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

Introduction
SEVERE ANAEMIA

Natural history and definition
Anaemia is defined as a reduction of the red blood cell volume or haemoglobin concentration below the range of values occurring in healthy children. Newborn infants have a relative high haemoglobin level which decreases and reaches its lowest value at the age of 2-4 months, followed by a gradual rise with age until adulthood. In addition to age, haemoglobin levels can be influenced by racial differences. Black children have levels about 0.5 g/dl lower than those of white and Asian children of comparable age and socioeconomic status.

Although a reduction in the amount of circulating haemoglobin decreases the oxygen-carrying capacity, few clinical disturbances occur until the haemoglobin level falls below 7-8 g/dl. Below this level, clinical symptoms of anaemia occur including pallor, weakness, tachypnea and tachycardia which are all consequences of several physiological processes aimed at increasing oxygen transport to the (vital) organs.

Severe anaemia refers to a condition in which the haemoglobin level decreases to such an extent that the compensatory mechanisms fail and/or a severe outcome can be expected. Children aged 6-60 months are most vulnerable to this condition and in this population WHO has defined severe anaemia as:
- All children with a haemoglobin level < 6 g/dl
- Children with a haemoglobin level between 4-6 g/dl and dehydration, shock, impaired consciousness, deep and laboured breathing, heart failure or very high malaria parasitaemia.

For practical reasons the old definition of severe anaemia will be used (haemoglobin concentration <5 g/dl).

Prevalence and mortality
Severe anaemia (haemoglobin concentration <5 g/dl) is a major cause of paediatric morbidity and mortality in sub-Saharan Africa. Population based studies have reported a severe anaemia prevalence of 3-5% in African children under five year of age. In different geographical settings 12-29% of children admitted to hospital were severely anaemic, with in-hospital mortality between 8-17%. In severely anaemic children following discharge from hospital an overall mortality of 30% has been reported.

Treatment and prevention
Severe anaemia is not a specific entity but results from many underlying pathogenetic and aetiological processes. The care for severely anaemic children may therefore exist of two
interventions: acute treatment of the anaemia (e.g. blood transfusion); and prevention or treatment of aetiologies and comorbidities.

The treatment of severe anaemia with blood transfusion, can be life-saving. However transfusions are expensive and logistically complex to deliver as only a minority of African children are transfused within six hours\textsuperscript{4,6,7}. Transfusion increases the risk of transmission of blood born diseases including HIV and viral hepatitis\textsuperscript{11}.

Since severe anaemia is a symptom rather than a disease, interventions should target prevention and treatment of the underlying causes\textsuperscript{12}. Yet surprisingly little is known concerning the aetiology and pathogenesis of severe anaemia in African children. Published studies mostly relate to malarial anaemia\textsuperscript{13}, or were limited by sample size\textsuperscript{7,14} or to a restricted set of etiological factors investigated\textsuperscript{4,5}. This has contributed to the current opinion that malaria, folate and iron deficiency are the most common causes of severe anaemia, and World Health Organisation-advocated treatment guidelines specifically address these\textsuperscript{12}.

Pathogenesis
Severe anaemia in African children may result from: a) an increased rate of destruction of red cells, due to either mechanical lysis or immune mediated destruction; b) acute or chronic blood loss, or c) impaired erythropoiesis with defective proliferation and/or maturation of red cell precursors.

The latter is probably the most important factor and may result from deficiencies of micronutrients (e.g. iron, folate), from chronic infections (e.g. malaria, HIV), or from an inappropriate host inflammatory response to intermittent infections\textsuperscript{15-18}. In experimental models, a toxic or infective stimulus elicits pro-inflammatory cytokines such as interferon-gamma (IFN\textsubscript{\gamma}) and tumour necrosis factor-\alpha (TNF), which modulate the production and effectiveness of erythropoietin, resulting in a suppression of erythroid progenitor cells\textsuperscript{19,20}. Studies in African children with severe (malarial) anaemia have demonstrated an inappropriate inflammatory response, with high serum levels of IFN-\gamma and TNF in the absence of normal counter-regulatory cytokine levels (IL-4 and IL-10)\textsuperscript{15,17,21}. In those children with persistent severe anaemia this cytokine imbalance persisted for weeks after effective antimalarial treatment\textsuperscript{22}. An inappropriate (un-modulated) chronic cytokine response may not only explain why only some children develop severe anaemia, but also why there was a striking additional post-hospital mortality found in Kenyan children following a severe anaemia episode\textsuperscript{23}. Very few studies have comprehensively studied the relative contribution of these mechanisms to the severe anaemia of African children.
Aetiology

Several aetiological factors may contribute to the development of severe anaemia which include nutritional factors, drugs, genetic disorders and infections.

Nutritional factors

There is a strong association between iron deficiency and mild to moderate anaemia in African children, but the attributable risk of iron deficiency for severe anaemia is uncertain. Only recently has a sufficiently powered study assessed the preventive value of iron supplementation against severe anaemia in African children. It showed a non-significant reduction in severe anaemia, but the study was stopped because iron supplementation was associated with an increased number of hospital admissions and deaths. The circumstantial evidence on iron status can be difficult to interpret as iron status mostly is assessed with proxy measures including serum ferritin, free iron, transferrin, and MCV which each can be altered by infection, malnutrition, or haemoglobinopathies, conditions that are common in African children. Examination of bone marrow iron stores is a more accurate measure that has rarely been used in studies of severe anaemia in African children. The most promising measure of body iron status could be a combination of sTfR and ferritin levels (measuring cellular iron needs and body iron stores), with an additional non-specific marker of inflammation (e.g. C-reactive protein). Again data on this in African children remains limited.

Vitamin B12 and folate deficiency lead to diminished DNA synthesis and can cause megaloblastic/macrocytic anaemia. The main dietary source for folate is green vegetables and fruits, whilst vitamin B12 is derived from animal sources. Both deficiencies are thought to frequently occur in developing countries. Although these deficiencies are generally considered to be causes of mild and moderate anaemia, little information is available on their contribution to severe anaemia in African children. Folate supplementation in anemic children with malaria failed to raise haemoglobin concentrations in a study from The Gambia. Vitamin B12 deficiency has mostly been studied in adults and this data suggests it may play a more important role than currently expected.

Vitamin A supplementation has been associated with the prevention of infections such as malaria and in deficient populations should prevent anaemia. Supplementation trials in women improved the haemoglobin levels of newborns, although the evidence in older children is limited. In fact the same study that showed a protective effect against malaria in children did not observe a protective effect against anaemia. Other micronutrients that may influence haemoglobin concentration include zinc, riboflavin and vitamin E. The data on these in relation to the prevention of severe anaemia, especially in children, is limited.
Drugs
Several drugs, mostly prescribed as treatment of infections, can cause anaemia in African populations. These include; antibiotics (mainly anti-folate drugs and chloramphenicol), tuberculostatics, and to a lesser extent anti(retro)virals (especially AZT), and cytostatic agents. Some of the mechanisms are known to relate to genetic polymorphisms affecting the red cell.

Genetic disorders
Glucose-6-phosphate dehydrogenase deficiency (G6PD), alpha-Thalassaemia and haemoglobinopathies such as sickle cell trait and haemoglobin C commonly occur in African populations and have a protective role against the development of malaria. However homozygosity as in sickle cell disease may lead to severe anaemia. There is increasing evidence that genes regulating the immune response may also play a role in host susceptibility to severe anaemia, one possible pathway being through genetic regulation of cytokine production. Recently polymorphisms in the tumor necrosis factor gene have been associated with severe malaria anaemia (TNF-238 A allele) and some polymorphisms in the IL-10 gene are associated with decreased IL-10 production. Whether this and other polymorphic markers are of true functional importance (directly affecting cytokine production) or are just linked genetic markers requires further investigation.

Infections
Malaria, a major aetiological factor for severe anaemia, causes haemolysis and impaired erythropoiesis. The relative contribution of both mechanisms to malaria related severe anaemia are poorly understood. Haemolysis may be intravascular through red-cell destruction by schizont rupture, or extravascular, aggravated by increased clearance of parasitized and non parasitized red-cells by the spleen. Recent detailed studies have indicated that even with high parasitaemia, the extravascular component is the dominant mode of haemolysis in malaria. Impaired erythropoiesis in children with ‘malaria related severe anaemia’ appeared not to be related to hypoxic damage due to parasite sequestration. The evidence was more suggestive of a non-specific activation of the inflammatory cytokine network related to infections. In areas with high malaria endemicity, malaria is likely to be the major contributor to the (chronic) infectious component, but other pathogens (e.g. HIV and chronic bacterial infections) may well have been underestimated (or under-diagnosed) in the past. Antimalarial prophylaxis can prevent severe anaemia in holoendemic areas but has been associated with increased susceptibility to malaria and increased risk of severe anaemia after the intervention was stopped.

Both Hookworm and Schistosoma are well known to be associated with the development of anaemia. Hookworm (Necator americanus and Ancylostoma duodenale) can be very prevalent in rural populations and this prevalence increases with age. Blood loss (and
iron deficiency) appears to be the main causal mechanism and the magnitude is closely related to the burden of infection\(^56\);\(^57\). Hookworm infections have been associated with severe anaemia\(^58\). In Schistosomiasis both blood loss and cytokine responses have been identified as main mechanisms leading to mild and moderate anaemia\(^59\);\(^60\).

HIV infection is associated with anaemia in African children\(^36\);\(^61\)–\(^63\), although its link to severe anaemia is less well described\(^37\). The pathogenesis of anaemia in HIV-infected adults, although multi-factorial, relates primarily to a reduced production of erythrocytes\(^64\)–\(^67\). This reduction is influenced by several aetiological factors including infection and neoplasms, drugs such as zidovudine, a direct effect of HIV on erythropoiesis, a blunted response to erythropoietin and nutritional deficiencies\(^64\)–\(^67\). Compared to adults there is very little information available about the association between HIV infection and anaemia in children. This evidence on anaemia and HIV in children is reviewed in detail in Chapter 5.

**HIV**

Until thirty years ago, HIV was an unknown disease and few persons had been infected\(^68\). By 2007 an estimated 33 million people are infected worldwide and another 25 million have died of AIDS\(^69\). Approximately 7.5% (2.5 million) of all infected persons worldwide are younger than 15 years of age. In 2007 16.8% of all new infections occurred in children and 15.7% of HIV-related mortality occurred in this age group. These numbers are striking given the fact that maternal-infant transmission, which is the main cause of new infections in this age group, can largely be prevented with minimal interventions

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**Figure 1.** Estimated number of new paediatric HIV infections globally. (source UNAIDS\(^9\))

**Estimated number of children (<15 years) newly infected with HIV, 2007**

<table>
<thead>
<tr>
<th>Region</th>
<th>Number of New Infections</th>
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<tbody>
<tr>
<td>North America</td>
<td>1600</td>
</tr>
<tr>
<td>Latin America</td>
<td>6700 (2000 - 2009)</td>
</tr>
<tr>
<td>Sub-Saharan Africa</td>
<td>370 000 (2000 - 2001)</td>
</tr>
<tr>
<td>Middle East &amp; North Africa</td>
<td>3500 – 4500</td>
</tr>
<tr>
<td>Western &amp; Central Europe</td>
<td>4000 – 5100</td>
</tr>
<tr>
<td>Eastern Europe &amp; Central Asia</td>
<td>3500 – 4600</td>
</tr>
<tr>
<td>East Asia</td>
<td>2100 (1990 – 2001)</td>
</tr>
<tr>
<td>South &amp; South East Asia</td>
<td>24 000</td>
</tr>
<tr>
<td>Total</td>
<td>420 000 (350 000 – 540 000)</td>
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</table>

**Total: 420 000 (350 000 – 540 000)**
which could be made accessible and cost-effective\textsuperscript{48/70/71}. In Western & Central Europe and North America less than 2500 children were infected in 2007, in sub-Saharan Africa the estimate was 370 000 (Figure 1).

**Viral Characteristics**

HIV is a human retrovirus which can be subdivided into two types: HIV-1, the most common variant throughout the world, and HIV-2 a related but less pathogenic and relatively uncommon type which is concentrated in West Africa. HIV-1 can be further subdivided into several subtypes which possess varying pathogenicity. Clade B is the most common subtype in Western Europe and the Americas. In sub-Saharan Africa subtype A was most common in the early epidemic. Nowadays the more virulent subtype C has become dominant and accounts for 55% of HIV-1 infections worldwide\textsuperscript{72}. Several other subtypes and recombinants exist and others are being discovered throughout the world.

In addition to this genetic distinction, HIV can be sub-divided by the type of co-receptor the virus uses to infect human cells. To infect a cell, the virus binds to both the CD4 receptor and to a second receptor, the so-called co-receptor, both located on the cellular membrane. Most HIV-strains have affinity for the CCR5 co-receptor and are called R5 or non-syncytium-inducing (NSI) viruses. Several other strains using different co-receptors exist of which the strain binding to the CXCR4 receptor, the X4 or Syncytium Inducing (SI) strain, is the most common\textsuperscript{73/74}. Most infections, and probably all perinatal infections, are thought to be caused by the R5 strain\textsuperscript{75}. During the course of infection some viruses may evolve to express X4 affinity\textsuperscript{72}. This viral switch is associated with a more rapid decline in CD4+ T-cells and disease progression\textsuperscript{73}. The switch to X4 affinity is a consequence of changes to the viral genome of the envelope protein gp120\textsuperscript{76}. It is best studied and most prevalent in subtype B infected persons. In subtype C infected persons the X4 strain is relatively uncommon and little studied\textsuperscript{76}.

**Natural course of HIV infection**

HIV can be transmitted sexually, through parenteral-exposure to blood or vertically from mother to child. In contrast to other viral infections, the risk of serious illness and death will increases over time in infected individuals. In adults the initial infection is followed by a symptom free interval which on average lasts 8 to 10 years. During this period CD4+ or T-helper cells, primary targets of HIV, decline. Once T-helper cells, which have an immuno-regulatory function, reach a low-threshold, the infected person becomes immunodeficient and several opportunistic infections and neoplasms may occur. This condition, called Acquired Immunodeficiency Syndrome (AIDS), will eventually, if left untreated, lead to death.
Although in children the infection follows a similar pattern, some differences are present. Firstly children are mainly infected perinatally, either in-utero, during delivery or through breast milk. All children born to HIV-infected mothers will carry antibodies which can persist up to the age of 18 months and may complicate diagnosis. If no preventive measures are taken 12-30% of exposed children in western settings will become infected. In developing countries these numbers are even higher with 25-52% of HIV-exposed children becoming infected.

Secondly children are known to have higher viral loads and more rapid progression to AIDS than adults. In western countries 15-25% of infected children die before the age of one. The majority of children (60-80%) survive longer (median 6 yrs) and a small group survives beyond the age of 10 (5%). In developing countries up to 85% of those infected follow a rapid progression and die within months after birth.

Thirdly a different classification system is used in children. Immunological staging, based on CD4+ T-cells, generally shows higher cell counts in children, is (therefore) expressed in % of total lymphocytes rather than cells per liter and uses age dependent cut offs. Clinical staging is done using guidelines specifically adapted to children. These were developed as some HIV-related conditions are relatively uncommon in children (e.g. toxoplasmosis and Cryptococcal meningitis), whilst others may not necessarily be restricted to immunodeficient children (e.g. oral candidiasis and recurrent upper respiratory tract infections). Overall these differences complicate diagnosis, staging and therefore timely and accurate treatment in HIV-infected children, especially in resource poor settings.

**Anti-Retroviral Therapy**

HIV cannot be eliminated from infected persons, and antiretroviral therapies (ART) are aimed at diminishing HIV replication. Four classes of antiretroviral agents directed against three steps in the HIV life cycle are currently available.

- **Reverse transcriptase enzyme (RT):** nucleoside and nucleotide analogs (NRTI’s) and non-nucleoside reverse transcriptase inhibitors (NNRTI’s) inhibit the function of this enzyme.
- **Fusion inhibitors** attach themselves to the envelope glycoprotein gp41 which prevent fusion between the virion and the host membrane.
- **Protease enzyme (Pro):** protease inhibitors (PIs) inhibit the function of the viral protease.

During the reverse transcription of HIV to proviral DNA many mutations occur (1/2000 bp) and drug resistance may follow. Therefore highly active antiretroviral therapy (HAART) with combinations of antiretroviral agents is applied, and the chance that the virus will develop drug resistance is minimised. Drug intolerance and drug toxicity
are significant problems for all drugs used to treat HIV infection\textsuperscript{81}. Monitoring patients based on the dynamics of CD4+ cells and viral load gives a good indication of potential resistance and disease progression\textsuperscript{78}. However these tests are costly and this restricts good monitoring of patients started on ART in sub-Saharan Africa\textsuperscript{82}. Reliable and yet affordable monitoring may be one of the most important challenges for the future of ART in Africa.

The drugs used in ART can also be applied to prevent in pregnant HIV-infected mothers transmission to their children. The PACTG076 study first described that zidovudine monotherapy reduced transmission drastically\textsuperscript{48}. Later several more practical regimens have been successfully tested in developing countries\textsuperscript{70,71,83}. Although elective caesarean section and bottle milk still are advocated in western countries, probably the most practical strategy in African countries may be maternal reduction of viral load by HAART. Despite a few gifts of ART can prevent a new infection, only a minority of pregnant women infected with HIV living in Malawi received this treatment in 2006\textsuperscript{84}.

METHODS

Study setting

Malawi is situated in south-east Africa and is bordered on the north by Tanzania, the west by Zambia, and the north and east by Mozambique (Figure 2). Malawi is approximately 900 kilometres in length and ranges in width from 80 to 160 kilometres. The country has a total area of 118,486 square kilometres, approximately three times the size of the Netherlands, of which 94,276 is land. The remaining area consists mainly of Lake Malawi, which is about 475 kilometres long and runs down Malawi’s eastern boundary with Mozambique. The climate is tropical continental with some maritime influences. Malawi has three seasons: a dry hot season from September to November, a rainy season from December to April, May to August is marked by colder dry weather. Temperature and rainfall vary with proximity to Lake Malawi and altitude. Some demographic indicators are presented in Table 1.

Landlocked Malawi ranks among the world’s least developed countries. The economy is predominately agricultural, with about 90% of the population living in rural areas. The stable diet consists of maize and to a lesser extent rice and cassava. According to the Malawi Annual and Economic survey (2001) the economy depends on substantial inflows of economic assistance from the IMF, the World Bank, and individual donor nations. Agriculture comprises 55% of gross domestic product (GDP). The main exports are tobacco, tea, coffee, peanuts and wood products.
Malawi’s health service consists of 23 District Hospitals which are surrounded by health centres that provide basic services. Health care is free of charge in the governmental institutions, although mission hospitals charge a small amount for their services. Both systems are constrained by lack of financial and human resources. Since 1998 medical doctors were able to fully complete their training in Malawi at the College of Medicine.

<table>
<thead>
<tr>
<th>Table 1. Demographic, developmental and health data on Malawi</th>
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<tbody>
<tr>
<td><strong>BASIC DATA</strong></td>
</tr>
<tr>
<td>Population</td>
</tr>
<tr>
<td>Surface area (km$^2$)</td>
</tr>
<tr>
<td>Density (pop/km$^2$)</td>
</tr>
<tr>
<td><strong>DEVELOPMENTAL AND ECONOMICAL DATA</strong></td>
</tr>
<tr>
<td>Illiteracy rate (%)</td>
</tr>
<tr>
<td>Subsistence farmers (%)</td>
</tr>
<tr>
<td>BNP per capita (US $)</td>
</tr>
<tr>
<td>Population $&lt;$ 5 years of age (%)</td>
</tr>
<tr>
<td><strong>HEALTH</strong></td>
</tr>
<tr>
<td>Life expectancy (yrs)</td>
</tr>
<tr>
<td>Under 5 mortality (1000 children)</td>
</tr>
<tr>
<td>HIV prevalence rate general population (%)</td>
</tr>
<tr>
<td>HIV prevalence rate children $&lt;$ 15 yrs (%)</td>
</tr>
<tr>
<td>Annual per capita budget on health (US$)</td>
</tr>
</tbody>
</table>

Malawi’s health service consists of 23 District Hospitals which are surrounded by health centres that provide basic services. Health care is free of charge in the governmental institutions, although mission hospitals charge a small amount for their services. Both systems are constrained by lack of financial and human resources. Since 1998 medical doctors were able to fully complete their training in Malawi at the College of Medicine.
in Blantyre. For nurses, medical assistants and clinical officers training facilities already existed in Blantyre and Lilongwe.

**Study sites**

*Blantyre* is the main commercial town of Malawi. Located in the southern region (Figure 3), it has a predominantly urban population of half a million people. At an altitude of 800m above sea level, malaria and anaemia are mainly seasonal. Its main hospital, the Queen Elizabeth Central Hospital, is not only a District and referral hospital, but also the main teaching hospital for Malawi’s only medical university: the College of Medicine. The hospital has recently renovated its Accidents and Emergency department (A&E) which caters for children presenting to hospital. During the rainy season 300 children are seen daily in this department of which 100 are admitted. All children who are admitted to hospital are routinely screened for malaria and anaemia. Reasons for admissions are presented in table 2. Individuals living in this area will endure approximately one infectious malaria bite per person per year (T Mzilahowa personal communication) and the overall HIV-prevalence in adults living in this urban area is 25%86. Blantyre was selected as a study site because of the large numbers of children with severe anaemia who present to the hospital. The SevAna study established a clinic within the A&E department staffed by two nurses, a clinical officer, two research assistants and a driver. Study patients were managed in this clinic during recruitment and follow-up visits.

*Chikwawa* district is, located 50km from Blantyre in the Shire valley (altitude 250m above sea level) and experiences malaria transmission year round (Figure 3.1). Individuals living...
in this area will endure approximately 170 infectious malaria bites per person per year (T Mzilahowa personal communication) and the adult HIV-prevalence in this rural area is estimated at 13%86. Schistosoma haematobium and hookworm infections are thought to be common among children living in this area88;89. The Chikwawa District Hospital caters for a predominantly rural population of approximately 400,000 people and its under-five clinic treats on average 86 children per day. Chikwawa was chosen as a study site due to its rural population and contrasting malaria transmission pattern to Blantyre. The SevAna study established a clinic within the paediatric ward where patients were recruited and followed-up. Several other studies on anaemia from our study group have been performed in this area.

**Study design and groups**

To improve our understanding of severe anaemia, we conducted a case-control study to assess causative factors in Malawian children with severe anaemia. Between July 2002 and July 2004 three groups of children, aged between 6 and 60 months were recruited: cases, hospital controls, and community controls. All children needed to be living within the pre-defined catchment area and should not have received a blood transfusion within four weeks prior to recruitment. Additional recruitment criteria per study group were:

**Cases:**
- Hb less than 5g/dl on admission
- Should not have trauma or malignancy as a recognised specific cause of severe anaemia

**Hospital Controls:**
- Hb greater or equal to 5g/dl on recruitment
- Should be presenting to hospital due to an illness

**Community controls:**
- Hb greater or equal to 5g/dl on recruitment
- Share same residency as a case
- Physically healthy child

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Malaria</td>
<td>32%</td>
</tr>
<tr>
<td>Respiratory tract infection</td>
<td>18%</td>
</tr>
<tr>
<td>Abscess</td>
<td>12%</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>8%</td>
</tr>
<tr>
<td>Gastro-enteritis</td>
<td>7%</td>
</tr>
<tr>
<td>Bacterial infection other</td>
<td>7%</td>
</tr>
<tr>
<td>Severe anaemia</td>
<td>4-12%</td>
</tr>
</tbody>
</table>
The aim of recruiting hospital controls was to control for factors that influence health-seeking behaviour in the population. Community controls were recruited to control for environmental factors that may influence development of severe anaemia.

Cases, hospital and community controls were recruited in a ratio of 1:1:1. Informed consent was requested from the guardians of the children if they fulfilled the inclusion criteria for the study. During recruitment, a questionnaire collecting demographic information was administered, followed by a medical history and full physical examination. Prior to administering a blood transfusion, venous blood was collected and a bone marrow aspirate was performed under local or general anaesthesia. Hospital controls were recruited the day following recruitment of a case. The first child at the front of the queue waiting to be seen by medical staff in A&E (Blantyre) and under-five clinic (Chikwawa) was selected. Community controls were recruited on discharge of a case. The case was escorted home by a member of the study team and the first child fulfilling the selection criteria at a distance of 100-1000 metres from the compound was selected. Informed consent, data and sample collection procedures were the same as in cases except for the bone marrow collection which was not performed in either control population.

Follow-up study
To determine outcome in children who experienced an episode of severe anaemia all children (also the control populations) were followed during a period of 18 months from recruitment. Follow-up consisted of scheduled visits to the study clinic (active follow-up) which took place on 1, 3, 6, 12 and 18 months from recruitment. If children did not report for their follow-up, families were visited by a study team member. In addition guardians were asked to present children in case of illness during the follow-up period (passive follow-up). At both visits a standardized history and physical examination was completed.

Main aims of severe anaemia study
To investigate in Malawian children with severe anaemia:

- Aetiological risk factors associated with progression from mild to severe anaemia.  
  **Hypothesis**: severe anaemia is not just one part of the anaemia spectrum, but has a distinctive pattern of causes.

- The contribution of the bone marrow inflammatory cytokine network to the pathogenesis of severe anaemia, and its relation to genetic determinants.

- The natural history of severe anaemia through longterm follow up after a documented episode.
  **Hypothesis**: children have differential susceptibility to severe anaemia, in relation to either inappropriate or normal bone-marrow cytokine response to infections.
Additional aims of this PhD
To study in Malawian children:

- If HIV is a risk factor for developing severe anaemia.
- The pathogenesis of HIV-associated severe anaemia.
- The use of severe anaemia as a potential predictor of mortality in HIV-infected children.
- The association between HIV and anaemia in children in different geographical settings; and to summarize currently available data on pathogenesis, aetiology and treatment.
- The erythroid response and importance of bone marrow apoptosis in severely anaemic HIV infected children.
- Potential aetiological factors of HIV-associated severe anaemia.
- Viral differences among severely or non-severely anaemic HIV infected children

Chapter overview
2. Severe anaemia in Malawian children.
5. HIV-associated anaemia in children, a systematic review from a global perspective.
7 Severe anaemia is not associated with HIV-1 env gene characteristics in Malawian children.
8 Discussion and summary.
REFERENCES


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