African children, responsible for many hospital admission and deaths. The aetiology is complex and as a consequence poorly studied. HIV may be amongst these causes, however little data on the association between severe anaemia and HIV in children has been published. The aim of this thesis is to investigate these causes and to understand their pathogenesis in order to identify possible targets to prevent and treat (HIV-associated) severe anaemia.

Severe anaemia aetiology

In Chapter 2, a large case control study identifying potential aetiological factors for severe anaemia in Malawian children is described. Bacteraemia, malaria, hookworm, HIV infection, G6PD, and deficiency of vitamin A or vitamin B12 were significantly associated with severe anaemia. Folate deficiency, sickle cell disease, Parvo B19 infection and laboratory indicators of an abnormal inflammatory response were uncommon. Iron deficiency occurred significantly less frequently in case-patients and was inversely associated with bacteraemia. The majority of hookworm infections were found in children aged less than two years.

These results provide new insights into the aetiology and of severe anaemia in children living in this area of sub-Saharan Africa. Current WHO guidelines focus on treatment of malaria and hookworm, in children over two years of age, and supplementation with iron and folic acid. These guidelines are mostly derived from data in adults or studies in children which have focussed on investigation of single aetiologies. Our results suggest that folate supplementation is less important than treatment of other causes or potentially harmful co-morbidities such as vitamin B12 deficiency, bacteraemia or hookworm infections in young children. The importance of these findings has not been previously recognised in children and the study emphasises the need to complete comprehensive assessments of anaemia co-morbidities. The data presented in this thesis is the first study in African children to undertake such a comprehensive assessment.

An unexpected and important finding which provides a critical new insight was the negative association between iron deficiency and severe anaemia. The structural equation model in Chapter 2 helps to explain this finding by indicating that iron deficiency was negatively associated with bacteraemia. This supports the hypothesis that iron deficiency protects against infection by creating an unfavourable environment for bacterial growth. It is also in agreement with observations of increased morbidity and mortality in children receiving iron supplementation in areas where bacterial infections are common. Although iron supplementation does help to prevent anaemia, it has recently been associated with increased morbidity and mortality in children in a population based supplementation trial in Pemba island Tanzania which was stopped prematurely by the Data Safety Monitoring Board because of the increased mortality risk. In severe malaria
anaemia iron supplementation has been shown to have no haematological benefits as well as increase morbidity risk. It is likely that routine iron supplementation in children living in sub-Saharan Africa can prevent mild anaemia; however its role in preventing live-threatening severe anaemia in populations with a high infectious pressure is less clear and may incur substantial health risk.

To assess these associations of causation of severe anaemia and its consequences, or comorbidities, properly powered intervention studies are essential. As several of these potential aetiological factors interact methodologies are required to unravel their associations (Chapter 2, structural equation model), and as many children suffer from more than one condition at a time (Chapters 2 and 3) longitudinal studies are also required to describe the natural history of anaemia. Perhaps because of the complexity of undertaking such studies several investigators have focussed on supplementation trials to prevent severe anaemia focussing on a single aetiology. An example of this is the vitamin A supplementation trial in Papua New Guinea. Children receiving vitamin A supplements had reduced incidence of malaria without any improvement in haemoglobin. Our results suggest that preventing anaemia using a single intervention may be an oversimplification of the biological processes underlying the condition and as a consequence will have limited success. The way forward to develop preventive strategies for severe anaemia is to evaluate combined intervention strategies based on a detailed understanding of pathogenesis and natural history.

We have concluded that current recommendations promoting iron and folate-supplementation and ignoring bacteraemia and vitamin B12 deficiency may not be applicable in severely anemic children. Even in the presence of malaria parasites, additional or alternative causes of severe anaemia should be considered, and without a more comprehensive approach limited effectiveness will be achieved with the current policy of prevention.

Pathogenesis of severe anaemia

The pathogenesis of anaemia is complex because several distinct mechanisms may lead to a reduced number of circulating red cells. In African children, mechanisms known to contribute to severe anaemia include haemolysis (intra or extra vascular), acute or chronic blood loss, and red cell production failure. Each of these mechanisms may be activated by a variety of aetiological factors and single aetiologies may affect more than one mechanism. Furthermore, a single aetiology may predominate in some patients, while in others multiple aetiologies and mechanisms may combine to result in severe anaemia.

In Chapter 3 we have applied a new approach to the assessment and description of severe paediatric anaemia by identifying both the mechanisms and aetiologies associated with
severe anaemia in Malawian children. Red cell production failure was the most important mechanism and was identified in 48% of cases, haemolysis occurred in 22% and direct or indirect evidence of blood loss was found in 7%. Infections were common as 59% had one, and 17% had two or more of the four infections which have previously been associated with severe anaemia (HIV, *P.falciparum* malaria, hookworm or bacteraemia). No differences in the prevalence of these mechanisms were observed amongst children suffering from either infections or those with micronutrient deficiencies.

These findings support the view that malarial anaemia is not primarily a consequences of increased destruction of red blood cells\(^{13}\). They also demonstrate that, especially in HIV-infected children with severe anaemia, co-infections are common (84%). This is the first report to confirm that in children HIV-associated anaemia may be a consequence of decreased red cell production as has previously been reported in adults\(^{14-17}\).

We have produced an assessment of the syndrome of severe anaemia in these children which shows that red cell production failure is the principle underlying mechanism which occurs in the presence of these various co-morbidities. Preventive strategies need to take this finding into consideration in order to promote ways of enhancing red cell production which, in severely anaemic children in this population, are unlikely to be based on single (micronutrient) initiatives.

**Long-term outcome of severe anaemia**

In different geographical settings in sub-Saharan Africa the in-hospital mortality of paediatric severe anaemia ranges between 8-17%\(^{12,18,19}\). Investigators in Kenya reported an unexpectedly high post-discharge mortality and recurrence of severe anaemia in children within two months of their severe anaemia episode\(^{20}\). There is a need to confirm these findings as the excess risk of death in this large group could contribute an important and potentially preventable component of the high mortality of young African children.

In **Chapter 4** we described the long term outcome of children recruited in the case-control study of severe anaemia. In severely anaemic children the in-hospital mortality was 6.4%. In severely anaemic children who were discharged from hospital the additional mortality during the 18-month follow-up period was 12.6%, which was significantly higher than in the hospital controls (2.9%) or community controls (1.4%), (p<0.001). The incidence of a (new) episode of severe anaemia during the follow-up period among the cases was 80 per 100 person-years, which was significantly higher than the rate of 5 per 100 person-years in both control groups combined (p<0.001).

HIV infection had the highest risk estimate for mortality (HR 10.5, 95% CI 4.0-27.2). Mortality was 60% among HIV-infected compared to 11% among HIV-uninfected severely anaemic children (p<0.001). Severe anaemia was significantly associated with
increased mortality in HIV-infected cases compared to controls (60% vs. 15%, p<0.001, Chapter 4). Currently ‘unexplained moderate anaemia (Hb<8g/dL)’ is a stage III criterion for commencing ART (WHO)21, but in practice this is often not used in deciding which children should be started on ART. If the finding of the very high HIV-related case fatality rate in severely anaemic children is confirmed in other studies, it may be appropriate to consider including severe anaemia in the criteria for stage IV disease in the WHO paediatric HIV staging system.

These data provide disturbing evidence of the consequences of severe anaemia on child health and survival. It is commonly thought that most deaths due to severe anaemia result from in-hospital mortality22. This study shows that there is an even higher mortality post-discharge. As severe anaemia is very common19, 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 – a significant reduction in child mortality – is to be achieved. In HIV-infected children severe anaemia should be considered an important predictor of mortality and future research should focus on the potential use of severe anaemia as a stage IV criterion in the WHO paediatric HIV staging system.

**HIV and severe anaemia**

Early in the HIV-pandemic anaemia appeared to be the most common haematological complication of HIV in infected adults23, and a positive association had been reported between prevalence of anaemia and severity of clinical disease24. Subsequently anaemia has been repeatedly identified as a strong, independent and reversible predictor of mortality in large studies in western settings25-27. In contrast to adults there is very little information available about the association between HIV infection and anaemia in children. This situation is not likely to improve because in western countries, which generate most of the research on this topic, paediatric HIV infection is declining28.

In **Chapter 2** we outlined results which showed that HIV infection occurred in 13% of cases and 6% of controls. The attributable risk of HIV for severe anaemia was 6.2% for both settings combined and 15% in the urban setting. To further study the association between HIV and (severe) anaemia in children, a systematic review was undertaken on this topic. In **Chapter 5** an overview is provided of the data on HIV-associated anaemia in children worldwide. This meta-analysis of anaemia prevalence and incidence data suggested that mild (haemoglobin <11 g/dl) and moderate (Hb <9 g/dl) anaemia were more prevalent with HIV infection and mean haemoglobin levels were lower. These differences were observed for both western and tropical settings. Anaemia incidence ranged between 0.41-0.44 per person-year. There was limited data available for severe categories of anaemia (Hb <7 g/dl or <5 g/dl).
We further reviewed data available relevant to the development of curative or preventive strategies. Failure of erythropoiesis appeared to be the most important mechanism causing anaemia in HIV-infected children. This conclusion is based on descriptive evidence showing that other pathogenetic mechanisms (blood loss\textsuperscript{29-35} or hemolysis\textsuperscript{31,35-38}) were not prominent features in HIV-infected children with anaemia. Only a few studies have directly assessed erythropoiesis and haematological findings in HIV-infected children\textsuperscript{31,33-35} and these studies were either retrospective\textsuperscript{31,34,35} or lacked a suitable control population for comparison\textsuperscript{33-35}.

Therapeutic options include highly-active-antiretroviral-therapy\textsuperscript{25,39}, and prevention or treatment of secondary infections. Erythropoietin can improve anaemia in children\textsuperscript{40}, but this has not been evaluated in tropical settings, although low values are reported in African children\textsuperscript{41,42}. Little data was available on the preventive effectiveness of supplementation with micronutrients other than iron. However, the available data does not suggest that routine supplementation may be an effective intervention to reduce anaemia in HIV-infected children though it may be helpful in individual children.

Definitive evidence on the contribution of iron deficiency to the anaemia of HIV-infected children and the effects and possible harm of iron supplementation\textsuperscript{5,43,44} was lacking as intervention trials have not been undertaken in children or adults infected with HIV. Given the data in Chapter 2 showing that a substantial number of severely anaemic children were not iron deficient, and the fact that presumptive supplementation is currently recommended for most children living in tropical countries\textsuperscript{45}, studies addressing the safety and efficacy of iron supplementation in HIV-infected children are urgently needed. The situation is complex as it is likely some children could benefit, whereas for others it would be detrimental. This dilemma might be resolved by improved understanding of how these distinct categories of children can be identified, and it may be necessary to take a much more cautious approach to blanket supplementation with iron, or to consider only very low dose strategies.

We have concluded that anaemia is a very common complication of paediatric HIV infection worldwide and is associated with a poor prognosis.

Areas for future research and which have been partly addressed in this thesis include:
The association between HIV and severe anaemia, a common diagnosis in tropical areas, which is associated with high morbidity and mortality (\textit{Chapter 2}).
The pathogenesis of anaemia in HIV-infected children (\textit{Chapters 3 and 6}).
The role of viral characteristics of HIV in the development of anaemia (\textit{Chapter 7}).
The use of haemoglobin to predict and monitor disease progression, and the effect of anaemia reduction for reversing disease progression in children infected with HIV in resource poor settings (\textit{Chapter 4 and ongoing work}).
The safety and efficacy of possible nutritional intervention strategies in children, including iron supplementation (Chapter 3 and ongoing work).

Haematology of HIV-associated severe anaemia

In the previous section we suggested that the unexplained association between HIV and severe anaemia in Malawian children (Chapter 5) may result from an increased cell death of erythroid precursors. In Chapter 6 results comparing haematological abnormalities among severely anemic children with and without HIV infection are described.

Bone marrow flow cytometry showed that HIV-infected children had fewer CD34+ haematopoietic progenitor cells (1.0% vs. 1.5%, p=0.04), erythroid progenitors (0.2% vs. 0.3%, p=0.05) and erythroid precursor cells (18% vs. 26%, p=0.06). Dyserythropoiesis and apoptosis of red cell precursors were not more common in HIV-infected than uninfected children (2.8% vs. 3.8%, p=0.12 and 9.3% vs. 12.3 %, p=0.23). There were no significant correlations between the proportion of dyserythropoietic or apoptotic cells, and peripheral blood levels of the cytokines TNF-α, IFN-γ, IL-10, erythropoietin, or vitamin A. Polychromatic erythroblasts, reticulocyte counts and peripheral blood erythrocytic indices were similar in both groups.

The finding that HIV-infected children had fewer CD34+ haematopoietic progenitor and erythroid progenitor cells in the bone marrow than uninfected children supports the hypothesis that red cell production failure is an important correlate of severe anaemia in HIV-infected children which may be caused by a reduced erythroid progenitor capacity. Despite the difference in CD34+ haematopoietic progenitors and erythroid progenitors the proportion of more mature erythroid precursor cells in bone marrow or peripheral blood (reticulocytes) did not differ between the two groups. Hence HIV-uninfected children with severe anaemia appeared to be less productive or efficient in their (subsequent) erythropoiesis than HIV-infected children. This explanation is supported by the trend, (although not significant), towards less dyserythropoiesis and apoptosis in HIV-infected children. This finding contrasts with other studies which reported that dyserythropoiesis was more common in the later stages of HIV disease leading to the conclusion that anaemia may be related to increased dyserythropoiesis.

In this study dyserythropoiesis or apoptosis were not associated with altered cytokine levels or Vitamin A deficiency which is in contrast to other reports. It is possible that more intensive investigations might identify cytokines which affect regulatory signals and could be therapeutic targets to reduce haemopoietic inhibition in HIV patients.

In conclusion the findings in these Malawian children indicate that despite an HIV-associated deficiency in early red-cell precursors, subsequent erythropoiesis is at least as efficient in HIV-infected as in uninfected children with severe anaemia. Apoptosis and
dyserythropoiesis were not more prominent mechanisms in HIV-infected than in HIV-uninfected children.

**Infections and HIV-associated severe anaemia**

Infections, as described in Chapter 5, were identified as potential contributory causes of anaemia in HIV-infected children. The contribution of opportunistic infections to severe anaemia in HIV-infected children in sub-Saharan Africa remains unclear. The role of malaria is unclear as, although in adults there is evidence in HIV-infected women that the prevalence of malaria parasitaemia is increased\(^50\), in children there is no evidence to support this finding\(^51-55\). However children suffering from both infections could have more severe anaemia than children with either infection alone\(^51,54\).

We found that the acute phase reactant CRP was raised (>10 mg/L) in HIV-infected children with severe anaemia (90%). In Chapter 2 we described that in severely anemic children, the prevalence of EBV (50% vs. 31% \(P=0.03\)), or bacteraemia (26% vs. 13% \(P=0.02\)) was increased in HIV-infected compared to uninfected children. The prevalence of malaria parasitaemia did not differ between these groups (59% and 59%, \(p=0.96\)) and hyperparasitaemia was less common (5% vs. 13%, \(P=0.09\)). CMV, Parvovirus B19 infection, hookworm and invasive mycobacterial infections were uncommon in severely anaemic HIV-infected children (0%, 3%, 6% and 0% respectively).

Our data does not identify malaria as a more important factor contributing to the development of severe anaemia in HIV-infected compared to uninfected children, which contrasts hypotheses from other publications\(^51,54\). This finding is in agreement with observations that malaria does not occur more frequently in HIV-infected children\(^51,55\). Parvo B19 was not identified as contributory to the development of severe anaemia in HIV-infected children in this setting, which agrees with previous studies in children\(^56\), but not adults\(^15\). Invasive mycobacterial infections were not identified from blood or bone marrow isolates, which conflicts with data on Malawian adults\(^57\), and other studies in HIV-infected children\(^36;57-63\).

EBV has been considered as a possible aetiological factor in HIV-associated anaemia in adults, although data on children are lacking\(^14\). It is uncertain whether this is a true aetiological factor or a co-morbidity. Bacteraemia, especially with nontyphoidal salmonella has been associated with both HIV infection and severe anaemia\(^64,65\). As this is a treatable and preventable factor, future studies should aim to identify if salmonella bacteraemia is a causal factor, or rather a consequence possibly resulting from increased availability of iron which occurs during haemolysis (Chapter 3)\(^3\).

We conclude that infections occur frequently in HIV-infected children with severe anaemia, including bacteraemias (with nontyphoidal salmonella) and EBV infection.
Future studies need to elucidate the role of these pathogens in the development (and prevention) of severe anaemia in these children.

**Viral aspects of HIV-associated severe anaemia**

In a Chapter 2 we observed an association between HIV and severe anaemia that could not be fully explained by secondary infection or micronutrient deficiency alone. The association may be largely explained by a direct effect of the virus (HIV) on erythropoiesis (Chapter 5). In vitro studies have suggested that specific HIV strains, such as X4 which uses the CXCR4 co-receptor on erythroid precursors, are associated with diminished haematopoiesis66-70. This co-receptor affinity is determined by changes in the hypervariable loop of the HIV-1 envelope genome71. We therefore explored the possibility that alterations in the V1-V2-V3 fragment of HIV-1 were associated with severe anaemia and these results are described in Chapter 7.

Phylogenetic analysis showed that HIV-1 subtype C, the predominant subtype in South-East Africa71,72, was present in all but one child. All V1-V2-V3 characteristics tested: V3 charge, V1-V2 length and potential glycosylation sites, did not differ between cases and controls. Using a computer model (C-PSSM)73 four children (7.8%) were identified to have an X4 strain. This prevalence was not different between study groups (p=1.00). The V3 loop characteristics for bone marrow and peripheral blood isolates in the case group were identical. None of the children identified as having the X4 strain developed a (new) episode of severe anaemia during follow up.

Early in an infection the HIV-1 population usually comprises a strain with the capacity to bind to both CD4 and the co-receptor CCR5 (R5 strain)74,75. Later in infection a broadening, or switch, occurs and HIV evolves to infect cells expressing CD4 and the co-receptor CXCR4 (R4 strains)74,76. This switch is thought to occur in 50% of infections and is associated with an accelerated loss of CD4+ T-cells and progression to AIDS77. Like the T-helper cells, erythropoietic stem cells express both CD4 and CXCR4 on their membrane78,79. Until recently productive infection was considered uncommon in erythroid precursor cells16, though a recent study reported that in contrast to HIV-1 subtype B16, the predominant subtype in western settings, subtype C may actually infect red cell precursors46;80. As this is a new observation, these results will require confirmation in settings and populations like ours.

A further explanation for the association of HIV infection with severe anaemia could be increased cell death of erythroid precursors. Viral proteins, such as tat, erythropoietin and cytokines may play a crucial role in this66,68-70. Our results related to these hypotheses are discussed in the section above titled ‘haematology of HIV-associated severe anaemia’.
We conclude from these results that the prevalence of X4 strains was high in these young HIV-1 subtype C infected children who were most likely vertically infected and naïve to anti-retroviral therapy. It is unlikely that V1-V2-V3 fragment characteristics and HIV co-receptor affinity was an important feature influencing their development of severe anaemia. Further research on the possibility of direct infection of erythroid progenitors in HIV-1 C-infected children is an area for future research.

**Micronutrient deficiencies and HIV-associated severe anaemia**

The contribution of micronutrient deficiencies and malnutrition to the development of severe anaemia in these children is a critical area for assessment. Micronutrient deficiencies which have been associated with HIV-infection and which could lead to anaemia include iron, folate, vitamin B12, vitamin A and zinc. Controlled studies do not indicate that iron deficiency is more common in HIV-infected children\(^41\);81;82. Iron intervention studies have not been undertaken and bone marrow iron status has not been associated with anaemia in HIV-infected children\(^31\). Folate and vitamin B12 deficiency were also not more frequent in paediatric HIV infection\(^30;33;34;83-86\). The hematopoietic effect of supplementation with these hematins has not been assessed in HIV-infected children. Data on vitamin A and zinc deficiency or supplementation is available but the results are conflicting\(^87-90\);91-95. This is an area for further detailed research (Chapter 5).

In our non-severely anaemic control populations wasting occurred in 13% of HIV-infected and 6% of uninfected children (p=0.09). Deficiencies of vitamin B12 (20% vs. 15%, p =0.40), vitamin A (57% vs. 65%, p =0.70) or iron (52% vs. 71%, p =0.07) were not more common in HIV-infected than uninfected children. This is in line with other studies suggesting that in general micronutrient deficiencies were not more common in HIV-infected children\(^30;33;34;41;81-86\).

Our data on severe anaemia (Chapters 2, 3 and 7) showed that wasting was present in 19% of HIV-infected cases and in 13% of HIV-infected controls (p=0.46). Vitamin B12 deficiency was found in 13% of HIV-infected cases compared to 20% of HIV-infected controls (p=0.40). Folate deficiency was not present in any child. Vitamin A deficiency was present in 96% of HIV-infected cases and in 57% of HIV-infected controls (p=0.01). Iron deficiency was found in 33% of HIV-infected cases and in 52% of HIV-infected controls (p=0.18).

The size of these associations with severe anaemia was similar for HIV-infected and uninfected children. Only vitamin B12 deficiency did not appear to be associated with severe anaemia in HIV-infected unlike in HIV-uninfected children. This could be a result from selection bias as severely anaemic children without HIV infection children may be more prone to have vitamin B12 deficiencies (possibly due to poor food intake), whereas the anaemia in HIV-infected children could be anaemic due to primarily due to other...
conditions such as infectious diseases (as indicated in Chapters 2 and 5). This hypothesis requires further examination which would entail obtaining detailed dietary evaluation for these different categories of children.

The evidence from this thesis supports the conclusion that nutritional deficiencies do not play a more important role in the development of severe anaemia in HIV-infected children than in uninfected children. Randomised controlled trials are required to establish the validity of this conclusion and an approach using multi-micronutrient supplementation would be the most convenient choice in developing these studies.

**CONCLUSIONS**

This study was the first to comprehensively investigate the aetiology, pathogenesis and consequences of severe anaemia in sub-Saharan African children from the same study population. We found that several independent yet overlapping conditions were associated with severe anaemia in these children including bacteremia, malaria, hookworm, G6PD deficiency, vitamin A and vitamin B12 deficiencies and HIV infection. Folate deficiency was uncommon and iron deficiency occurred less frequently in case-patients and was inversely associated with bacteremia.

The pathogenesis data suggests that in these populations, red cell production failure is the most important mechanism, with haemolysis and blood loss contributing relatively little to the development of severe anaemia. It should be stressed that these conditions were frequently overlapping and that, especially in HIV-infected children with severe anaemia, co-infections were common (84%).

We identified an unexpected high mortality after discharge from hospital in severely anaemic children (12%), and this mortality was 60% in they were HIV-infected, indicating that severe anaemia should be interpreted as an important predictor of mortality in HIV-infected children.

A meta-analysis of anaemia prevalence and incidence data suggested that mild (haemoglobin <11 g/dl) and moderate (Hb <9 g/dl) anaemia were more prevalent with HIV infection and that mean haemoglobin levels were lower. These differences were observed for both western and tropical settings. We concluded that anaemia was a very common complication of paediatric HIV infection worldwide and associated with a poor prognosis. There was limited data available on more severe anaemia (Hb <7 g/dl or <5 g/dl), and little data on the pathogenesis and aetiology of HIV-associated anaemia. The research in this thesis aimed to address these gaps in knowledge.
The pathogenesis study suggested that despite an HIV-associated deficiency in early red-cell precursors, subsequent erythropoiesis is at least as efficient in HIV-infected as uninfected children with severe anaemia. Apoptosis and dyserythropoiesis are not more prominent mechanisms in HIV-infected children than in HIV-uninfected children.

We found that infections occurred more frequently in HIV-infected children with severe anaemia, especially bacteraemias (with nontyphoidal salmonella) and EBV infection. Future studies should focus on the potential contributory role of these pathogens in the development (and prevention) of severe anaemia in these children.

Our study of HIV characteristics indicated that there was a high prevalence of X4 strains in young Malawian children with HIV-1 subtype C infection compared to previous results from Malawi. It is unlikely that V1-V2-V3 fragment characteristics and HIV co-receptor affinity is an important feature in the development of severe anaemia in Malawian children. Direct infection of erythroid progenitors in HIV-1 C-infected children may be an area for future research.

The data from the control population and case-control study showed that micronutrient deficiencies were not significantly associated with HIV infection, nor that nutritional deficiencies played a more important role in the development of severe anaemia in HIV-infected than uninfected children. Intervention studies would be required to prove the validity of these statements.

There are multiple independent as well as overlapping causes of severe anaemia in Malawian children. The current preventive and therapeutic guidelines may not be applicable with respect to folate and iron supplementation in these children. HIV is a prevalent, independent and important factor associated with severe anaemia in these children and is associated with a very poor prognosis. Red cell production failure appeared to be the main pathogenetic mechanism in HIV-associated severe anaemia. This may result from a reduced number of erythroid progenitor cells rather than as a consequence of increased apoptosis or dyserythropoiesis in red cell precursors. Infectious diseases causing bacteraemia and EBV were especially prominent amongst HIV-infected children with severe anaemia in contrast to micronutrient deficiencies, or HIV V1-V2-V3 fragment characteristics.
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