Iron deficiency and malaria as determinants of anaemia in African children

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Proefschrift
Ter verkrijging van de graad van doctor
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Propositions

1. The benefits of intermittent iron supplementation in anaemic children probably outweigh the associated risks of adverse effects caused by malaria.

   - This thesis.

2. Inflammatory processes and the accompanying redistribution of iron to body stores are unlikely to play important roles in the pathogenesis of anaemia associated with asymptomatic malaria.

   - This thesis.

3. Until continuous use of contraceptive tablets is proven sufficiently safe, monthly menstruation should be considered as the Holy Grail of womanhood ;-)

   - Against: Thomas SL, Ellertson C. Nuisance or natural and healthy: should monthly menstruation be optional for women? Lancet 2000; 355: 922-24.

4. It is unethical, of course, for a scientist to do a physician's job. Experience shows that it is also unethical for a physician to do a scientist's job.

   - In response to: Altman DG. The scandal of poor medical research. BMJ 1994; 308: 283-84.

5. Research does not have to be original to be worthwhile.

   - In response to: Sommer A. Moving from science to public health programs: lessons from vitamin A. Am J Clin Nutr 1998; 68 Suppl: 513-16.

6. Statistics is an anvil on which the scientific mind is bashed into shape.

7. Development aid fails because those who receive will never own.

8. The cage of poverty and repression is usually held shut by those inside.

Propositions pertaining to the thesis 'Iron deficiency and malaria as determinants of anaemia in African children'

Hans Verhoef
Wageningen, 7 September 2001

Stellingen

1. De baten van regelmatig (elke 4 weken) toedienen van ijzer aan kinderen met bloedarmoede zijn waarschijnlijk zwaarwegender dan de risico's van bijwerkingen als gevolg van malaria.

   - Dit proefschrift.

2. Onstekingsprocessen spelen waarschijnlijk geen belangrijke rol bij het ontstaan van bloedarmoede van asymptomatische malaria. Dit geld evenzeer voor de herverdeling van lichaamsijzer naar opslagcompartimenten waarmee ontsteking vergezeld kan gaan.

   - Dit proefschrift.

3. Totdat is aangetoond dat het doorlopend gebruik van de pil voldoende veilig is, dient maandelijkse ongesteldheid beschouwd te worden als de Heilige Graal van vrouw-zijn ;-)

   - Tegen: Thomas SL, Ellertson C. Nuisance or natural and healthy: should monthly menstruation be optional for women? Lancet 2000; 355: 922-24.

4. Het is uiteraard onethisch voor een wetenschapper om het werk te doen van een arts. De ervaring leert dat het ook onethisch is voor een arts om het werk van een wetenschapper te doen.

   - Naar aanleiding van: Altman DG. The scandal of poor medical research. BMJ 1994; 308: 283-84.

5. Onderzoek dat niet origineel is, kan toch de moeite waard zijn.

   - Naar aanleiding van: Sommer A. Moving from science to public health programs: lessons from vitamin A. Am J Clin Nutr 1998; 68 Suppl: 513-16.

6. Statistiek is een aambeeld waarop de wetenschappelijke denkwijze zijn beslag krijgt.

7. Ontwikkelingshulp faalt omdat zij die krijgen nooit zullen bezitten.

8. De kooi van armoede en onderdrukking wordt gewoonlijk dichtgehouden door hen die daarin opgesloten zijn.

Stellingen behorend bij het proefschrift 'Iron deficiency and malaria as determinants of anaemia in African children'

Hans Verhoef
Wageningen, 7 september 2001

Abstract

Iron deficiency and malaria as determinants of anaemia in African children.
PhD thesis by Hans Verhoef, Division of Human Nutrition and Epidemiology,
Wageningen University, The Netherlands, 7 September 2001.

Approximately three quarters of east African children <5 y of age suffer from anaemia, which is due, at least in part, to malaria and iron deficiency. In children in areas of seasonal malaria, the benefits of iron supplementation may not outweigh possible inherent risks of adverse effects caused by malaria. Intermittent administration of sulfadoxine-pyrimethamine (SP) might improve haemoglobin concentrations while allowing children to develop protective immunity against severe disease and subsequent death caused by malaria. With a view to contribute to the development of programmes for anaemia control in preschool children in Africa, the immediate objectives of this thesis were as follows: 1) to measure the efficacy in improving haemoglobin concentrations in children aged 2-36 mo of intermittent iron supplementation and intermittent administration of SP, either alone or when given in combination; 2) to develop and evaluate survey methods for rapid assessment at community level of the burden of anaemia and its risk factors; 3) to contribute to improved methods for diagnosis of anaemia, iron deficiency, and malaria; 4) to evaluate the role of impaired erythropoiesis in the pathogenesis of malarial anaemia.

In randomised controlled trial (n=328) in anaemic, asymptomatic children aged 2-36 mo, the effect on change in haemoglobin concentration in the group receiving iron plus sulfadoxine-pyrimethamine relative to the placebo group, adjusted for prognostic factors at baseline, was 12.5 g/L (95% CI: 8.5 to 16.4 g/L). In the former group, the estimated prevalence of anaemia reduced from 100% at baseline to 36% at 12 w, and prevalence of iron deficiency reduced correspondingly from 66% to 8%. Administration of SP in addition to iron supplementation gave no haemoglobin response. Survival analysis indicated no evidence of increased risk of malaria following iron supplementation. Iron supplementation over a 12-week period resulted in a marked improvement of haemoglobin concentrations.

Analysis of haematological indicators from both the trial and a cross-sectional study (n=318) suggested that malaria-induced haemolysis is accompanied by increased erythropoiesis, which seemed adequate for the resulting degree of anaemia. Serum transferrin receptor concentration is not useful to detect iron deficiency in individuals with malaria. Inflammation probably plays no or a minor role in the pathogenesis of anaemia associated with asymptomatic malaria.

A new and inexpensive colour scale to facilitate anaemia diagnosis in developing countries performed satisfactory and was for many purposes superior to all other methods for detection of anaemia at primary care level. The methodology presented may facilitate development of strategies for its use in various target groups. A large proportion of unnecessary treatments for febrile diseases can be avoided by teaching mothers to palpate their child's forehead, and by confirming the presence of fever by thermometer.

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1

Introduction

1. Infection and malnutrition in African children

Few children from poor families in developing countries escape severe health challenges posed by infections and malnutrition. Both infections and inadequate supply of nutrients may initiate a vicious circle of deteriorating health (**figure 1**). Infections lead to growth faltering and malnutrition by causing anorexia, loss of nutrients, changes in metabolism and malabsorption, and changes in feeding practices. Conversely, protein-energy malnutrition and deficiency in micronutrients such as iron, vitamin A and zinc are known to adversely affect immunity (Dallman 1987, Ross 1996, Wellinghausen et al. 1997, Semba 1998, Shankar and Prasad 1998, Fraker et al. 2000). This in turn can increase the incidence, severity and duration of disease.

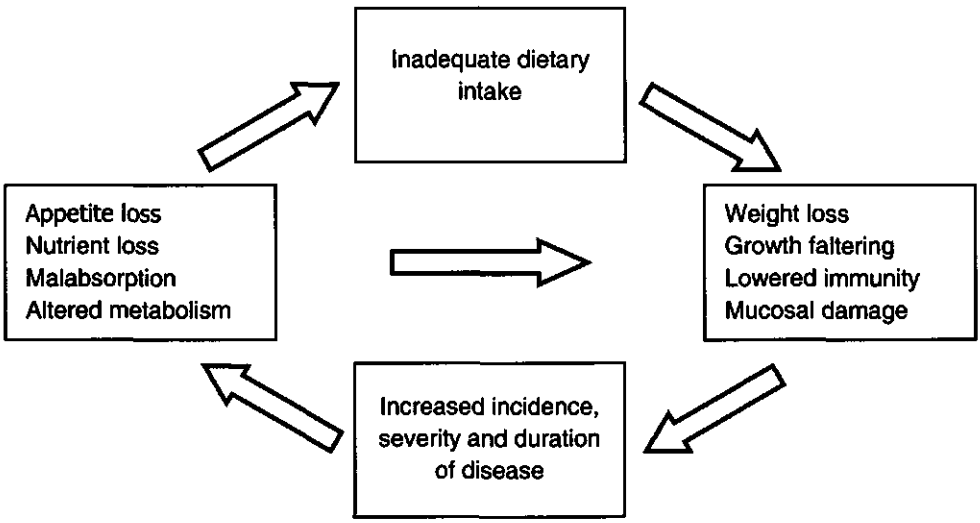


FIGURE 1. The infection/malnutrition cycle (Tomkins and Watson 1989)

One in 16 children born in developing countries dies before the age of 1 y, and one in 11 dies before the age of 5 y. In sub-Saharan Africa, these probabilities are one in nine and one in six, respectively (1998 data; UNICEF 2000). The infection/malnutrition complex typically accounts for much of this mortality. Of the estimated 12 million deaths in children under 5 y in developing countries, 50% are due to four conditions – acute respiratory infections, diarrhoea, measles and malaria – and 54% have been attributed directly or indirectly to malnutrition (**figure 2**).

The health consequences of malnutrition and infections depend on the nutrients and pathogens involved. This chapter examines iron deficiency and malaria as determinants of anaemia and growth faltering in African children. For this purpose, the following topics will be reviewed: the public health importance of growth faltering, anaemia, iron deficiency,

and malaria; the causal relationships between these factors, especially in relation to the aetiology and pathogenesis of anaemia; and the possibilities for interventions directed at solving the anaemia problem. Particular emphasis will be given to preschool children living in Kenya, because these are the subjects of studies described in subsequent chapters. Anaemia and malaria in pregnant and lactating women are discussed as determinants of anaemia and growth of their children.

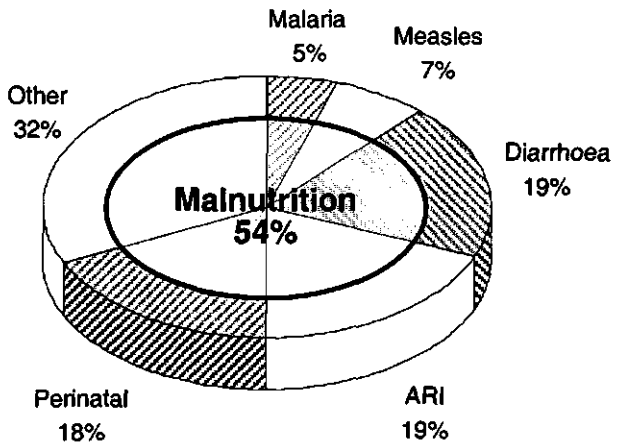


FIGURE 2. Relative contribution of infections and malnutrition to mortality in children below 5 y of age (Gove 1997)

2. Growth faltering

Anthropometric indicators of child growth

Children in developing countries generally fail to achieve their genetically determined potential height because of poor diet and infection (Martorell et al. 1988, Ulijaszek 1994, Waterlow 1994). Because short stature and thinness are believed to indicate poor health, they are often referred to as stunting and wasting, respectively. For example, growth retardation has been associated with an increased incidence, severity and duration of disease (Scrimshaw et al. 1968, Black et al. 1984).

To compare height and weight distribution between populations, children are usually compared to a reference population of similar age and sex who have grown up in a relatively well-nourished and infection-free environment (the NCHS population: WHO 1983). Thus, shortness and thinness can be measured by height-for-age and weight-for-height z-scores (HAZ and WHZ), respectively. A z-score with value 0 (nought) indicates the median of the NCHS reference population, while children with HAZ or WHZ below -2 standard deviations of the median of the NCHS reference population are generally considered to be moderately to severely stunted or wasted, respectively (WHO Working Group 1986, WHO 1995).

Stunting and wasting are used as markers of malnutrition (**figure 3**). As such they are justifiable targets for intervention. Their role in causing morbidity and mortality has long been suspected, but is difficult to demonstrate (Waterlow 1994).

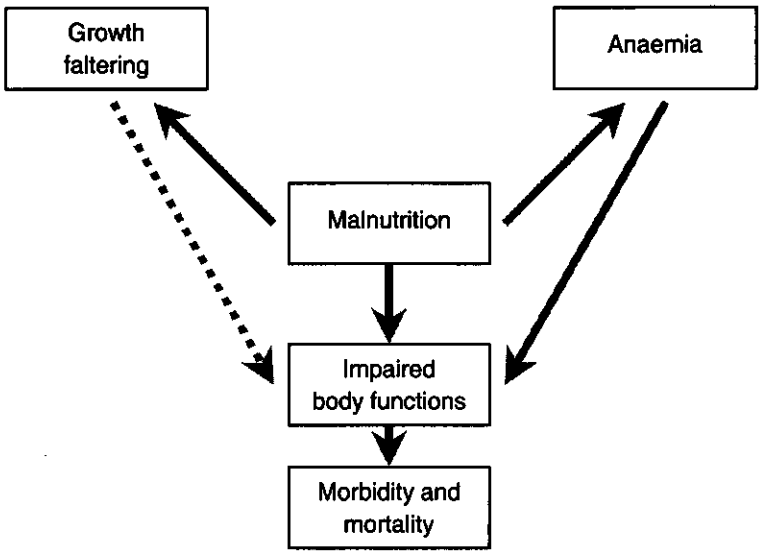


FIGURE 3. Hypothesised role of growth failure, anaemia and malnutrition as associated causes of morbidity and mortality

Both growth failure and anaemia are considered to be outcomes and markers of malnutrition. There is solid evidence that both malnutrition and anaemia may contribute to morbidity and mortality by impairing body functions. Such a causal role has also been suspected for growth failure, but is difficult to demonstrate. Note the absence of a causal link between growth failure and anaemia (see text for further explanation).

Magnitude of the problem

An estimated 182 million children – or one-third of all children <5 y of age in developing countries – are stunted (ACC/SCN 2000). The highest prevalence is found in eastern Africa, where 48% of children in this age range is stunted, and which is the only region in the world where its prevalence is rising (ACC/SCN 2000). Because severe wasting is usually a transient condition, its frequency of occurrence is preferably measured by its incidence rather than by its prevalence. Unfortunately, no such incidence estimates are available.

Causes and mechanisms

Stunting and wasting represent different processes of malnutrition (WHO Working Group 1986, WHO 1995). Stunting indicates slowing in skeletal growth, which may not be evident for years. It takes a long time to correct even in favourable environments, and is the cumulative effect of low or inadequate intake of energy, macronutrients or micronutrients over a long period, or results from chronic or frequent infections. Stunting does not

necessarily indicate low growth rates, as shown by the fact that stunted school children usually grow at rates similar to the NCHS reference population. The stunting process starts in the first few months of life and slows down at around 3 y of age (WHO 1995).

Wasting results from failure to gain weight or from weight loss. It usually occurs after recent and severe starvation and/or disease, and peaks in the second year of life (WHO 1995). Severe wasting can develop rapidly and generally can be restored easily after refeeding (Young and Jaspers 1995). Wasting and stunting do not necessarily result from the same nutritional deficiencies, as is evident from the fact that stunting usually starts earlier in life than wasting.

3. Anaemia

Manifestations in children

Anaemia occurs when bodily functions are impaired as a result of low haemoglobin concentrations. It develops when the rate of red cell destruction exceeds the capacity of the bone marrow to mount a compensatory increase in production. In children aged 6-60 mo, anaemia is indicated and has been operationally defined by haemoglobin concentrations <110 g/L when measured at sea level (WHO/UNICEF/UNU 1997).

The main function of red cells is to carry haemoglobin-bound oxygen to tissues and return carbon dioxide from tissues to the lungs. Symptoms of anaemia are usually shortness of breath, weakness, lethargy, palpitations and headaches. Anaemia causes decreased physical activity, fitness and work capacity (Haas and Brownlie 2001). The severity of symptoms depends on the degree of anaemia and the rate of its development. Adaptation may occur in the cardiovascular system (with increased heart rate and stroke volume) and in the dissociation characteristics of haemoglobin, so that oxygen is more readily given up to the tissues (Hoffbrand and Pettit 1993). Rapid haemolysis – as may occur during acute febrile malaria attacks – may cause severe anaemia with little time for adaptation. Children with severe anaemia may eventually die. Although it is commonly believed that breathing difficulty in a severely anaemic child is due to 'congestive heart failure', recent studies in Kenyan children hospitalised for malaria suggest that death in severely anaemic children may be rather due to acidosis (English et al. 1997, English 2000, WHO 2000c). Particularly in developing countries, blood transfusions administered to patients with severe anaemia also carries an increased risk of HIV infection (Hedberg et al. 1993, Fleming 1997, Jager et al. 1990, Ryder et al 1992). Mild anaemia often produces no symptoms. In areas of high malaria endemicity even children with haemoglobin concentrations <60 g/L can be asymptomatic, presumably after prolonged exposure to chronic or repeated infection with low levels of parasitaemia (see below).

Magnitude of the problem

Approximately half of children <12 y and women of child-bearing age in developing countries suffer from anaemia. Children <5 y and pregnant women are at highest risk with estimated prevalences of 51% and 59%, respectively. In eastern Africa, the prevalence of anaemia in children under 5 y has been estimated at 75% (DeMaeyer and Adiels-Tegman

1985). Anaemia is an important cause of childhood death in developing countries, and estimates that 20% of maternal deaths in Africa are attributable to anaemia (Ross and Thomas 1996) are probably conservative (Gillespie 1998).

Causes and mechanisms

There is little support for the common misinterpretation that anaemia is a cause of poor growth. Anaemia usually indicates malaria or other underlying infections, deficiencies of iron and other nutrients, or genetic disorders. These factors may cause serious adverse health effects, including impaired physical growth and mental development, reduced physical activity, and death. Thus, anaemia is both an outcome and marker of malnutrition, and also contributes directly to morbidity and mortality (**figure 3**). Successful interventions to control anaemia are therefore assumed to lead to better health.

Anaemia in developing countries may be caused by deficiencies in iron, folate, vitamins A and B_{12}, infections such as malaria, HIV infection and tuberculosis and inherited red cell disorders (Fleming 1994). Their relative contribution to anaemia depends on age, season, geographical area, and other factors, reflecting local and time-dependent patterns in infection and nutrient availability. For most of these determinants, major gaps exist in our understanding of the pathogenic mechanisms involved, and in our knowledge about the degree to which they interact in producing anaemia. The following sections will first discuss normal erythropoeisis and iron metabolism, and then review iron deficiency and malaria as determinants of anaemia in African children.

4. Erythropoiesis and iron metabolism

Red cell development and its regulation

The life expectancy of red cells is normally about 120 d, and in adults the marrow must normally produce 200 billion red cells every day to replace those lost to senescence or damage (Hoffbrand and Pettit 1993). Erythropoiesis is the continuous process whereby primitive erythroid progenitor cells proliferate and differentiate into mature, circulating red cells (reviewed by Brittenham 1994). This process is principally regulated by erythropoietin, a hormone predominantly produced in the kidney in response to hypoxia. In normal conditions, increased production of erythropoietin results in erythroid hyperplasia and reticulocytosis. The biological effects of erythropoietin and other growth factors are mediated through specific receptors on target cells. One action of erythropoietin is to stimulate survival of erythroid progenitor cells by inhibiting apoptosis (gene-directed cell death).

The uptake of serum iron that is required for haem synthesis within the erythroblast is mediated and regulated by a specific receptor located on the outer cell membrane (Cook et al. 1994, 1996). The increased expression of these receptors on the membrane of the developing red cells follows the increased expression of erythropoietin receptors but precedes the onset of cellular haem synthesis. Erythropoiesis is not entirely efficient, because 10-15% of developing erythroblasts normally die within the marrow without producing mature cells, whereupon they are ingested by marrow macrophages. Red cells

are taken out of circulation via phagocytosis by macrophages belonging to the mononuclear phagocyte system and resident mainly in the spleen, but also in the liver, marrow and muscle.

Iron metabolism and movement

Body iron comprises metabolically active iron that is required for normal functions, storage iron that constitutes a reserve upon which the body can draw, and transport iron (**figure 4**). Most body iron is incorporated into haemoglobin. The remainder of the metabolically active iron is found as myoglobin iron in muscles and as iron-containing or iron-dependent enzymes throughout the cells of the body. Iron released by macrophages from degraded haemoglobin into serum is bound to the transport protein transferrin and can be recycled for erythropoiesis or used for other functions. Alternatively, macrophages can hold iron in storage by binding it to the proteins ferritin or haemosiderin. Storage iron in the mononuclear macrophage system is derived almost entirely from phagocytosis of senescent red cells or defective developing red cells. The liver also holds ferritin-bound iron in reserve in hepatocytes. Most of the iron used for erythropoiesis in the bone marrow is derived from recycled iron, and iron supplied by absorption through the intestine makes up only a small fraction of the total amount of iron that is used for this purpose. No physiological means of iron excretion exist. Iron absorption from the gastrointestinal tract is the sole means of regulating iron stores.

Cellular uptake of iron from the circulation takes place by binding of the transferrin-iron complex to receptors in the cell membrane, followed by receptor-mediated endocytosis and by dissociation of iron from apotransferrin and its transport across the vesicle membrane into the cytoplasm by unknown carrier mechanisms (Pollack 1992a). Endocytic vesicles recycle to the cell surface where apotransferrin dissociates from transferrin receptors. The number of transferrin receptors expressed on the cell membrane is the prime determinant of cellular iron supply. Cellular synthesis and expression of transferrin receptors is primarily regulated by intracellular iron concentration, and is coupled to ferritin synthesis (Casey et al. 1988, Melefors and Hentze 1993). In cellular iron deficiency, translation of ferritin-coding mRNA is repressed, whilst translation of mRNA coding for transferrin receptors is accelerated. Conversely, when cellular iron is in excess, synthesis of ferritin is increased and production of transferrin receptors is decreased. In addition, there is evidence that erythropoietin increases iron uptake into erythroid progenitor cells via post-transcriptional induction of transferrin receptor expression on the cell surface (Weiss et al. 1997).

Iron status during infancy and childhood

The intra-uterine environment is characterised by relative hypoxia, which results in high haemoglobin concentrations at birth. Thereafter, oxygenation of the blood improves markedly, leading to a cessation of erythropoiesis within days after delivery (Halvorsen 1963, Clark and Roche 1973). Haemoglobin concentrations drop dramatically over the first 2 mo of life – from 166 g/L to 112 g/L (Saarinen and Siimes 1978) – as a result of growth-related haemodilution and elimination of red cells that have reached the end of their life

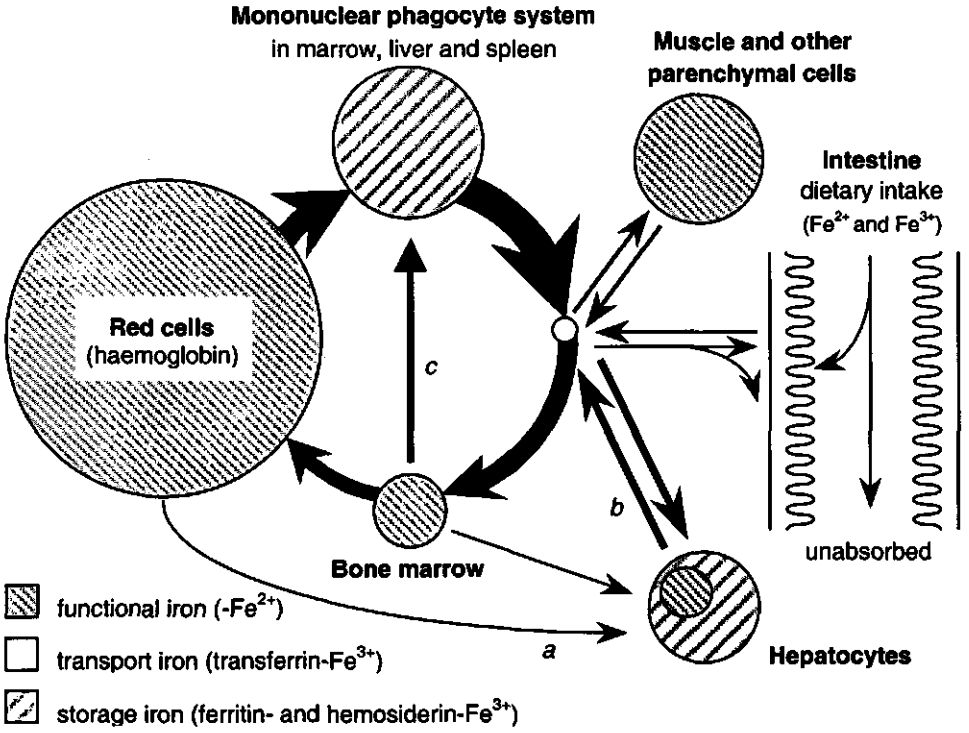


FIGURE 4. Body iron distribution and movement in a healthy adult (adapted from Brittenham 1994)

The area of each circle is proportional to the amount of iron contained in the compartment, and the width of each arrow is proportional to the daily flow of iron from one compartment to another. The mononuclear-phagocyte system is part of the immune system and comprises a diffuse network of macrophages originating from circulating monocytes and attached to the epithelium of various organs where they act as a 'sieve' for pathogens and foreign antigens. Note that most of the iron used for erythropoiesis in the bone marrow is derived from recycled iron, and that iron supplied by absorption from the intestine makes up only a small fraction of the total amount of iron that is used for this purpose. Normally, less than 0.5% of the total body iron is acquired or lost each day. Also note that the storage iron in the mononuclear phagocyte system is derived almost entirely from phagocytosis of senescent red cells or defective developing red cells.

Malaria can influence iron distribution and movement in several ways. Haemoglobin iron lost because of malaria-induced haemolysis can be bound to haptoglobin or haemopexin and removed from the plasma by hepatocytes (a) for eventual return to plasma transferrin (b). In addition, ineffective erythropoiesis may occur, whereby developing red cells are prematurely phagocytosed (c).

span. Dietary iron absorption is minimal during this period (FAO/WHO 1988). Iron stores, as indicated by serum ferritin concentrations, rise in the first few weeks of life as haemoglobin is broken down, but then drop as the child continues to grow and more iron

is needed for erythropoiesis and other functions. By the age of 4-6 mo, iron stores are marginal or become depleted. Iron deficiency seldom occurs before that period unless the child is born prematurely, or if the mother had severe iron deficiency anaemia during pregnancy. Mild maternal iron deficiency anaemia has few repercussions on the iron status of the newborn (NAS 1991). Normally after 4-6 mo, iron absorption and iron loss become the critical factors for maintaining normal haemoglobin concentrations. In infancy, all the bone marrow is erythropoietic and reddish in appearance, but during childhood this is progressively replaced by fat in the long bones. This fatty marrow is capable of reversion to haemapoiesis under conditions of anaemia (erythroid hyperplasia).

5. Iron deficiency

A large proportion of the world's children lives in a state of precarious iron balance because of low dietary intake of absorbable iron. Relatively large amounts of iron are needed for erythropoiesis during periods of bodily growth, while iron supply is limited by the intestinal capacity to absorb iron. The decline in body iron is first marked by the depletion of iron stores, which indicates the onset of iron deficient erythropoiesis. At this stage, iron absorption is insufficient for the bone marrow to mount a compensatory increase in haemoglobin production to counter growth-related haemodilution and losses due to physiological and pathological processes. Haemoglobin synthesis starts to become impaired and haemoglobin concentrations fall. When anaemia ensues, the iron deficiency may be severe enough to lead to distortion of newly produced red cells, with microcytosis and hypochromia.

Iron demand may be further increased by losses due to chronic bleeding from intestinal wounds caused by schistosomal or intestinal worms. School children are most likely to suffer the heaviest worm infections and the associated morbidity. Considerable variation has been observed in African preschool children from different geographical locations in the prevalence of intestinal worm infections (Brooker et al. 1999, Hautvast et al. 2000, Stoltzfus et al. 2000, Tshikuka et al. 1997). This variation is probably due to differences in exposure frequency as determined by behaviour, soil microclimate and other factors.

Manifestations in children

Iron deficiency in children, when sufficiently severe to cause anaemia, has been associated with decreased growth rates (Allen 1994, Chwang et al. 1988, Latham et al. 1990, Lawless et al. 1994), decreased appetite (Lawless et al. 1994), impaired immune function (Dallman 1987, Brock 1994, Oppenheimer 2001) which probably leads to an increased risk of certain infectious diseases, reduced physical activity (Haas and Brownlie 2001) and possibly impaired performance in a range of mental and physical coordination functions (Gillespie 1998, Grantham-McGregor and Ani 2001). The latter include cognition (awareness and judgement abilities), social and emotional development and school achievement (Draper 1997, Nokes et al. 1998). Impaired mental and physical coordination functions may be more difficult to correct in infants and toddlers than in older children (Draper 1997), and possibly develop already in children suffering from iron deficient erythropoiesis (Bruner et al. 1996). Girls with deficiencies in iron and other nutrients may have insufficient pelvic

development, which has been associated with increased risks of subsequent obstetric complications and maternal mortality (Konje and Ladipo 2000). There is little empirical evidence of the importance of iron deficiency anaemia as a cause of death in African children, in part because of the difficulties in separating these deaths from those caused by malaria-associated anaemia.

Magnitude of the problem

Young children and women of childbearing age are at greatest risk of iron deficiency, particularly those living in areas where cereals are the staple food, little meat is eaten, dietary intake of ascorbic acid is low, and high intakes of phytates (e.g. in unprocessed cereals) and polyphenols (e.g. tannates in tea) impair iron absorption. A recent estimate of more than 3.5 billion people being affected by iron deficiency globally (UNICEF/UNU/WHO/MI 1999) is probably an overestimate (Stoltzfus 2001), and may furthermore be inaccurate because of difficulties of measuring iron status in developing countries (Cook 1994).

6. Malaria

Malaria in humans is caused by four species of protozoa. *Plasmodium falciparum* is by far the most pathogenic and accounts for the majority of infections, whereas the other three species seldom lead to death. *P. malariae* and *P. ovale* only have local or regional importance, while *P. vivax* occurs rarely in sub-Saharan Africa. Contrary to *P. vivax* and *P. ovale*, there are no dormant liver stages of *P. falciparum* that upon reactivation lead to recurrences. Chemotherapy aimed at asexual blood stages can successfully eradicate *P. falciparum* infection. Recrudescence or persistence of parasitaemia and symptoms may occur, however, following insufficient intake or malabsorption of antimalarial drugs, or following treatment of patients infected with drug-resistant parasite strains. Prophylactic or therapeutic use of antimalarials in individuals with drug-resistant strains probably contributes to anaemia as a public health problem. Chloroquine resistance emerged in east Africa in 1978 and is now widespread (Charmot et al. 1991). Several countries in the region, including Kenya, have included sulfadoxine/pyrimethamine (SP) in their policy for first-line treatment of uncomplicated malaria, but this is not yet widely practised. Resistance to SP has been reported in many areas, but the occurrence and level of resistance vary between and within countries.

Life cycle

Malaria parasites are transmitted with the saliva of biting mosquitoes of the genus *Anopheles*. Within half an hour following inoculation by the mosquito, the parasites disappear from the blood and enter parenchymal cells of the liver, where they multiply and re-enter the blood circulation as merozoites. They then enter red cells, where they develop into trophozoites that feed on haemoglobin. Following parasite multiplication, infected erythrocytes burst and newly released merozoites invade fresh erythrocytes, in which a subsequent generation of parasites is produced by the same process. Some merozoites may develop into sexually differentiated forms (gametocytes), which are taken up by

mosquitoes, where they undergo further development, mate and eventually make their way to the salivary glands.

Manifestations of malaria

African children are protected to some degree from malaria by maternal antibodies that are conveyed during pregnancy, or by immunity that develops following repeated or chronic exposure to infection. There is evidence that immunity against severe disease and subsequent death from malaria develops much faster than that against fever and parasite densities (Snow and Marsh 1998, Gupta et al. 1999). Thus, in endemic areas, the most vulnerable period occurs during early childhood, when maternal antibodies have waned and protective levels of immunity have not yet developed (Snow and Marsh 1998). For the same reason, parasitaemia may occur without symptoms or signs other than splenomegaly and some degree of anaemia (McElroy et al. 2000). For example, in western Kenya (Asembo Bay, Nyanza Province), where children are typically exposed to 100-300 infective mosquito bites per year, 70% of children aged 2-36 mo were parasitaemic (Ter Kuile, personal communication, 2001). In less endemic areas, the vulnerable age starts earlier and ends later, and the prevalence of parasitaemia is considerably less. Little is known about the functional consequences of mild-to-moderate anaemia caused by malaria other than that it probably leads to reduced physical activity (Haas and Brownlie 2001).

Uncomplicated malaria is characterised by febrile attacks that may be accompanied by symptoms of low specificity such as chills, headache, dizziness, pain in the back and limbs, malaise, anorexia, nausea, sweating and vomiting (Gilles and Warrell 1993). The level of parasitaemia is variable but has been associated with the severity of symptoms. Anaemia is a common feature, and may continue to develop for up to two weeks after parasite clearance (Menendez et al. 2000). When left untreated, uncomplicated falciparum malaria may rapidly (<48 h) progress to severe (acutely life-threatening) malaria and death. Demonstration of parasitaemia has low diagnostic value in highly endemic areas, because many diseases resemble malaria in their manifestations and asymptomatic infection is common. Several new, rapid dipsticks allow for detection of proteins specifically produced by *P. falciparum*, and of proteins produced by all four human malaria parasite species (WHO 2000b).

Malarial deaths have been contributed mostly due to two overlapping syndromes, namely cerebral malaria and severe anaemia (Beales et al. 2000). Hospitalisation for severe anaemia occurs mostly in children <3 y of age, whilst peak hospitalisations for cerebral malaria occurs somewhat later, at 2-7 y of age (Brewster et al. 1990, Snow et al. 1994). Findings from recent studies indicate that metabolic acidosis plays a major role in the pathogenesis of severe disease and is particularly important in the overlap between the aforementioned syndromes (Marsh et al. 1995, Marsh and Snow 1997, English 2000). In cerebral malaria, persisting coma and death follow a history of convulsions, impaired consciousness, and other manifestations of central nervous dysfunction. Severe malarial anaemia may develop after fulminant infection of relatively short duration, or following chronic or repeated infection. In the latter case, considerable adaptation may have taken place (see above). For this reason, severely anaemic but asymptomatic children may be found in community surveys, often with low levels of parasitaemia. The incidence of severe

malarial anaemia is probably increasing with the spread of parasite strains that are resistant to antimalarial drugs (Bloland et al 1993, Campbell 1991).

Magnitude of the problem
Malaria has been estimated to cause 300-500 million symptomatic attacks and over 750.000-1 million deaths per year globally (Snow et al. 1999, WHO 2000a). Perhaps 90% of malarial attacks and deaths occur in children in sub-Saharan Africa (WHO 1998). Most community-based studies have shown a malaria-associated reduction in haemoglobin concentration of 10-20 g/L (Brabin 1992).

7. The pathogenesis of malaria-associated anaemia

Several pathogenic mechanisms have been described to explain the anaemia of malaria (Weatherall et al. 1982, 1983, Menendez et al. 2000), although detailed knowledge is lacking and the relative importance of these mechanisms in various presentations of malaria is poorly understood.

Genetic red cell disorders
Abnormal variants exist of haemoglobin and certain enzymes involved in red cell metabolism. In tropical areas, these variants may occur in polymorphic gene frequencies, presumably because they confer some protection against malaria in heterozygote individuals. The degree to which some variants may cause anaemia depends on genotype and environmental factors.

Glucose-6-phosphate dehydrogenase (G6PD) is needed in red cells to prevent oxidative damage. The presence of certain abnormal genetic variants of G6PD in red cells is associated with low activity or concentration of this enzyme, and this may cause oxidative stress in the presence of certain oxidant nutrients, drugs or infections. When induced by malaria parasites, this oxidative stress is believed to be the prime mechanism whereby infected red cells are selectively cleared and G6PD deficiency confers partial protection against malaria. Two abnormal variants (G6PD types A and A^-) occur in high frequencies in sub-Saharan Africa (Bienzle 1981). The A^--variant occurs in gene frequencies of 10%-25%. Only these genotypes have markedly lower erythrocyte G6PD activity, but even individuals with homozygote or hemizygote G6PD A^- deficiency have little chronic haemolysis and only marginally lower haemoglobin concentrations (May et al. 2000). Chloroquine does not usually cause haemolysis in G6PD A^- Africans (Abdalla and Weatherall 1987). Thus, it would appear that G6PD deficiency – at least in the absence of abnormal causes of stress – is not an important cause of anaemia in sub-Saharan Africa.

Sickle cell disorders occur in high frequencies in parts of Africa. The manifestations of sickle cell anaemia result from the presence of two abnormal β-chains in the haemoglobin molecule (HbSS). The prevalence of sickle cell anaemia in community surveys is low in developing countries because children born with this condition usually die at an early age. Sickle cell trait (HbAS) is believed to confer partial protection against malaria, and its prevalence may reach 40% in some areas that are highly endemic for malaria (Beutler

1995). This condition is usually asymptomatic and has been associated with only marginal degrees of anaemia (Rana et al. 1993).

Thalassaemias are inherited disorders of the rate of synthesis of globin chains of haemoglobin. Although β- and α^0-thalassaemia are relatively rare in Africans and their descendants outside Africa, α^+-thalassaemia is prevalent in high frequencies in parts of Africa (Falusi et al. 1987, Hill 1992, Mockenhaupt et al. 1999, Mouele et al. 2000, Muklwala et al. 1989, Serjeant et al. 1986). A study on the Kenyan coast found two-gene deletion α-thalassaemia in 26 of 57 subjects (46%) studied without sickle cell disorders (Ojwang et al. 1989). Detection of both heterozygotes and homozygotes for α^+-thalassaemia is difficult, because neither present with symptoms or signs other than perhaps mild anaemia (Mockenhaupt et al. 1999). Such anaemia can be microcytic and hypochromic, and is easily mistaken for iron deficiency (Mockenhaupt et al. 1999, Weatherall and Provan 2000). Conventional electrophoresis of blood samples does not detect these conditions, and the diagnostic tests required are specialised and available only in some laboratories. Thus, although the importance of α-thalassaemia is often overlooked, it probably contributes to the burden of anaemia in large parts of Africa, and may lead to overestimates of the prevalence of iron deficiency anaemia.

Increased red cell destruction

Haemolysis occurs intravascularly following rupture of parasitised red cells and by sequestration and phagocytosis not only of parasitised but also non-parasitised red cells in the mononuclear phagocyte system in the spleen and other organs. With uncomplicated falciparum malaria, anaemia usually develops within 48 h after onset of fever (Menendez et al. 2000). A moderate reduction in survival of red cells has been observed in various inflammatory conditions, and is believed to play a minor role in the anaemia of chronic disease (Sears 1992). This appears to be due to increased clearance of non-senescent red cells rather than to intrinsic red cell defects (Jurado 1997, Feelders 1999). In addition, fever may contribute to decreased red cell survival (Karle 1974).

P. falciparum preferentially but not necessarily exclusively invades young red cells (Pasvol et al. 1980, Pasvol and Wilson 1982), and animal studies have shown that reticulocytosis may favour the proliferation of malaria parasites (Zuckerman 1958). This has several important implications (Weatherall and Abdalla 1982). First, it may provide a mechanism that contributes to the observed low incidence of malaria in the first few months of life: erythropoiesis is markedly reduced in the first two months after birth and hence there are relatively few young red cells in circulation. Second, individuals with raised reticulocyte counts, which may occur following iron supplementation, may be particularly prone to malaria attacks. Third, transient depression of bone marrow activity as seen in chronic infections (see below) may serve to limit parasite proliferation.

With mild haemolysis, iron released from red cells at the end of their shortened life span is usually effectively recycled by phagocytic macrophages for erythropoiesis. There is erythroid hyperplasia but delivery of mature red cells to the circulating blood is effective. When there is substantial haemolysis, free haemoglobin or haem released into the blood stream are bound to haptoglobin and haemopexin, respectively. Haemoglobin-haptoglobin

and haem-haemopexin complexes are removed from the plasma by hepatocytes (**figure 4**; line *a*), and iron thus recovered can eventually be returned to plasma transferrin and recycled. Free haemoglobin rapidly saturates serum haptoglobin, in which case the excess free haemoglobin exceeds the renal reabsorptive capacity. Thus, haemoglobin can be excreted through urine (haemoglobinuria), but iron loss through this mechanism is probably of minor importance in most acute infections.

Decreased red cell production

Malaria causes a shift of iron distribution from functional towards storage compartments. Thus, serum iron concentrations, iron-binding capacity and serum transferrin saturation are all decreased (Das et al. 1997), while – contrary to iron deficiency – serum ferritin concentrations are increased (Das et al. 1997) and stainable iron may be present in bone marrow (Abdalla 1990a, Phillips et al. 1986). Reticulocyte counts are normal or increased (Abdalla et al. 1980, Abdalla 1990a, Abdalla and Wickramasinghe 1988, Phillips et al. 1986), but appear inappropriately low for the degree of anaemia (Abdalla et al. 1980). These characteristics all indicate that erythropoiesis is decreased, or increased but inappropriately low for the degree of anaemia. This is further supported by ferrokinetic studies and morphological studies on bone marrow aspirates in patients with acute malaria (Srichaikul et al. 1969, Abdalla et al. 1980, Abdalla 1990b, Wickramasinghe et al. 1982).

Anaemia and the laboratory findings described in the preceding paragraph are observed in malaria and a range of other infections. This suggests that the pathogenic mechanisms underlying the anaemia of chronic disease (Sears 1992, Means and Krantz 1992, Konijn 1994, Jurado 1997, Weiss 1999, Spivak 2000) also play a central role in the development of malarial anaemia. The anaemia of chronic disease is usually mild but can be severe (Sears 1992) and – despite the adjective 'chronic' – may develop rapidly (Olivares et al. 1989). Evidence is accumulating that it is a consequence of simultaneous suppression of both erythropoietin production and the ability of erythroid progenitor cells to proliferate in response to erythropoietin (Means and Krantz 1992, Spivak 2000). Both effects are probably mediated by proinflammatory cytokines such as tumour necrosis factor (TNF) and interleukin (IL)-1.

It is commonly assumed that infections lead to iron sequestration in the mononuclear phagocyte system, thus resulting in iron-limited erythropoiesis. There are several reasons to believe that this is not the case. First, the observed presence of iron in bone marrow corroborates experimental evidence (Spivak 2000) that iron therapy in patients with rheumatoid arthritis, inflammatory bowel disease, cancer or AIDS does not result in correction of anaemia, whereas therapy using recombinant erythropoietin can correct the anaemia of chronic disease but not of iron deficiency (Mean and Krantz 1992). A partial response to iron therapy is possible in patients with coexisting anaemia of chronic disease and iron deficiency (Sears 1992). Second, contrary to a state of iron deficiency, iron absorption is normal or somewhat decreased in the anaemia of chronic disease (Jurado 1997). Third, absorbed iron is effectively incorporated in the erythron (Pollack 1992b). Instead of being incorporated into haemoglobin, however, it appears that iron taken up in developing red cells is stored in ferritin and haemosiderin (Pollack 1992b). Fourth, expression of transferrin receptors on individual erythroblasts is increased in iron

deficiency but decreased in anaemia of chronic disease (Feelders et al. 1993, Kuiper-Kramer et al. 1997, 1998).

Thus, the distinctive abnormalities in body iron distribution as observed in the anaemia of inflammation are not central to its pathogenesis (Mean and Krantz 1992, Pollack 1992b, Spivak 2000). Iron sequestration in the mononuclear phagocyte system appears a by-product of the mechanisms that also cause decreased erythropoiesis (Walter et al. 1997). The evidence presented in the preceding paragraph also suggests that iron therapy might be less effective in improving haemoglobin concentrations in individuals with malaria – particularly those with persistent, asymptomatic infections and relatively high production of proinflammatory cytokines – than in those without malaria.

Observational evidence further supports a possible role of proinflammatory cytokines in the pathogenesis of malarial anaemia (**figure 5**). Serum erythropoietin concentrations are increased in acute malaria (Burchard et al. 1995, Burgmann et al. 1996, Kurtzhals et al. 1997) but appear lower than expected for the degree of anaemia (Vedovato et al. 1999). Proinflammatory cytokines such as TNF, IL-1 and IL-6 are released by monocytes and macrophages in response to schizont rupture (Kwiatkowski 1995). They are thought to suppress erythropoietin synthesis in adults with malaria (Clark and Chaudri 1988, Clark et al. 1989). Similar observations have been made in children with persistent, asymptomatic falciparum malaria (Kurtzhals et al. 1999). In the latter study, children with malaria and who were asymptomatic and who remained so during 4 mo of observation had lower haemoglobin concentrations and higher plasma concentrations of ferritin and TNF at the end of the observation period than children who remained uninfected throughout this period. In the same study, plasma erythropoietin concentration appeared higher in children with asymptomatic and symptomatic malaria than children who remained uninfected throughout (Kurtzhals et al. 1999: figure 1a).

Morphological studies of bone marrow have also shown that patients with malaria often have ineffective erythropoiesis, whereby defective erythroid precursors are prematurely phagocytosed (Abdalla et al. 1980, Abdalla 1990b). This has been described in acute malaria (Dörmer et al. 1983, Phillips et al. 1986, Wickramasinghe et al. 1989) and chronic malaria (Abdalla et al. 1980), but appears particularly important in the latter group (Abdalla 1990b, Das et al. 1999). Little is known about the underlying causal mechanisms (Abdalla 1990b, Phillips and Pasvol 1992). Ineffective erythropoiesis is occasionally observed in the anaemia of rheumatoid arthritis (Gerard Vreugdenhil, St Joseph Hospital, Veldhoven, The Netherlands: personal communication, 2001). A study in mice suggests that TNF may play an important role (Clark and Chaudri 1988).

High serum concentrations of IL-10 and IL-12, which counteract TNF, IL-1 and IL-6, might prevent the development of severe malarial anaemia (Othoro et al. 1999). Insufficient IL-10 production in response to high plasma TNF concentrations have been reported in African children with severe malarial anaemia (Kurtzhals et al. 1998), whereas defective IL-12 production has been shown experimentally to be a mechanism causing fatal rodent malarial anaemia (Mohan and Stevenson 1998, Mohan et al. 1999).

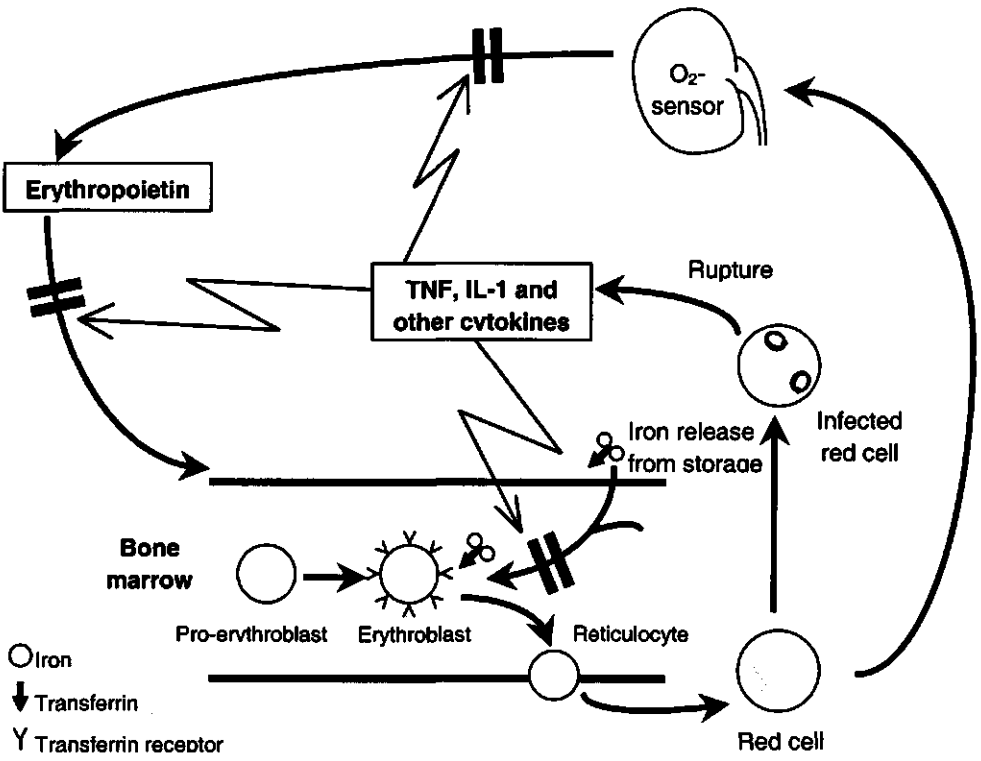


FIGURE 5. Pathophysiological mechanisms determining the anaemia of malaria

Malaria-induced haemolysis of parasitised and non-parasitised red cells leads to a decline of haemoglobin concentrations. The ensuing hypoxia is detected and followed by the increased production of erythropoietin in the kidneys, which in turn enhances the proliferation and differentiation of developing red cells in the marrow. Inflammatory cytokines released by monocytes and macrophages in response to schizont rupture lead to decreased production of erythropoietin, decreased responsiveness of red cell precursors in the marrow to erythropoietin, and decreased release by macrophages of iron from degraded haemoglobin. See text for further explanation.

8. Measurement of iron status

Iron deficiency and anaemia of chronic disease

Diagnosis of iron deficiency is often difficult in African children, because – as described above – it may coexist with changes in body iron distribution that are associated with malaria and other infections. The gold standard for iron deficiency in persons without infection or inflammation is the demonstration of absence of stainable iron stores in macrophages in bone marrow aspirates or biopsy specimens. However, this is traumatic for patients, relatively expensive and generally deemed unethical in asymptomatic individuals. The deficit of functional iron is already profound before red cell abnormalities

become manifest (Baynes 1994). As a consequence, mean red cell volume has low sensitivity for detecting mild iron deficiency (Skikne et al. 1990). Serum ferritin concentration is normally measured as an indirect indicator of the degree of iron sufficiency. During infections, however, serum ferritin concentration is increased under influence of proinflammatory cytokines (see above). Low serum ferritin concentrations almost always have a fairly straightforward interpretation, as concentrations <15 µg/L are highly predictive for absent iron stores (Worwood 1994). High values in the absence of anaemia normally indicate adequate iron stores or even iron overload. When coupled with anaemia, normal or high serum ferritin concentrations indicate the anaemia of chronic disease, but this may mask coexisting iron deficiency (Cook 1994, Feelders et al. 1999). In the latter situation, a higher cut-off point of serum ferritin can be used to demonstrate iron deficiency, but this approach has limited value because of differences in serum ferritin measurements between test kits, and because of its poor diagnostic performance (Guyatt et al. 1990, Coenen et al. 1991). Several alternative laboratory measures, such as serum iron-binding capacity and zinc protoporphyrin concentration in circulating red cells, have been evaluated but these have limited capacity to distinguish between iron deficiency and anaemia of chronic disease (Sears 1992, Hastka 1993).

Serum soluble transferrin receptor concentration

A soluble form of transferrin receptors occurs in serum that provides a new method for assessment of iron status (Cook et al. 1993, 1994, 1996; Feelders et al. 1999). The physiological function of these serum receptors is not clear, but they presumably result from cellular degradation of membrane-bound receptors. Proteolytic cleavage of membrane-bound transferrin receptors occurs both at the outer cell surface and intracellularly during endocytosis of the iron-transferrin complex.

The number of transferrin receptors in non-erythroid tissues is constant under normal circumstances. In vitro studies have shown that most serum soluble transferrin receptors (sTfRs) originate from erythroblasts and reticulocytes. The concentration of sTfR has furthermore been shown to be proportional to erythron iron turnover, a ferrokinetic estimate of erythroid marrow mass (Huebers et al. 1990). These findings are explained as follows. An increased rate of erythropoiesis – such as may occur following haemolysis – is accomplished by accelerated red cell development and hyperplasia of the erythroid marrow mass. This results in higher sTfR concentration and an increased rate of iron uptake. Hence, the rate of erythropoiesis is the major determinant of the total number of transferrin receptors. The only notable exception to the rule that the sTfR concentration is proportional to erythron iron turnover is in iron deficiency anaemia. The expression of receptors on the cell surface can be modified to reflect cellular iron requirements, resulting in an increased density of surface transferrin receptors in iron-deficient cells and a decreased density of these receptors in iron-replete cells (Cook et al. 1994, Klausner et al. 1993).

Hence, sTfR concentration is increased as a result of haemolysis and iron deficiency, but not by infection-induced inflammation. In the absence of haemolysis or other causes of increased erythroid iron turnover, increased sTfR concentrations reliably indicate iron deficiency (Ferguson et al. 1992). Conversely, in the absence of iron deficiency, increased

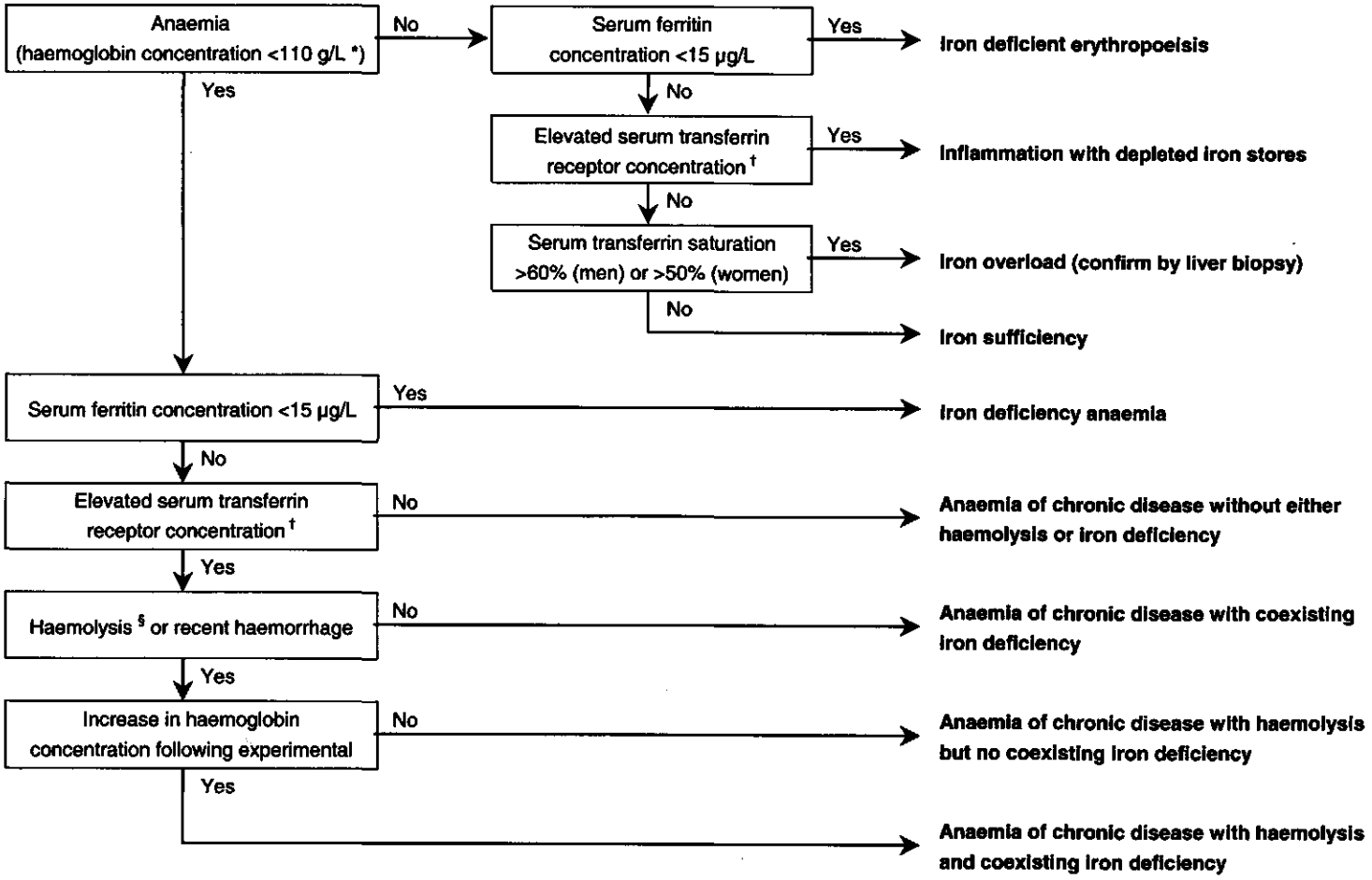
sTfR concentrations indicate an increased rate of erythropoiesis. Concentration of sTfR is furthermore more reliable than zinc protoporphyrin or mean corpuscular volume for the detection of early tissue iron deficiency (Skikne et al. 1990). In the presence of haemolysis, sTfR concentration has no value in diagnosing iron deficiency, because it may also indicate increased erythroid iron turnover. In those cases, iron deficiency can be excluded by the observation of the absence of a response in haemoglobin concentration to experimental iron therapy (**figure 6**).

Measurement of sTfR concentration requires little blood that can be obtained through finger punctures, which is convenient when used in children in developing countries (see below). Various commercial enzyme-linked immunosorbent assay test kits are now available to measure sTfR concentration, but at least some of the disparity between the results of these assays (Åkesson et al. 1999) could be eliminated by using a common standard (Cook 1993, Kuiper-Kramer et al. 1996).

FIGURE 6 (OPPOSITE PAGE). Decision tree for determining iron status in rural African children

* Different cut-off values are require to define anaemia in older age groups (WHO/UNICEF/UNU 1997); † cut-off values have not been defined because commercial tests presently available to determine serum concentration of transferrin receptor are not standardised, see text); § haemolysis may possibly be assessed by serum concentrations of haptoglobin, lactate dehydrogenase or bilirubin.

The proposed procedure is primarily designed to demonstrate the diagnostic value of serum transferrin receptor concentration as an indicator of iron status. The scheme may be used to classify young children by their iron status in community-based studies in rural Africa. Considerations of cost-efficiency were not taken into account. In the design of the scheme, it was presumed that small volumes of blood are available, so that few diagnostic tests can be carried out (see text). Larger volumes of blood can be collected through venipunctures, in which case additional tests may be carried out and the scheme may be refined. In clinical practice, additional tests (including symptoms and signs) are required to diagnose causes underlying anaemia or iron deficiency.


Anaemia
(haemoglobin concentration <110 g/L *)
No
Serum ferritin
concentration <15 µg/L
Yes
Iron deficient erythropoiesis
No
Elevated serum transferrin
receptor concentration †
Yes
Inflammation with depleted iron stores
No
Serum transferrin saturation
>60% (men) or >50% (women)
Yes
Iron overload (confirm by liver biopsy)
No
Iron sufficiency
Yes
Serum ferritin concentration <15 µg/L
Yes
Iron deficiency anaemia
No
Elevated serum transferrin
receptor concentration †
No
Anaemia of chronic disease without either haemolysis or iron deficiency
Yes
Haemolysis § or recent haemorrhage
No
Anaemia of chronic disease with coexisting iron deficiency
Yes
Increase in haemoglobin
concentration following experimental
No
Anaemia of chronic disease with haemolysis but no coexisting iron deficiency
Yes
Anaemia of chronic disease with haemolysis and coexisting iron deficiency

9. Strategies to reduce anaemia

Even when successful, public health programmes to control iron deficiency and malaria will not eliminate anaemia as a public health problem. Depending on geographical location and characteristics of target groups, primary care workers may be faced with other possible causes of anaemia such as deficiencies in folate and vitamins A and B_{12} , HIV infection and tuberculosis. Thalassaemias and other inherited red cell disorders may be regionally important. Better tools and strategies are needed to detect anaemia at primary care levels and to differentiate between its underlying causes. Nevertheless, increasing understanding and awareness of the public health consequences of iron deficiency and new opportunities for its control should lead to renewed efforts to develop anaemia control policies in young children and pregnant women. Several of the interventions that can be used in an integrated programme will be discussed below.

Reproductive and obstetric interventions

Efforts to improve iron status in infants by improving iron status of pregnant or lactating women probably have limited efficacy unless these women are severely anaemic (Bothwell et al. 1989, Allen 1997, Mahomed 2000). On the other hand, erythrocyte transfer and thereby iron transfer to new-born infants can be substantially increased if the umbilical cord is not clamped and ligated until it stops pulsating (Grajeda et al. 1997). Meat-products should be introduced with weaning foods from 4-6 months onwards (Yip 1994), although for economic, cultural or religious reasons this is seldom practised.

Helminth control

Heavy helminth infections are unlikely to occur in infants even in areas that are highly endemic, but even moderate hookworm infection may double iron requirements of children and menstruating women (Pawlowski et al. 1991, Stoltzfus and Dreyfuss, 1998). Annual presumptive therapy is effective and safe in reducing parasite loads and anaemia in school children (Adams et al. 1994, Stephenson et al. 1990, 1989, 1993a,b). More frequent treatment may be needed in highly endemic areas (Albonico et al. 1995). In populations with endemic hookworm, antihelminthic therapy has been recommended presumptively to anyone with severe anaemia, because treatment is considered safe and less expensive than diagnosis (Stoltzfus and Dreyfuss, 1998).

Food-based measures to improve iron status

Iron absorption depends on the form in which iron occurs in ingested food. When bound to haem, as in red meat, up to 20-30% of iron is absorbed (FAO/WHO 1988, IOM 2000). This absorption is relatively independent of other food substances. Absorption of non-haem iron is enhanced by ascorbic acid, or inhibited by plant factors such as phytates in cereals and tannins in tea. For non-haem cereal-based diets, as in developing countries, iron absorption may be as low as 5% (Gillespie 1998). In preschool children, this may at best be adequate to just prevent iron deficiency anaemia, but not to prevent iron deficiency, or to treat iron deficiency anaemia (Gillespie 1998). Thus, food-based approaches need to focus not only on increasing iron content of the diet but also on increasing iron bioavailability. Economic barriers to increased meat consumption and, in some areas, the

lack of iron-containing food items form additional constraints to improving iron status through dietary improvement.

Iron fortification, when feasible and if the right food is selected, is appealing because high coverage of target groups that can be achieved, its cost-effectiveness compared to supplementation (Lofti et al. 1996), the absence of gastro-intestinal side effects, and because little cooperation of individuals is warranted. The choice of centrally processed and widely consumed foodstuffs is limited, however, and iron salts – with the exception of iron EDTA (INACG 1997) – can readily spoil the appearance or taste of many foods. As with dietary improvement, iron fortification may be sufficient to prevent iron deficiency anaemia, but is likely to be insufficient for its treatment in populations with high physiological requirements (Stoltzfus and Dreyfuss 1998).

Iron supplementation

There is growing consensus that iron supplementation should be a component of programmes to control iron deficiency in preschool children. Such programmes should not be constrained by concerns for iron overload (Gillespie 1998, UNICEF/UNU/WHO/MI 1999). UNICEF and WHO recommend preventive iron supplementation for all infants and young children where the prevalence of iron deficiency anaemia is >30% (UNICEF/WHO 1994), and several expert groups subsequently issued similar recommendations (Nestel and Alnwick 1997, Stoltzfus and Dreyfuss 1998, UNICEF/UNU/WHO/MI 1999). Current guidelines for mass programmes recommend a daily regimen of supplementation (**table 1**). Iron is usually given as ferrous sulphate tablets, which are relatively low-cost and easy to transport and store. Liquid forms of iron may be more appropriate for children <2 y because they are easier to administer and may give fewer adverse gastro-intestinal effects. At the time of design of the studies described in this thesis, there was considerable debate but little empirical evidence about the efficacy and effectiveness of daily compared with weekly or biweekly iron supplementation (Wright and Southon 1990, Galloway and McGuire 1994, 1996, Viteri et al. 1995, Cook and Reddy 1995, Viteri 1996, Cook 1996, Solomons 1995, Stephenson 1996).

Measures to control malaria

Personal protection measures comprise prevention of mosquito bites through the use of repellents, protective clothing or mosquito nets. Community protection measures comprise environmental management for mosquito control and the use of insecticide-impregnated nets. In areas of high endemicity, incidence and severity of symptomatic malaria are determined by immunity rather than vector densities or the rate of infection. In such areas, environmental management and other vector control measures aimed at a reduction of the rate of transmission therefore have limited effect in reducing malarial morbidity. A meta-analysis of three randomised trials among African children aged 1-59 mo showed that the use of insecticide-treated mosquito nets prevented 6 deaths per 1000 protected children per year for the three trials, corresponding to a reduction of overall mortality by 17% (Lengeler 2000). The use of insecticide-impregnated mosquito nets can also markedly improve haemoglobin concentrations or haematocrit levels, either in areas of seasonal (D'Alessandro et al. 1995a,b) or stable malaria (Premji et al. 1995, Fraser-Hurt et al. 1999,

TABLE 1. Guidelines for iron supplementation * to children aged 6-24 mo (Stoltzfus and Dreyfuss 1998)

Prevalence of anaemia †	Birth-weight	Age when supplements should be provided
<40%	Normal	6-12 mo
	Low (<2,500 g)	2-24 mo
≥ 40%	Normal	6-24 mo
	Low (<2,500 g)	2-24 mo

* The dosage is 12.5 mg elemental iron plus 50 µg folic acid daily based on 2 mg iron d^{-1} kg body $weight^{-1}$

† Measured in children 6-24: if prevalence is unknown, it is assumed to be similar to the prevalence of anaemia in pregnant women in the same population

Marbiah et al. 1998, Shiff et al. 1996). Currently, the greatest challenge for research is to show that these nets are effective under real life conditions.

There is little support for chemoprophylaxis as a routine health policy for young children in malaria-endemic areas, mainly because of the limited choice of safe and effective drugs, fear of selection and spread of drug resistance, poor compliance and the inherent logistic problems, and impairment of acquired immunity (WHO 1993). Several studies have shown an increased occurrence of malaria attacks following the cessation of chemoprophylaxis (Greenwood et al. 1995, Menendez et al. 1997). The mainstay of malaria control in Africa is early diagnosis and prompt treatment. Despite the occurrence of drug resistance, malaria remains a curable disease, although the number of suitable drugs is limited and decreasing.

Recent findings from studies in Papua New Guinea that oral zinc and vitamin A supplementation may result in 32% and 30% reductions, respectively, of malaria-attributable fevers in children aged 6-60 mo (Black 1998, Shankar et al. 1999, 2000) may offer prospects for control but require confirmation of effect in Africa.

Iron supplementation in malaria-endemic areas

Several issues must be addressed when considering iron supplementation in malaria-endemic areas. First, as argued above, iron therapy might be less effective in improving haemoglobin concentrations in individuals with malaria – particularly those with persistent, asymptomatic infections – than in those without malaria.

Second, despite considerable evidence for impaired immunity and increased risk of infections in iron deficiency (Dallman 1987, Brock 1994), several studies have reported increased parasite densities and frequency of attacks following parenteral and oral iron supplementation to individuals with asymptomatic malaria infections (Oppenheimer et al.

1986, Oppenheimer 1989a, 2001, INACG Expert Panel 1999). In a pooled analysis of these studies, it was found that such an effect in preschool children cannot be ruled out, but that the evidence is weak (INACG Expert Panel 1999). The usefulness of this meta-analysis is limited because of severe flaws in design, implementation and analysis in most of the individual studies. In addition, pooling of estimates from individual study results may be inappropriate because the presumed effects of iron supplementation are likely to depend on the level of immunity in the population studied, with greatest effects to be expected in young children living in areas of seasonal malaria.

Several mechanisms have been proposed to explain increased malaria following iron supplementation. First, the shift in body iron distribution towards storage compartments and reduced iron absorption that are observed in anaemia of chronic disease have been regarded as a non-specific immunological defence by the host, conferring protection from pathogen proliferation (Jurado 1997, Kent et al. 1994, Weinberg 1992, 1993). This 'iron-withholding' has also been presumed to be a defensive mechanism employed by the host against malaria, and, vice versa, iron supply can possibly increase iron availability to parasites and thereby increase the risk of malaria (Oppenheimer 1989b, Kent et al. 1994, Weinberg 1993, Scrimshaw 1991). This might hold truth if iron is supplied in massive doses and in relatively labile forms, as when administered parenterally (Brock 1994, 1999, Weinberg 1993), but – considering the considerable reserves of iron-binding capacity that both transferrin and ferritin have in individuals with the anaemia of chronic disease (Brock 1999) – this is unlikely to occur when iron is administered orally (Brock 1994). In addition, although iron is an essential element for the development of *Plasmodium* species, there is little doubt that the intra-erythrocytic parasite acquires iron from intracellular sources, most likely from degraded ferriprotoporphyrin IX (Ginsburg 1999).

These mechanisms might nevertheless play a role in severely malnourished individuals. Broad impairment of protein synthesis might result in decreased serum transferrin concentrations (Sears 1992). Increased iron intake in wasted individuals may result in the available serum transferrin being overwhelmed. Thus, it has been suggested that in severely malnourished individuals a combination of low serum transferrin and a large rise in serum iron following treatment may have enhanced iron availability to parasites (Brock 1994).

A second and more likely mechanism that has been proposed (Oppenheimer 1989b) concerns an increased reticulocytosis following iron supplementation, which might favour the proliferation of *P. falciparum* and *P. vivax* (see previous sections). Thirdly, iron supplementation might increase a small labile pool of iron that may exist in red cells (Loyevski et al. 1999) and that possibly provides the parasite with iron (Oppenheimer 2001).

The above findings indicate the continued need for a randomised controlled trial with sufficient size and a homogenous study population to assess the effects on malaria of supplementation in young children living in an area of seasonal transmission, and to confirm evidence for such an effect from an earlier trial in severely malnourished individuals (Murray et al. 1978).

10. The study site in Kenya

The studies described in this thesis were carried out near the town of Mtito Andei in Eastern Province, located halfway along the road and rail link between Nairobi and Mombasa (**figure 8**). Before independence from British colonial rule (1963), this area was a national park with few permanent settlements. The present inhabitants almost exclusively belong to the Akamba tribe and were resettled in the 1970s and 1980s from the highland area around Machakos, a city located 70 km east of Nairobi. No epidemiological or entomological studies on malaria had been carried out previously in the study area, and no research infrastructure was present.

The area was selected because it was our purpose to conduct the studies in conditions of seasonal malaria transmission. In addition, this study site allowed us to collaborate with an existing local network of community health workers who had been trained in preceding decades by the African Medical and Research Foundation (AMREF). A similar research project to ours but not further described in this thesis was carried out simultaneously under conditions of stable, perennial malaria transmission in Western Kenya (coordinator: Feiko ter Kuile, University of Amsterdam/Academic Medical Centre, Amsterdam, in collaboration with the Kenya Medical Research Institute/Centers for Disease Control and Prevention, Atlanta, GA, USA).

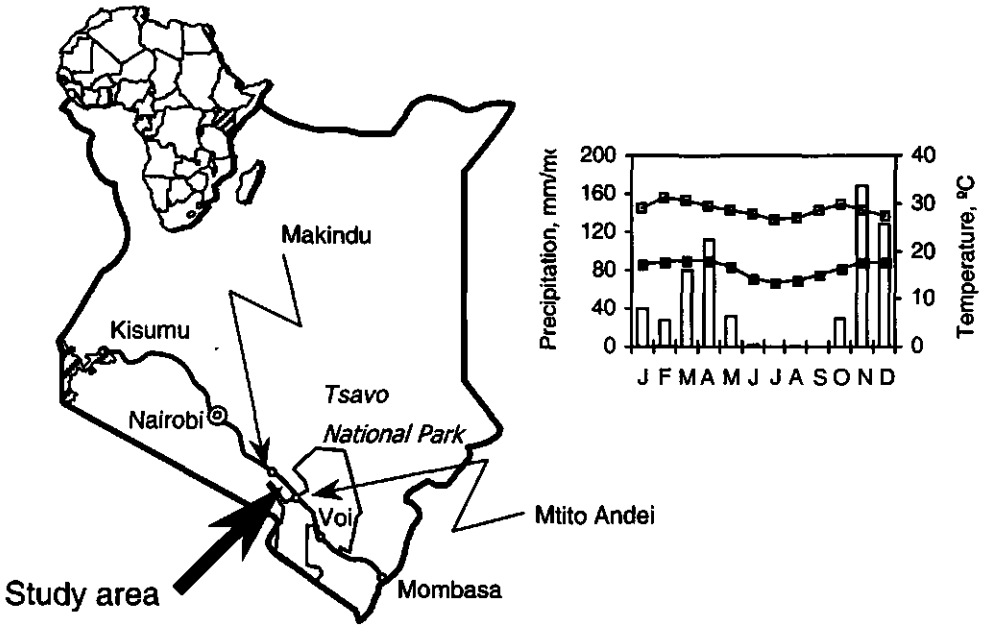


Figure 8. Location and weather characteristics of the study area

Source of weather data: FAOCLIM Global Weather Database, release 20 July 1990 (Makindu meteorological station; precipitation indicated by bars; maximum and minimum temperatures indicated by lines with open en closed squares, respectively).

11. Aims and outline of the thesis

Rationale and design considerations

The age between the first few months of life and 60 mo is the broad range at which children are at greatest risk of iron deficiency, malaria, anaemia and stunting, and for which successful interventions would give great health benefits. When designing the studies described in this thesis, it was decided to settle on children aged 2-36 mo, because this range was expected to provide some degree of homogeneity in the population studied, while for practical reasons it was expected not to be overly restrictive. It was furthermore hoped that at this age worm infections would not yet interfere with our intention to focus our studies on iron deficiency and malaria as causes of anaemia. Care was taken to sample all resident children in a fairly large study area, so that inferences from the studies could be expected to be reasonably valid for similarly aged children living in areas of seasonal malaria transmission.

Iron supplied in pharmaceutical preparations is readily absorbed. Hence, when administered in placebo-controlled randomised trials, iron supplementation is useful to determine efficacy, i.e. to measure maximum effect achieved under highly controlled conditions. No other study design can achieve this. Observational studies – although indispensable for rapid appraisal and to explore hypotheses along the path to proving causality – are also more amenable to bias due to confounding factors. Randomised controlled trials can thus be used to set goals that can possibly be achieved by micronutrient control programmes using more realistic approaches and under 'real-life conditions' (effectiveness).

It might be argued that intervention studies to determine whether or not iron deficiency and malaria are causes of anaemia in Africa are redundant. Such an argument is both correct and beside the point. The prime reason to measure efficacy is to evaluate interventions in terms of health benefits that can be achieved. Thus, the main objective of our randomised trial was not formulated in terms of hypothesis testing, but with a view to give adequate precision in estimating gains that can be achieved in haemoglobin concentration.

Another particular aspect of this trial concerns its 2×2 factorial design. We chose this design because it allows for effect estimation of combined iron supplementation and malaria control, and theoretically it allows for testing whether such an effect is greater (or less) than the sum of individual interventions (interaction). However, for practical reasons, the number of people that can be included is commonly limited in randomised trials. As a result of this drawback, the trial described in this thesis was necessarily limited in its power to detect existing interaction between interventions, and in its power to measure possible adverse effects of iron supplementation.

Objectives and outline of the thesis

The long-term objective of this thesis was to contribute to the development of programmes for anaemia control in preschool children in Africa.

The immediate objectives were as follows:

1. to measure the efficacy in improving haemoglobin concentrations in children aged 2-36 mo of intermittent iron supplementation and intermittent administration of sulfadoxine-pyrimethamine, either alone or when given in combination;
2. to develop and evaluate survey methods for rapid assessment at community level of the burden of anaemia and its risk factors;
3. to contribute to improved methods for diagnosis of anaemia, iron deficiency, and malaria;
4. to evaluate the role of impaired erythropoiesis in the pathogenesis of anaemia that is associated with asymptomatic malaria.

The fieldwork undertaken comprised two studies that were conducted in 1997-2000. First, a cross-sectional study was carried out to pursue objectives 2-4, and in preparation for further studies. Its results are described in chapters 2-4 of this thesis. Second, a randomised controlled trial was undertaken to achieve objectives 1 and 4; its results are described in chapters 5 and 6 respectively. Several sub-studies were undertaken as part of the cross-sectional and intervention studies in fulfilment of objective 3; their results are presented in chapters 7-9. One spin-off from the cross-sectional study relevant to the occurrence and prevention of anaemia concerned a study into the use of antimalarial drugs. The findings of this study are given in chapter 10. Finally, the main findings and conclusions of the above studies are discussed in chapter 11, together with our recommendations for health policies and further research. Other spin-offs which are not necessarily relevant for the study objectives are described in annexes 1-2.

12. Ethical considerations

Methods of blood collection

Consideration for individual study children and cultural perceptions of blood collection require only limited volumes to be drawn. Collection of venous blood can be particularly difficult in young children who often have ample baby fat, and parents may refuse consent or children may be lost to follow-up when venous blood collection is repeated in the same children. On the other hand, sufficient blood is needed to arrive at reliable study conclusions, which is an ethical goal by itself. These conflicting demands were balanced by using collection techniques by finger puncture that may yield up to 1 mL whole blood, by thoroughly explaining and discussing procedures with local health personnel, community leaders and parents before informed consent was obtained, and by using diagnostic tests requiring a minimum volume of blood.

Use of placebo groups

A heated and on-going debate in the medical literature started when Lurie and Wolfe (1997) questioned the ethics of using placebo groups in intervention studies undertaken in developing countries. Their criticism was triggered by ongoing placebo-controlled trials to prevent mother-to-child transmission of human immunodeficiency virus (HIV), despite evidence that zidovudine had been clearly shown to substantially prevent vertical transmission. Others commented that antiretroviral agents because of their cost and logistic constraints could never become the standard of care for developing countries (Merson 1998) and that 'the inclusion of placebo controls [will determine] the value of the intervention being studied compared to the local standard of care' (Varmus and Satcher 1997) – they were subsequently supported by some (Gambia Government/Medical Research Council Joint Ethical Committee 1998) and criticised by others (Angell 1997). The ensuing discussion centred in large part around the interpretation of the word 'best' as contained in the following statement in the guiding principles of ethical research, the Declaration of Helsinki (WMO 1989): 'In any medical study, every patient – including those of a control group, if any – should be assured of the best proven diagnostic and therapeutic method.'

On the basis of the existing evidence at the time of study design, we had doubts whether the benefits of iron supplementation in terms of gains in haemoglobin concentrations outweighed the possible negative effects posed by malaria. The occurrence and severity of possible adverse effects on malaria of iron supplementation depend on the level of acquired immunity as determined by geographical locations and age. Also in the case of malaria, there is general doubt about whether the benefits of chemoprophylaxis outweigh the possible adverse effects (see above). Some children were left untreated for asymptomatic malaria during the intervention period, which is associated with mild anaemia and possibly with an increased risk of subsequent malaria; on the other hand, these children are acquiring immunity that protects them from severe disease and death. In this context, we considered the use of placebos in our trial to be justified.

Perhaps most importantly, we believe that all children taking part in our studies, including those in the placebo groups, were better off than children not taking part, and that all received substantially better care than practically attainable in the host country (Anonymous 1999). This belief is grounded in the fact that intensive supervision was given to ensure that any common childhood illness was detected and treated promptly. Lastly, all children received treatment for common infections or diseases immediately after the intervention period.

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ANNEX. Glossary of terms used in this thesis

Chronic malaria	Malaria with persistent or repeatedly recurring parasitaemia, which may occur in low densities over long periods of time and often without symptoms
Erythroblast	Human red cell that contains a nucleus
Erythroid hyperplasia	Increase in the number of developing red cells, usually confined to the marrow
Erythroid progenitor cell	Stem cell giving rise to red cell
Mononuclear phagocyte system	(formerly referred to as the reticuloendothelial system) group of phagocytic white cells derived from bone marrow stem cells and located outside the vascular system in tissues of many organs; their function is to engulf particles, including infectious agents, and destroy them
Preschool children	Children <5 y of age
Sign	Manifestation of disease as observed during medical examination by health personnel
Symptom	Manifestation of disease as reported by patients or their caretakers

2

Variance estimation when using cluster surveys in developing countries to assess exposure-health outcome relationships

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ABSTRACT

Purpose: Cluster sampling as advocated by the World Health Organization is by far the most common design for health surveys in developing countries. Methodological limitations have limited the use of these surveys to descriptive statistics. We evaluated whether they can be used to assess relationships between exposures and health outcomes.

Methods: Data were used from a cluster survey on malaria, anemia and anthropometric indices in Kenyan children. SUDAAN software was specifically designed for analysis of survey data and can take effects of weighting and clustering on variance estimates into account.

Results: When assuming cluster sampling with replacement, estimated design effects ranged from 0.8 to 2.7, showing that standard statistical software can lead to biased variance estimates. Our findings suggest smaller design effects for regression coefficients than for means and proportions. SUDAAN may not yield variance estimates for large sampling fractions, but this is a design rather than a software problem. It can be overcome by assuming sampling with replacement, although this will yield overestimates of standard errors and confidence intervals.

Conclusions: SUDAAN greatly expands existing analytical capabilities, including regression modeling. In most surveys, the sampling fraction of the total population of clusters is small, and SUDAAN will yield valid variance estimates.

Submitted for publication

Surveys form attractive tools for health managers who want a rapid assessment at relatively low cost of the occurrence of disease or the utilization of health services in a population. In the past, surveys in developing countries typically assumed random sampling when analysing data from all or a random sample of individuals living in arbitrarily selected communities. This yields inaccurate estimates, because it does not take into account that variability in health outcomes originates from variability between individuals, but also from variability between communities.

A major improvement was the introduction of the cluster sample methodology, which was designed to estimate the proportion of immunized children (Henderson and Sundaresan 1982, Lemeshow and Robinson 1985), but which has also been adopted for measuring other health outcomes (Birmingham et al. 1997, Chirambo et al. 1986, Ferrinho et al. 1992, Hercberg et al. 1986, Hlady et al. 1994, Katz et al. 1988, 1993, 1994, Legetic et al. 1996, Materia et al. 1995, Sikosana 1994, Stetler et al. 1980). In the first sampling stage of this design, a systematic sample is drawn from all communities ('clusters') in the geographical area of interest with probability proportional to size. At the second stage, households are selected in a random-like fashion within the selected communities. All children within the eligible age range living in these households are included, and selection of households continues until a predetermined number of children has been examined. In the statistical analysis, it is assumed that each child in the target population has an equal probability of being included in the sample, so that valid point and variance estimates can be obtained using unweighted data.

We evaluated whether the cluster survey can also be used for analytical purposes, i.e. to assess relationships between exposures and health outcomes at a single point in time. Variance estimation should rely on specialized sample survey software to take this design into account (Brogan et al. 1994). Survey data on malaria, anemia and anthropometric indicators of nutritional status in Kenyan children were used to compare estimates of arbitrarily selected parameters and their corresponding standard errors when using three different statistical software packages. Clustered data can be described using Epi Info software, and analysed using SUDAAN software. SPSS is a commonly used standard statistical software, which may lead to biased variance estimates when applied to clustered data. Biological interpretation of estimates will be published elsewhere (Verhoef et al. 1999; accepted). Although we incorporated modifications in the sampling procedures (Bennett et al. 1991, Brogan et al. 1994), we tried to retain the simplicity of the original design as much as possible.

Subjects and methods

Study area and subjects

The study was conducted in the first annual rainy season (April to June) of 1997 in Mtito Andei Division, Eastern Province, Kenya. This area comprises approximately 720 km^2 and is located halfway on the road and rail link between Nairobi and Mombasa. The inhabitants live in widely scattered homesteads. The target population of our study consisted of all

resident children born between 15 April 1994 and 15 February 1997, with no symptoms reported by mothers or carers of malaria or anemia.

Local government administrators, community leaders and auxiliary health workers (community health workers, traditional birth attendants and traditional herbalists) were explained the purpose of the survey and asked to identify communities, and to update a household survey that had previously been conducted in each community in the study area. For enumeration purposes and as the basic sampling unit, we defined a household as a group of people living on the same premises and whose food is prepared by the same person(s). Each household was identified by the name of its head, and the number of its members was listed. No names or vital data were recorded for individual household members at this stage. Small communities were considered jointly with other communities, so that their total number of households was greater than the number to be drawn in the second sampling stage. This yielded a complete list of all households in the areas of interest (7,141 households living in 79 communities, with a total population of more than 40,000 inhabitants). A systematic sample of 45 communities was drawn from a geographically (north to south) ordered list, with probability proportional to size (measured as the number of households per community), excluding 'urban' centers, and using procedures described by Bennett et al. (1991). The number of households in three out of these 45 communities was marginally higher than the sampling interval. In such cases, it is possible that the same community is selected twice, in which case the procedure foreseen calls for double sampling within the communities concerned (Bennett et al. 1991, Lemeshow and Robinson 1985). However, this did not occur in our study.

From each of the selected communities 12 households were randomly sampled with replacement, and for each of these households, the resident children were listed together with their dates of birth as ascertained from the child health card. All children thus identified without symptoms of malaria or anemia and within the desired age range were selected for the study (n=302). Children who in the course of the study migrated with some household members were excluded without replacement. Children who migrated with all household members, or were still missing after repeated visits, or had parents who refused consent were replaced where possible by children from randomly selected households within the same community.

Mothers and study children were invited to pre-arranged meetings in or close to their resident communities for anthropometric measurements and a medical examination. Study procedures during the examination and laboratory procedures will be described elsewhere. Children received medical treatment as necessary. The study was approved by the African Medical and Research Foundation and the Kenya Medical Research Foundation whose ethical standards were followed.

Response and missing values

A total of 302 children were selected for study, of whom 16 were double-selected at the second sampling stage (**figure 1**). Observations for these 16 children were weighted twice. Thus 318 children were included in the study, of whom 35 did not participate or fully participate for the following reasons: refused consent (26); not home or temporarily

absent (7); hospitalized for burns (2). Of these 35 children, 14 were replaced by random selection, which brought the total number of children included in the analysis up to 297. When non-participating children were not replaced, weighting was used to maintain the validity of assuming an equal probability sample. Thus, observations on those who participated within the same cluster were inflated by weighting with a multiplication factor calculated as the number of selected children in that cluster divided by the number of participating children (Bennett et al. 1991, Brogan et al. 1994). This brought this number back to 318 children; sample sizes reported below this value are due to missing values.

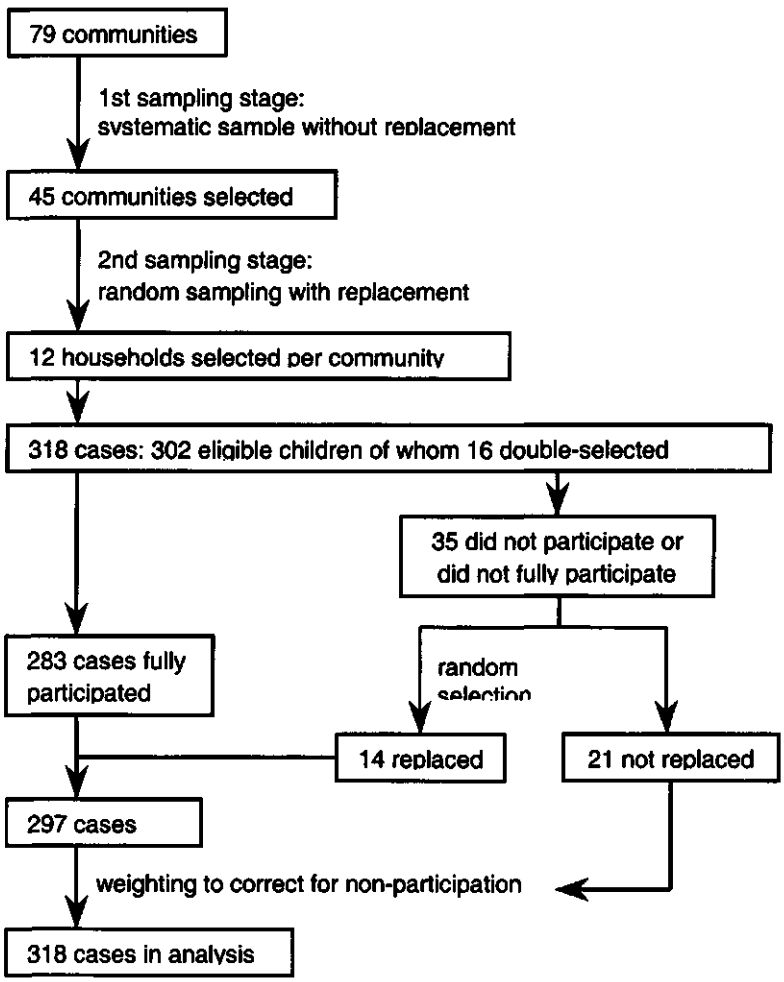

FIGURE 1. Framework for selection and analysis

Statistical analyses

Data were entered into the computer and cleaned using SPSS software (v7.5 for Windows; SPSS Inc., Chicago Ill, USA). Anthropometric indices were calculated using Epi Info (Dean 1994, Dean et al. 1994) (v6.04; CDC, Atlanta GA, USA). The SPSS data set was also imported for calculations by SUDAAN (Shah et al. 1997) (stand-alone v7.5.2A for Windows; Research Triangle Institute, Research Triangle Park NC, USA) or Epi Info.

Estimates were calculated assuming: 1) cluster sampling without replacement at the first stage (computations in SUDAAN, DESIGN=UNEQWOR statement); 2) cluster sampling with replacement (or with small sampling fractions) at the first stage (computations either in SUDAAN with the DESIGN=WR statement, see **annex 1**, or in Epi Info); and 3) simple random sampling (calculations by SPSS or, when computing prevalence ratios, by spreadsheet).

The DESIGN=UNEQWOR design statement in SUDAAN requires the specification of a matrix naming the single and joint inclusion probability for each community and each pair of communities. A description of our procedures for calculating this matrix is given in **annex 2**.

By convention, the design effect is defined as the ratio of the actual variance of an estimated parameter to the variance based on a simple random sample of the same size. It indicates the proportional change in sample size that would yield the same precision of the estimated parameter as when using simple random sampling.

When calculating descriptive statistics, estimates of standard errors using ordered lists of clusters (El-Bindari-Hammad and Smith 1992) did not appreciably alter the values obtained for non-ordered lists (Bennett et al. 1991), so that only the latter are reported here, thus enabling values obtained by SUDAAN and SPSS to be compared.

When estimating prevalence ratios and the corresponding standard errors under the assumption of simple random sampling, the data from all sampled clusters were pooled and tabulated in a 2×2 frequency table cross-classifying binary exposure and health outcome variables. Estimates were computed using the iterative procedures by Fleiss (1981). This provides somewhat larger, but more accurate standard error estimates than those based on the log prevalence ratio (Fleiss 1981).

Results

When assuming without replacement cluster sampling at the first stage (DESIGN=UNEQWOR statement), SUDAAN did not yield estimates of standard errors. The Yates-Grundy formula used in this procedure for variance estimation incorporates the term $P_i*P_j - P_{i,j}$ where P_i and P_j are single inclusion probabilities for each community, and $P_{i,j}$ denotes the joint inclusion probability for each pair of communities. Whenever this term is negative, variance contribution is negative. Thus because estimated standard errors were negative in our study, they were not reported by SUDAAN (Dr B Shah, Research Triangle

Institute, Research Triangle Park NC, USA; personal communication). Estimates obtained with the remaining two design assumptions (with replacement cluster sampling and simple random sampling) are shown in **tables 1-4**. Point estimates were identical under both procedures.

Descriptive statistics
Estimates of standard errors were identical whether calculated by SUDAAN, Epi Info or as in Bennett et al. (1991). Estimates of standard errors were greater, and confidence intervals wider, when assuming cluster sampling than when assuming simple random sampling (**table 1**). Consequently, the design effects of prevalences and means varied between 1.4 for the prevalence of anemia and 2.7 for the prevalence of children who tested positive for chloroquine. The corresponding values for the intracluster correlation coefficient, ρ, varied between 0.06 to 0.29. Epi Info and SUDAAN were found to give slightly lower estimates of design effects than reported in **table 1** (not shown).

Differences in means
Standard errors of the differences in means were all larger, and confidence intervals wider, when assuming cluster sampling than when assuming simple random sampling (**table 2**).

Prevalence ratios
Standard errors for prevalence ratios were smaller or larger under cluster sampling with replacement than under simple random sampling, depending on the ratio which was examined (**table 3**). As a result, confidence intervals were smaller or wider, respectively.

Multiple regression
Results of linear regression of hemoglobin and log ferritin concentrations are given in **table 4**. Again, depending on the point estimate to be examined, standard errors were smaller or greater when assuming cluster sampling than assuming simple random sampling, resulting in design effects below or above 1, respectively.

Discussion

Our study illustrated differences between estimates of standard error when assuming cluster or simple random sampling. Design effects varied between 0.8 and 2.7. It also showed the use of SUDAAN for estimating measures of association. SUDAAN can take weighting and clustering effects of study design into account. When applied to cluster surveys of the Expanded Programme on Immunization, however, it did not yield variance estimates when assuming sampling from finite populations of clusters.

The cluster survey of the Expanded Programme on Immunization was originally designed to estimate the prevalence of a certain characteristic in a population under study within a desired level of precision (Henderson and Sundaresan 1982). It is used increasingly for new applications or objectives requiring higher levels of accuracy. For example, the success of immunization programs in many developing countries has resulted in relatively

TABLE 1. Comparison of estimates of population characteristics assuming cluster and simple random sampling

Measure	n	Cluster sampling			Simple random sampling *	
		Estimate §	[95% CI]	Design effect † (ρ)	Estimate	[95% CI]
Prevalence of anemia, %	318	71.2 ± 3.0	[65.4 to 77.0]	1.4 (0.06)	71.2 ± 2.5	[66.2 to 76.2]
Prevalence of stunting, %	311	38.7 ± 3.4	[32.0 to 45.4]	1.5 (0.09)	38.7 ± 2.8	[33.2 to 44.1]
Prevalence of wasting, %	311	4.7 ± 1.6	[1.6 to 7.8]	1.8 (0.13)	4.7 ± 1.2	[2.3 to 7.0]
Prevalence of malaria, %	318	17.6 ± 3.2	[11.3 to 23.8]	2.2 (0.20)	17.6 ± 2.1	[13.4 to 21.8]
Prevalence of chloroquine use ‡, %	314	37.5 ± 4.5	[28.6 to 46.3]	2.7 (0.29)	37.5 ± 2.7	[32.1 to 42.9]
Mean height-for-age ‖, z-score	311	-1.79 ± 0.12	[-2.02 to -1.55]	2.5 (0.25)	-1.79 ± 0.08	[-1.94 to -1.63]
Mean haemoglobin concentration, g/L	318	102.1 ± 1.1	[99.9 to 104.2]	1.8 (0.13)	102.1 ± 0.8	[100.4 to 103.7]

Point estimates ± standard error; CI: confidence interval; ρ: intracluster correlation coefficient.

Estimates by * SPSS; § SUDAAN, Epi Info and formulae by Bennett et al. (1991) give identical estimates.

† Formulae by Bennett et al. (1991); estimates by Epi Info and SUDAAN are slightly but consistently lower.

‡ As detected by enzyme-linked immunosorbent assay of whole blood (Verhoef et al. 1999).

‖ Height-for-age is an indicator of linear growth retardation expressed in z-scores (the more growth has been retarded, the lower the z-score).

TABLE 2. Comparison of estimated means assuming cluster and simple random sampling

Measure	n	Cluster sampling			Simple random sampling *	
		Estimate §	[95% CI]	Design effect †	Estimate	[95% CI]
Hemoglobin concentration, g/L						
- In stunted children	120	99.7 ± 2.0			99.7 ± 1.5	
- In non-stunted children	191	103.7 ± 1.0			103.7 ± 1.0	
Difference		*4.0 ± 2.2*	*[-0.3 to 8.2]*	*1.3*	*4.0 ± 1.7*	*[0.7 to 7.4]*
- In children with malaria	56	92.7 ± 2.6			92.7 ± 2.4	
- In children without malaria	262	104.1 ± 1.0			104.1 ± 0.8	
Difference		*11.3 ± 2.5*	*[6.4 to 16.3]*	*1.1*	*11.3 ± 2.5*	*[6.3 to 16.3]*
- blood chloroquine test positive	118	98.6 ± 1.9			98.6 ± 1.4	
- blood chloroquine test negative	196	104.2 ± 1.0			104.2 ± 1.0	
Difference		*5.6 ± 1.9*	*[1.9 to 9.4]*	*1.3*	*5.6 ± 1.7*	*[2.3 to 8.9]*
Height-for-age , z-score						
- In wasted children	15	-2.64 ± 0.54			-2.64 ± 0.45	
- In non-wasted children	297	-1.74 ± 0.12			-1.74 ± 0.08	
Difference		*0.89 ± 0.54*	*[-0.16 to 1.95]*	*1.5*	*0.89 ± 0.36*	*[0.19 to 1.60]*

Means or difference in means ± standard error, SE. Estimates by * SPSS; § SUDAAN, DESIGN=WR; Epi Info gives identical estimates; † calculated as the ratio of $SE^2_{(cluster\ sampling)}$ and $SE^2_{(simple\ random\ sampling)}$.

TABLE 3. Comparison of prevalence ratio estimates assuming cluster and simple random sampling

Measure	n	Cluster sampling			Simple random sampling *	
		Estimate §	[95% CI]	Design effect †	Estimate	[95% CI]
Anaemia in stunted and non-stunted children	311	1.13 ± 0.08	[0.96 - 1.33]	1.9	1.13 ± 0.07	[0.97 - 1.29]
Malaria in children with and without anaemia	318	2.35 ± 0.40	[1.05 - 5.25]	1.1	2.35 ± 0.39	[1.15 - 5.21]
Malaria in stunted and non-stunted children	311	1.12 ± 0.24	[0.69 - 1.79]	0.8	1.12 ± 0.27	[0.66 - 1.87]
Chloroquine use in children with and without anaemia	314	1.59 ± 0.18	[1.1 - 2.29]	0.8	1.59 ± 0.20	[1.09 - 2.41]
Chloroquine use in children with and without malaria	314	1.74 ± 0.17	[1.23 - 2.46]	1.3	1.74 ± 0.15	[1.25 - 2.27]

Point estimates ± standard error (natural logarithm of prevalence ratio); CI: confidence interval.
Estimates by * SUDAAN; Epi Info gives near identical estimates; † computing procedures by Fleiss (1981).

TABLE 4. Comparison of estimates obtained by multiple linear regression of serum transferrin receptor concentrations, assuming cluster and simple random sampling

Variable	Log (serum transferrin receptor concentration, mg/L)			Serum transferrin receptor concentration, mg/L	
	Cluster sampling ($\beta \pm$ SE)	Design effect	Simple random sampling ($\beta \pm$ SE)	Cluster sampling (10^{β} [95% CI])	Simple random sampling (10^{β} [95% CI])
Hemoglobin concentration, g/L					
<100	–	–	–	–	–
100-110	- 0.120 ± 0.041	1.4	- 0.120 ± 0.035	0.76 [0.63 to 0.91]	0.76 [0.65 to 0.89]
≥100	- 0.139 ± 0.043	1.5	- 0.139 ± 0.036	0.73 [0.60 to 0.88]	0.73 [0.62 to 0.85]
Malaria					
Negative	–	–	–	–	–
Positive	0.146 ± 0.066	2.7	0.146 ± 0.040	1.40 [1.04 to 1.89]	1.40 [1.17 to 1.68]
Serum ferritin concentration quartile, µg/L					
≥30.7	–	–	–	–	–
14.7-30.7	0.033 ± 0.047	1.5	0.033 ± 0.038	1.08 [0.87 to 1.34]	1.08 [0.91 to 1.28]
6.0-14.7	0.045 ± 0.035	0.8	0.045 ± 0.040	1.11 [0.95 to 1.30]	1.11 [0.78 to 1.33]
<6.0	0.129 ± 0.038	0.9	0.129 ± 0.041	1.35 [1.13 to 1.60]	1.35 [1.12 to 1.62]

Serum transferrin receptor concentrations were normalized by decimal logarithm transformation; effect estimates ± standard error were first obtained for regression coefficients (β) indicating changes in log serum transferrin receptor concentration; the effect estimates reported for re-transformed values (10^{β}) are proportional instead of additive. Thus, these factors indicate the proportional change in sTfR concentration relative to children with haemoglobin concentration <100 g/L, without malaria and with serum ferritin concentration ≥ 30.7 µL.

high levels of coverage, and many countries now wish to measure relatively small increases in coverage beyond levels that are already high (Brogan et al. 1994). Thus, a number of improvements have been proposed in the original design. Bennett et al. (1991) and Brogan et al. (1994) proposed selection of a fixed number of households at the second sampling stage, resulting in a variable number of children per cluster, rather than a fixed number of children as in the original design (Henderson and Sundaresan 1982). They demonstrated how clustering effects can be taken into account in the estimation of means and prevalences, the latter calculated either as a proportion of a fixed number of subjects, or as an index with a variable number of children as the denominator.

Formulae are also available to calculate standard error estimates when, prior to first-stage sampling, clusters are ordered according to a criterion that is related to the outcome of interest (El-Bindari-Hammad and Smith 1992). This has the effect of stratifying the systematic sample and should lead to an increased precision in the estimated prevalence.

The assumption made in household surveys of equal inclusion probability in the sample for each individual child may be violated by non-response. This can be corrected for by weighting the observations in the analysis (Bennett et al. 1991, Brogan 1998, Brogan et al. 1994).

The procedures developed so far for this survey design had limited capability for analytical purposes. Harris and Lemeshow (1991) provide methods for calculating prevalence ratios taking the cluster sampling design into account. Katz et al. (1993, 1994) used a new technique, called alternating logistic regression (Carey et al. 1993), to examine associations between binary variables while allowing for adjustment for other variables; however the expertise required is not usually available to workers in the field. SUDAAN offers analytic tools for descriptive data analysis, cross-tabulation, and for multivariate linear and logistic regression.

Three effects of our study design should be taken into account, namely weighting, clustering and sampling from a finite population. Standard statistical software packages such as SPSS or SAS (SAS Institute Inc., Cary NC, USA) are capable of weighted analysis and may yield valid point estimates of population parameters. However, estimates of standard errors may be biased because these packages presume independent observations as obtained by random sampling (Brogan 1998), whereas cluster sample survey data are usually dependent. The health status of individuals in the same household or the same community is often more alike than that of individuals from different households or communities. This may be due to infections spreading through contact between individuals, genetic predisposition to certain diseases within families or within communities with a high degree of consanguinity, or to common environmental or behavioral factors leading to an increased prevalence of the health outcome within certain households or communities (Katz et al. 1993). Hence, the observed variability between individuals is partly due to variability within clusters, and partly due to variability between clusters. By ignoring this issue, standard statistical software packages generally underestimate the variance of point estimates by a degree that depends on the spatial clustering of the health outcome under consideration. Our data suggest much smaller design effects for

regression coefficients than for means and proportions. This has also been suggested in a previous health examination survey conducted by the US National Center for Health Statistics (1976).

Although designed to handle observations drawn from finite populations, this option failed to work in SUDAAN. In our example, we selected 45 out of 79 communities at the first sampling stage. Taking this large sampling fraction into account in the analysis should have led to greatly reduced variance estimates. The inability to incorporate a finite population correction factor in variance estimates is not limited to SUDAAN, but seems inherent to the Yates-Grundy formula that was used for variance estimation in our design (**annex 2**). Many pairs of communities have a considerable overlap in position on the shifted interval (**figure 1**). As a result, their joint inclusion probabilities were larger than the product of the single inclusion probability. Our study had an unusually large sampling fraction. We expect that in most other surveys this is not the case, so that valid variance estimates are obtained when assuming cluster sampling with replacement at the first stage.

Our study may have been unusual because village elders in the entire area of interest had enumerated all households in their communities a year before for the purpose of emergency food distribution. This list could easily be updated before drawing a sample of clusters. In other circumstances, some approximate measure of the number of households or residents per community is needed, whereby relative size is more important than absolute size. Such information can often be obtained from censuses that were carried out previously, from local government or from non-governmental organizations that are locally active.

Calculation of sample size poses more difficulties with cluster sampling than with simple random sampling. Ideally, one decides on the precision required and then calculates the required number of children, as well as the required number of clusters, m, and the required number of households per cluster, h. Decisions as to the exact values of m and h to use should be tailored to the outcome under consideration and to assumptions on the spatial distribution of this outcome in the study area. To some degree, such decisions can be guided by knowledge on the variability of the outcome between clusters, which can be expressed as the intracluster correlation coefficient, ρ (the variability between clusters as compared to the variation within clusters), or the design effect, D (Bennett et al. 1991). Because we could not find previously published values for these parameters for malaria, anemia, or anthropometric indicators of nutritional status, we let ourselves be guided by practical considerations. Preliminary analysis of census data suggested that 12 sampled households should be included per cluster to arrive at the target of 7 children per cluster, which was judged to be the average number that we could comfortably handle in half a day of field work. The number of sampled clusters (45) was made as large as possible within the operational constraints of the study.

Although there is no good way in SUDAAN to account for implicit stratification by ordering clusters according to the north-south gradient, this could be specified as a categorical covariate when analyzing the data.

Conclusions and recommendations

SUDAAN is versatile software that greatly expands the existing capabilities for data analysis of the cluster survey methodology. It can take weighting and clustering effects of study design into account. The inability to incorporate finite population corrections in variance estimation is not limited to SUDAAN, but is unlikely to be an issue in most surveys. Should this problem nevertheless occur, then variance estimates can be computed in SUDAAN assuming cluster sampling with replacement at the first stage, which will yield overestimates of standard errors and confidence intervals.

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ANNEX 1 Example of SUDAAN design statements

The left-hand column shows sample input to specify a cluster sample design assuming with replacement sampling at the first sampling stage.

Statements	Description
PROC descript	- Requests the procedure for estimating descriptive statistics.
DATA = "c:\\survey.sav"	- Specifies the file location of the data set
FILETYPE = SPSS	- Identifies the used data set as an SPSS file
DESIGN = WR;	- Specifies sampling with replacement at the first stage
WEIGHT weight;	- Specifies the variable with the weight for each observation
NEST _one_ village;	- Specifies that, at the first sampling stage, one stratum was used from which clusters were drawn, with sampled clusters denoted by the variable 'village

ANNEX 2. Procedure for calculating the JOINTPROB matrix

The procedure to select a systematic sample of clusters has been described by Bennett et al. (1991). In brief, communities are listed, together with their size and cumulative size, where size is preferably measured by the number of households (Brogan et al. 1994). The total household size is then divided by the number of clusters to be selected to obtain the sampling interval. By selecting a random number within this interval, the first cluster to be sampled is identified as the one which includes this number in the cumulative list. Additional communities to be included are subsequently identified by successively adding the sampling interval. Using this procedure, it is possible that the same community is selected twice. If this occurs, then the number of households should be doubled at the second sampling stage within the communities concerned (Bennett et al. 1991, Lemeshow and Robinson 1985).

The use of without-replacement sampling at the first stage, as specified in the DESIGN=UNEQWOR design statement, requires the specification in SUDAAN of a matrix in a 'jointprob' statement naming the single and joint inclusion probability for each community and each pair of communities. To illustrate the calculation of these probabilities, consider 11 communities C1-C11, with sizes 50, 50, 75, 50, 50, 25, 125, 75, 25, 50, 25 households, respectively. The cumulative size of all households would be 600. If 6 communities were drawn, then the sampling interval would be 100 (i =600/6). First, communities are fitted, in order of appearance of the original list, onto the shifted interval, as in **figure 2.** The single inclusion probability for each community is calculated as the proportion of the shifted interval taken up by that community. For example, the single inclusion probabilities for communities C1 or C7 are 0.50 (=50/100) and 1.00 (=100/100), respectively. To calculate the joint inclusion probabilities, we first determine the interval where pairs of communities overlap on the shifted interval. For example, for communities C2 and C5 this interval is 25, namely on their positions 50-75 and 250-275, respectively, (see **figure 2**). Hence, the joint inclusion probability of communities C2 and C5 is 0.25 (=25/100). Similarly, there is no overlap for communities C1 and C11, so that their joint inclusion probability is zero. Inclusion of the single and joint inclusion probabilities in the matrix depends on the randomly selected starting point. For example, if 64 was selected as the starting point, this would result in a 6x6 matrix with the probabilities for the villages 2, 3, 5, 7, 8 and 10 (see vertical dotted arrow in **figure 2**).

An example of computerized calculation of such a matrix in SAS software, based on the survey described in this article, is available upon request from the authors.

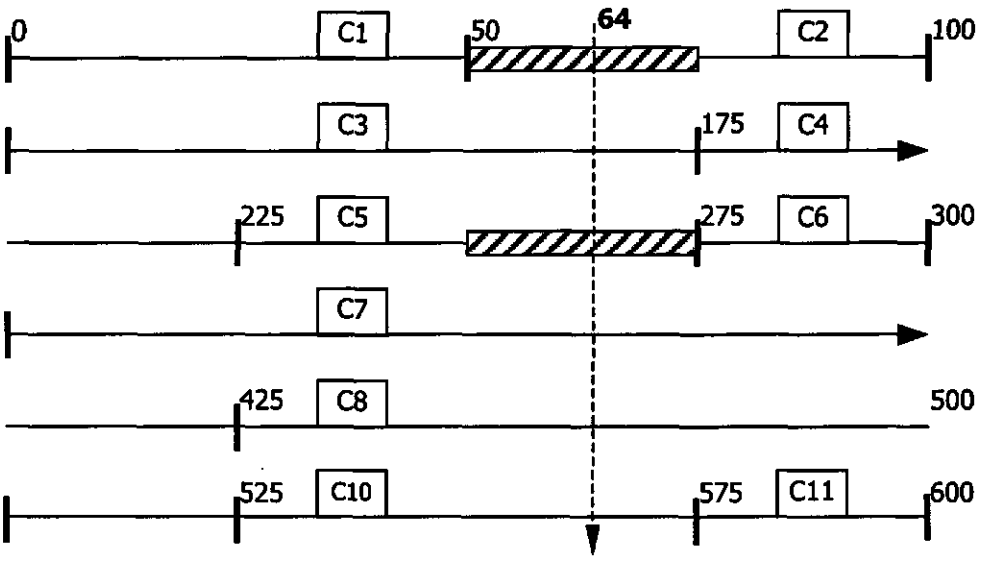

FIGURE 2. Visual representation for calculation of JOINTPROB matrix (see text)

Vertical bars: boundaries of successively listed communities with numbers indicating their cumulative size; C1-C11: identification labels of communities; horizontal shaded bars: overlap between C2 and C5 in their relative positions on the sampling interval.

3

Serum transferrin receptor concentration indicates increased erythropoiesis in Kenyan children with asymptomatic malaria

Hans Verhoef, Clive E West, Paul Ndeto, Jan Burema, Yves Beguin, Frans J Kok

ABSTRACT

Background: Serum transferrin receptor concentration indicates both erythropoietic activity and the deficit of functional iron in the erythron. Contrary to the serum concentration of the iron storage protein ferritin, it is not or only marginally influenced by the inflammatory response to infection.

Objective: To assess iron status and the relationship between serum transferrin receptor concentration and malaria in children aged 2-36 mo asymptomatic for malaria.

Design: Community-based cluster survey (n=318).

Results: Prevalences of malaria, anemia (hemoglobin concentration<110 g/L), iron deficiency (serum ferritin concentration <12 µg/L) and iron deficiency anemia were 18%, 69%, 53% and 46%, respectively. Malaria was associated with lower mean hemoglobin concentrations (92.7 compared with 104.1 g/L; p=0.0001), and higher geometric mean serum concentrations of transferrin receptor (11.4 compared with 7.8 mg/L; p=0.005), ferritin (21.6 compared with 11.9 mg/L; p=0.05) and C-reactive protein (12.5 compared with 6.8 mg/L; p=0.004). There was no evidence for an association between serum concentrations of C-reactive protein and transferrin receptor. Children with malaria had higher serum transferrin receptor concentrations than expected for the degree of anemia, also when adjusting for inflammation indicated by serum C-reactive protein concentration quartiles (p=0.02).

Conclusions: Our findings are consistent with the notion that malaria-induced hemolysis is accompanied by increased erythropoiesis. Serum transferrin receptor concentration is not useful to detect iron deficiency in individuals with malaria. Individuals with high concentrations of serum C-reactive protein or similar acute phase reactants should be excluded if serum ferritin concentrations<12 µg/L are to be used for measuring iron deficiency in malaria-endemic areas.

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Iron deficiency and malaria are likely causes of anemia in African children (Latham et al. 1990, Premji et al. 1995, Shiff et al. 1996, Menendez et al. 1997), but little remains known about the pathogenic mechanisms involved. Malaria leads to hemolysis, impaired erythropoiesis (Abdalla 1990) and possibly iron sequestration and iron deficiency (Brabin 1992). The relative contributions of these mechanisms is likely to vary with the burden, activity and duration of infection. Ferritin is an iron storage protein, and serum concentrations <12 µg/L are highly predictive of iron deficiency, defined by absence of iron stores (Cook 1994). Serum ferritin also reacts as an acute phase protein (Konijn and Hershko 1977). Chronic infections generally cause anemia and a shift of iron distribution from functional towards storage compartments (Sears 1992), as reflected by increased serum ferritin concentrations. The resulting hypoferremia may occur despite the presence of sufficient iron stores (Sears 1992). Until recently, it was believed that anemia of infection is primarily due to impaired release by macrophages of iron from degraded hemoglobin (Konijn 1994, Lee 1983). Recent evidence suggests rather that the prime cause of anemia of infection is decreased responsiveness of erythroid cells to erythropoietin and relatively impaired erythropoietin production, both under influence of inflammatory cytokines (Means and Krantz 1992, Spivak 2000).

Observations that serum soluble transferrin receptor (sTfR) concentrations are not or only marginally influenced by the inflammatory response to infection (Weiss 1999, Feelders et al. 1999) are consistent with the notion that hypoferremia in infection is a non-specific consequence of activation of inflammatory cytokines (Spivak 2000) and that erythroblasts are not deficient in iron (Kuiper-Kramer et al. 1997). As reviewed by Feelders et al. (1999), sTfR concentrations are closely correlated to the number of receptors expressed on the surface of erythroblasts, where they transport transferrin-bound iron into the cell. An expansion of the erythroid mass results in increased sTfR concentrations, and increased transferrin receptor expression takes place on the surface of iron-deficient erythroblasts. Hence, sTfR concentrations measure both erythropoietic activity and the deficit in functional iron in the erythron.

We hypothesized that malaria-induced hemolysis results in increased erythropoietic activity under influence of stimulated erythropoietin production, and that these and possibly other mechanisms lead to increased sTfR concentrations. We also hypothesized that serum ferritin concentrations are increased in malaria independent of their association with hemoglobin concentration. Using data from a survey among preschool children in an area with seasonal malaria transmission, we assessed the iron status of the children and tested whether our data are consistent with these hypotheses.

Subjects and methods

Study area

The study was conducted in the first annual rainy season (April to June) of 1997 in three administrative areas (Kathekani, Muthingiini and Mangelete) in Mtito Andei Division, Kenya. These areas together comprise approximately 720 square kilometers at an altitude of 800-900 m above sea level, located halfway on the road and rail link between Nairobi and

Mombasa. The inhabitants belong almost exclusively to the Akamba tribe, and live in widely scattered homesteads. Like elsewhere in rural Africa, communities are defined as administrative units, rather than as physically recognizable groups of houses. The vast majority of the population are engaged in subsistence farming, with maize and beans cultivated as staple foods, and have a diet poor in meat. Malarial infections reported at the clinical facilities in the area are exclusively due to *Plasmodium falciparum*. There is no malaria control program active in the area, and no epidemiologic or entomologic studies on malaria have been carried out previously.

Study population and sampling procedures

The target population comprised children living in the study area and aged 2-36 mo, with no sickness with symptoms suggesting malaria or anemia reported by mothers or carers. Children were selected using a cluster sampling procedure (Henderson and Sundaresan 1982), incorporating modifications proposed by Bennett et al. (1991) and Brogan et al. (1994). A household survey, conducted with the assistance of local government administrators, community leaders and auxiliary health workers, including community health workers, traditional birth attendants and traditional herbalists, listed a total of more than 40,000 inhabitants spread over 79 communities in the study area. For enumeration purposes and as the basic sampling unit, we defined a household as a group of people living on the same premises and whose food is prepared by the same person(s). Each household was identified by the name of its head, and the number of its members was listed. The sample was drawn in two successive stages (**figure 1**). At the first stage, a systematic sample of 45 communities was drawn from a north to south ordered list of all 79 communities ('clusters') in the study area, with sampling probability proportional to size, and excluding urban centers (Mtito Andei town).

From each of the selected communities, 12 households were randomly sampled with replacement, and for each of these households, the resident children were listed together with their dates of birth as ascertained from the child health card. All resident children thus identified with no symptoms of malaria or anemia and within the desired age range were selected for the study (n=302). Children who migrated with only some household members between the time of the census and the time of examination were considered not to be eligible and were excluded without replacement. Children who migrated with all household members, were still missing after repeated visits, or had parents who refused consent were replaced where possible by children from randomly selected households within the same community.

Field procedures

Mothers and their children were invited to pre-arranged meetings in or close to their resident communities. Containers for collection of stools were distributed by the auxiliary health workers on the day before the clinical examination and stools were examined for parasites on the day of the medical examination. For those children who could not produce stools on that day, mothers were asked to collect and deliver samples the next day. Community leaders, locally active auxiliary health workers and parents of eligible children were informed in their preferred language about the purpose and procedures of the study and prior written consent was obtained from the parents. Children were treated as deemed

necessary upon completion of the survey. Because such treatment occurred after all observations were made and samples collected, this had no influence on the data collected. The study was approved by the African Medical and Research Foundation and the Kenya Medical Research Foundation whose ethical standards were followed.

Capillary blood samples were taken using finger or heel punctures and an aliquot was collected in containers (Microtainer without additives, Becton-Dickinson, Franklin Lakes, NJ, USA). Serum was stored in liquid nitrogen (-196°C) within 12 h after blood collection and kept on solid carbon dioxide or frozen (-79°C) during and after transport to Europe for subsequent biochemical analysis.

The accuracy of the hemoglobinometer (HemoCue Inc., Ängelholm, Sweden) was checked every 4 hr during measurements using a control cuvette. Thick and thin blood films were stained using Field's stain and examined by experienced microscopists on the day of collection and cross-checked independently later. At least 100 high power (x 1000) fields of the thick films were examined for malaria parasites before a slide was considered negative. Stool samples were collected and examined on the day of stool production in saline solution. Additional stool samples were used for making Kato-Katz smears (Ash et al. 1994) and examined for *Schistosoma* infections, or stored in 10% formal saline until examination for the presence of hookworm, *Ascaris lumbricoides* or *Trichuris trichiura* (Ash et al. 1994).

Ferritin concentrations were measured by radioimmunoassay (Ciba-Corning, Brussels, Belgium). Concentrations of sTfR were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis MN, USA). C-reactive protein concentrations were measured as indicators of the activity of infection by a standard turbidimetric method. The intra- and interassay coefficients of variation were as follows: serum ferritin concentration, 3.7-5.9% and 4.9-9.1%, respectively; sTfR concentration, 4.3-7.1% and 5.4-6.4%, respectively; and serum C-reactive protein concentration, 3.1-4.4% and 2.6-5.7%, respectively.

Response and missing values

A total of 302 children were eligible and selected for study, of whom 16 were double-selected at the second sampling stage (**figure 1**). Observations for these 16 children were weighted twice. Thus 318 cases were included in the study, of which 35 did not participate or fully participate for the following reasons: refused consent (26); not home or temporarily absent (7); hospitalized for burns (2). Of these 35 children, 14 were replaced by random selection, which brought the total number included in the analysis to 297. In the case of non-participating children who were not replaced, weighting was used to maintain the validity of assuming an equal probability sample. Thus, observations on those who participated within the same cluster were inflated by weighting with a multiplication factor calculated as the number of selected children in that cluster divided by the number of participating children (Bennett et al. 1991, Brogan et al. 1994). This brought the number back to 318 children; sample sizes reported below this value are due to missing values. Only a limited volume of capillary blood could be collected; in the biochemical analysis, priority was given to the determination of serum concentration of transferrin

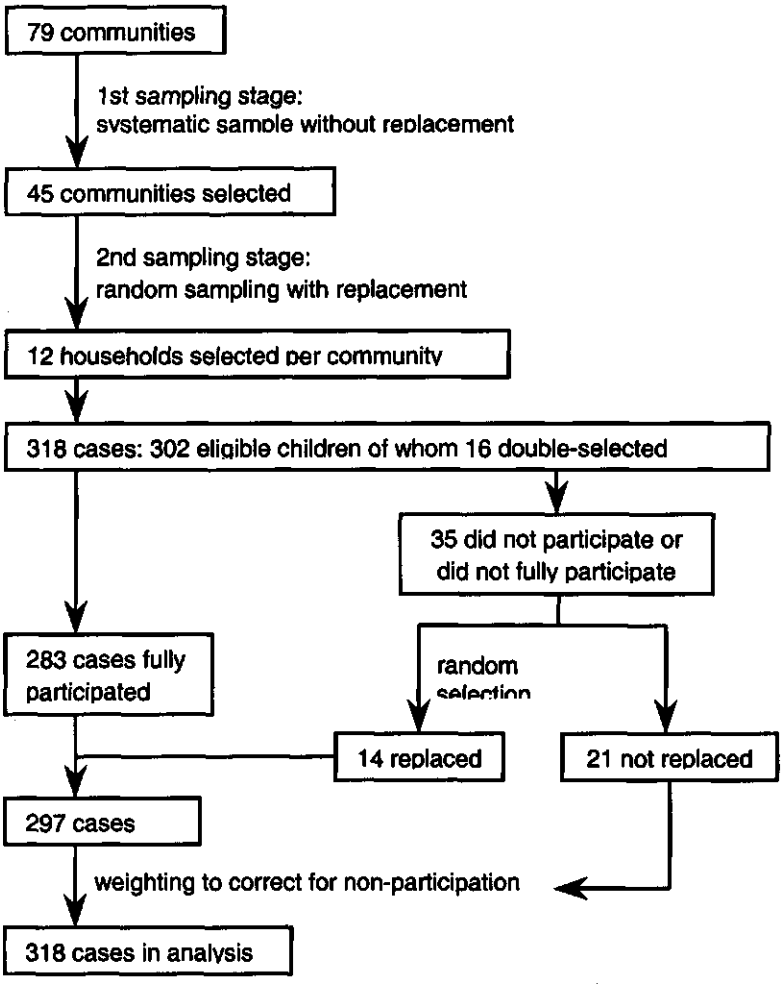

FIGURE 1. Framework for selection and analysis

receptor, ferritin and C-reactive protein (in this order). Thus, missing values occurred more frequently with the latter two variables.

Statistical analysis

Data were entered into the computer, cleaned and managed using SPSS v7.5 for Windows (SPSS Inc., Chicago IL, USA) and analyzed using SUDAAN v7.5.2a for Windows (Research Triangle Institute, Research Triangle Park NC, USA), assuming two-staged cluster sampling with replacement at the first sampling stage. The variance estimates under this assumption do not take into account that clusters were sampled from a finite population. Thus the standard error values and confidence intervals reported here are overestimates,

and the statistical tests are conservative in detecting existing associations. Recalculation of variance estimates for descriptive statistics using ordered lists of clusters (El-Bindari-Hammad and Smith 1992) only led to marginally smaller values (not shown).

Anemia was defined by hemoglobin concentrations <110 g/L (Scrimshaw et al. 1968). Intestinal worm infections or schistosomiasis were analyzed as a positive stool test result for a particular parasite in any stage of its development, detected by direct saline stool examination, or Kato-Katz smear or by formal concentration technique.

To assess the associations between malaria and indicators of iron status, and to determine the activity of the infection, we compared children with and without malaria with respect to their hemoglobin concentration and serum concentrations of transferrin receptor, ferritin and C-reactive protein, respectively. Distributions of serum concentrations of transferrin receptor, ferritin and C-reactive protein were normalized by decimal logarithmic transformation prior to analysis.

The associations between age and indicators of iron status were assessed by estimating the prevalences of anemia, iron deficiency and iron deficiency anemia (hemoglobin concentration <110 g/L and serum ferritin concentration <12 µg/L) in three age classes (2-11, 12-23 and ≥24 mo). Prevalences for anemia were given separately for children with and without malaria. Since serum ferritin concentration is increased in infections, this was controlled for in two different ways: namely, by excluding children with malaria and by excluding those with serum C-reactive protein concentration ≥8 mg/L, respectively. Individuals without infections usually have serum C-reactive protein concentrations <8 mg/L (Hoffbrand and Pettit 1993).

To assess if the activity of infection was associated with indicators of iron status, we compared children with relatively low and high serum concentrations of C-reactive protein with respect to their hemoglobin concentration and serum concentrations of transferrin receptor and ferritin.

Values of sTfR concentration were normalized by decimal logarithm transformation for use as a dependent variable in linear regression models, and re-expressed in their natural units for reporting. Malaria-associated changes in the rate of erythropoiesis may depend on immune status and therefore on age (Kling et al. 1998). This was examined by testing the product term malaria × age class in a multiple regression model that also included malaria and age class as main terms.

To further explore the association between sTfR concentration and malaria, geometric mean sTfR concentrations were estimated for each of the cross-tabulated categories of the variables malaria and hemoglobin concentration class. Multivariate linear models were subsequently used to assess this association when adjusting for possible effects of inflammation. Thus, a dependent variable indicating log-transformed sTfR concentrations was modelled as a function of hemoglobin concentration class, malaria, and serum C-reactive protein concentration quartiles. Exclusion of the latter variable did not result in different conclusions or substantially different effect estimates. Hemoglobin concentration

was entered as a categorical variables with three classes (<100 g/L, 100-110 g/L, ≥110 g/L) so as not to impose linear relationships with the outcome. Cut-off values for hemoglobin concentration were chosen to optimize sample size distribution between categories. Sex, age (categorized as above) and intestinal worm infections were considered as potential confounding variables, but their inclusion led to similar conclusions and coefficient estimates for the effects of malaria and hemoglobin concentration class (not shown), so that these terms were deleted in the final model (model 1; n=88).

Similar procedures were followed to assess the association between serum ferritin concentrations and malaria. The model (model 2; n=90) adjusted for possible effects on serum ferritin concentration of inflammation by including serum C-reactive protein concentration quartiles. Although exclusion of age class did not substantially affect the effect estimate of malaria on serum ferritin concentration, this variable was retained in the model because iron stores are known to rapidly decline during and following infancy as a result of body growth and associated hemodilution (Yip 1994).

Results

Descriptive statistics

Descriptive statistics for the study population are provided in **table 1**. All reported malaria infections were due to *P. falciparum*. The prevalence of malaria (17.6%) indicates a population of children exposed to seasonal, unstable malaria. Anemia was highly prevalent but moderate in degree. Iron deficiency as measured in the total sample occurred in 41.5% of children. The highest prevalence for any intestinal infection was for *A. lumbricoides*, namely 4.4%. The parasite load for this roundworm was low, ranging between 20 and 1100 eggs/g stool. No hookworm infection was found.

Univariate analyses

Compared with those without malaria, children with malaria had substantially lower hemoglobin concentrations (mean 92.7 compared with 104.1 g/L; **table 2**), higher sTfR concentrations (geometric mean 11.4 compared with 7.8 mg/L), higher ferritin concentrations (geometric mean 21.6 compared with 11.9 µg/L) and higher C-reactive protein concentrations (geometric mean 12.5 compared with 6.8 mg/L). The prevalence of children with serum ferritin concentrations <12 µg/L was 45.1% and 22.4% in children without and with malaria, respectively (difference: 22.6%; 95% CI: 6.6-38.7%; p=0.008). Children with malaria also had slightly higher axillary temperatures compared to those without malaria (36.9°C compared with 36.5°C; difference 0.4°C; 95% CI: 0.1-0.6°C; p=0.01).

Hemoglobin concentrations were not associated with sex (not shown). Of the three age classes examined, children aged 12-23 mo had the highest prevalence of anemia, iron deficiency and iron deficiency anemia (**table 3**). The prevalence of anemia increased with malaria in all age categories. Exclusion of children with serum C-reactive protein concentration ≥8 mg/L led to higher estimated prevalences of iron deficiency and iron deficiency anemia than exclusion of those with malaria.

TABLE 3. Iron status of children in various age categories

Iron status indicator	Age	n	Prevalence	(95% CI*)	p	n	Prevalence	(95% CI*)	p
		Children without malaria				*Children with malaria*			
Anemia (hemoglobin concentration <110 g/L)	2-11 mo	80	62	(50 - 73)	0.01	12	90	†	0.83
	12-23 mo	94	79	(70 - 88)		22	86	†	
	≥24 mo	88	55	(44 - 67)		23	82	†	
	All ages	262	66	(59 - 72)		56	85	(75 - 96)	
		Excluding children with malaria‡				*Excluding children with CRP ≥ 8 mg/L§*			
Iron deficiency (serum ferritin concentration <12 µg/L)	2-11 mo	57	26	(12 - 40)	0.006	19	49	(25 - 72)	0.02
	12-23 mo	53	63	(48 - 78)		20	77	(59 - 96)	
	≥24 mo	57	47	(31 - 63)		17	31	(9 - 54)	
	All ages	168	45	(36 - 54)		55	53	(39 - 67)	
		Excluding children with malaria‡				*Excluding children with CRP ≥ 8 mg/L§*			
Iron deficiency anemia (hemoglobin concentration <110 g/L and serum ferritin concentration <12 µg/L)	2-11 mo	57	21	(9 - 33)	0.005	19	38	(15 - 60)	0.01
	12-23 mo	53	55	(40 - 70)		20	72	(54 - 90)	
	≥24 mo	57	25	(13 - 37)		17	25	(4 - 45)	
	All ages	168	33	(26 - 41)		55	46	(32 - 59)	

*CI: confidence interval calculated using Normal approximation except when np or n(1-p) <5; in these cases† the conventional approximation using the hypergeometric distribution is not allowed because data were clustered. Serum ferritin concentration reacts as an acute phase protein; this was controlled for by excluding ‡ children with malaria or with § serum C-reactive protein concentration ≥8 mg/L (see text). Sample sizes are based on weighted observations and are not necessarily integers; due to rounding-off, the summed values of the three age categories may yield a slightly different value from the total given for all ages.

Compared to those with low serum C-reactive protein concentrations (<8 mg/L), children with high serum C-reactive protein concentrations (≥8 mg/L) had greatly increased serum ferritin concentrations (geometric mean of 25.1 compared with 9.4 µg/L; difference: 15.7 µg/L; 95% CI: 4.4-36.1 µg/L; p=0.002). Concentrations of sTfR were somewhat increased in children with high serum concentrations of C-reactive protein (9.3 compared with 7.6 mg/L) but this difference was not statistically significant (difference: 1.6 mg/L; 95% CI: -4.0 to 4.2 mg/L; p=0.13) and disappeared altogether when adjusting for malaria (not shown). Serum concentrations of C-reactive protein did not correlate with hemoglobin concentration.

STfR concentration was inversely related to hemoglobin concentration. Geometric mean sTfR concentrations in children with hemoglobin concentrations <100 g/L, 100-110 g/L and ≥110 g/L were 10.7 mg/L, 7.5 mg/L and 6.8 mg/L, respectively (p<0.0001).

Multivariate analyses

The malaria-associated increase in sTfR concentrations did not vary with age class (p=0.60). **Figure 2** shows the estimated geometric mean concentrations of both sTfR and ferritin for each of the cross-tabulated categories of the variables malaria and hemoglobin concentration class.

In children without malaria or inflammation, hemoglobin concentration was inversely associated with sTfR concentration (**figure 2, top, table 4**), and positively associated with serum ferritin concentration (**figure 2, bottom**). When adjusted for malaria and serum C-reactive protein concentration quartile, children with hemoglobin concentrations of 100-110 g/L and ≥110 g/L had sTfR concentrations proportionally decreased by factors 0.77 and 0.67 relative to the reference class of children with hemoglobin concentration <100 g/L, whilst their serum ferritin concentrations were elevated by factors 1.31 and 1,49, respectively (**table 4**). In these multiple regression models, malaria was associated with elevated serum concentrations of both sTfR and ferritin (**table 4**). Serum C-reactive protein concentration showed no consistent or substantial association with sTfR concentration, but serum ferritin concentrations were substantially elevated when serum C-reactive protein concentration was ≥8.4 mg/L (**table 4**). Children aged 12-23 mo and > 24 mo had serum ferritin concentrations proportionally decreased by a factor 0.48 and 0.72, respectively, relative to the reference class of children aged 2-11 mo. **Table 5** shows geometric mean sTfR concentrations that were calculated on the basis of the model presented in **table 4**.

Discussion

Malaria is associated with increased sTfR concentrations, both when assessed by crude analysis and when the effects of hemoglobin concentration and serum C-reactive protein concentration are taken into account. It is also associated with decreased hemoglobin concentrations. These findings are consistent with our hypothesis that malaria-associated hemolysis results in increased erythopoiesis. The inflammatory response to malaria is reflected by increased serum concentrations of C-reactive protein and ferritin.

TABLE 4. Multiple regression models of serum concentrations of soluble transferrin receptor (model 1; n=88) and ferritin (model 2; n=90)

Variables	Factor *	(95% CI †)	Wald F	df ‡	p
Outcome: ***serum soluble transferrin receptor concentration, mg/L***					
Hemoglobin concentration, g/L			5.3	2	0.01
<100	–	(–)			
100-110	0.77	(0.62 - 0.96)			
≥110	0.67	(0.53 - 0.86)			
Malaria	1.50	(1.07 - 2.10)	5.6	1	0.02
Serum C-reactive concentration quartile, mg/L			0.2	3	0.92
<4.7	–	(–)			
4.7-6.3	1.10	(0.83 - 1.44)			
6.3-8.4	1.04	(0.83 - 1.32)			
≥8.4	1.00	(0.82 - 1.22)			
Outcome: ***serum ferritin concentration, µg/L***					
Hemoglobin concentration, g/L			1.0	2	0.37
<100	–	(–)			
100-110	1.31	(0.70 - 2.44)			
≥110	1.49	(0.86 - 2.56)			
Malaria	2.13	(1.05 - 4.30)	4.4	1	0.04
Serum C-reactive protein concentration quartile, mg/L			6.0	3	.002
<4.7	–	(–)			
4.7-6.3	2.37	(0.98 - 5.76)			
6.3-8.4	2.13	(0.92 - 4.92)			
≥8.4	5.54	(2.33 - 13.20)			
Age, mo			2.2	2	0.13
2-12	–	(–)			
12-23	0.48	(0.24 - 0.96)			
> 24	0.72	(0.39 - 1.33)			

* Effect estimates were first obtained for the regression coefficient (β) indicating changes in ^{10}log sTfR concentration; the effect estimates reported here (10^{β}) are proportional instead of additive. Thus, factors indicate the proportional change in sTfR concentration relative to children with hemoglobin concentration <100 g/L and without malaria and in the lowest serum C-reactive protein concentration quartile, or the proportional change in serum ferritin concentration relative to infants with hemoglobin concentration <100 g/L, without malaria and in the lowest serum C-reactive protein concentration quartile; † CI: confidence interval; ‡ df: degrees of freedom.

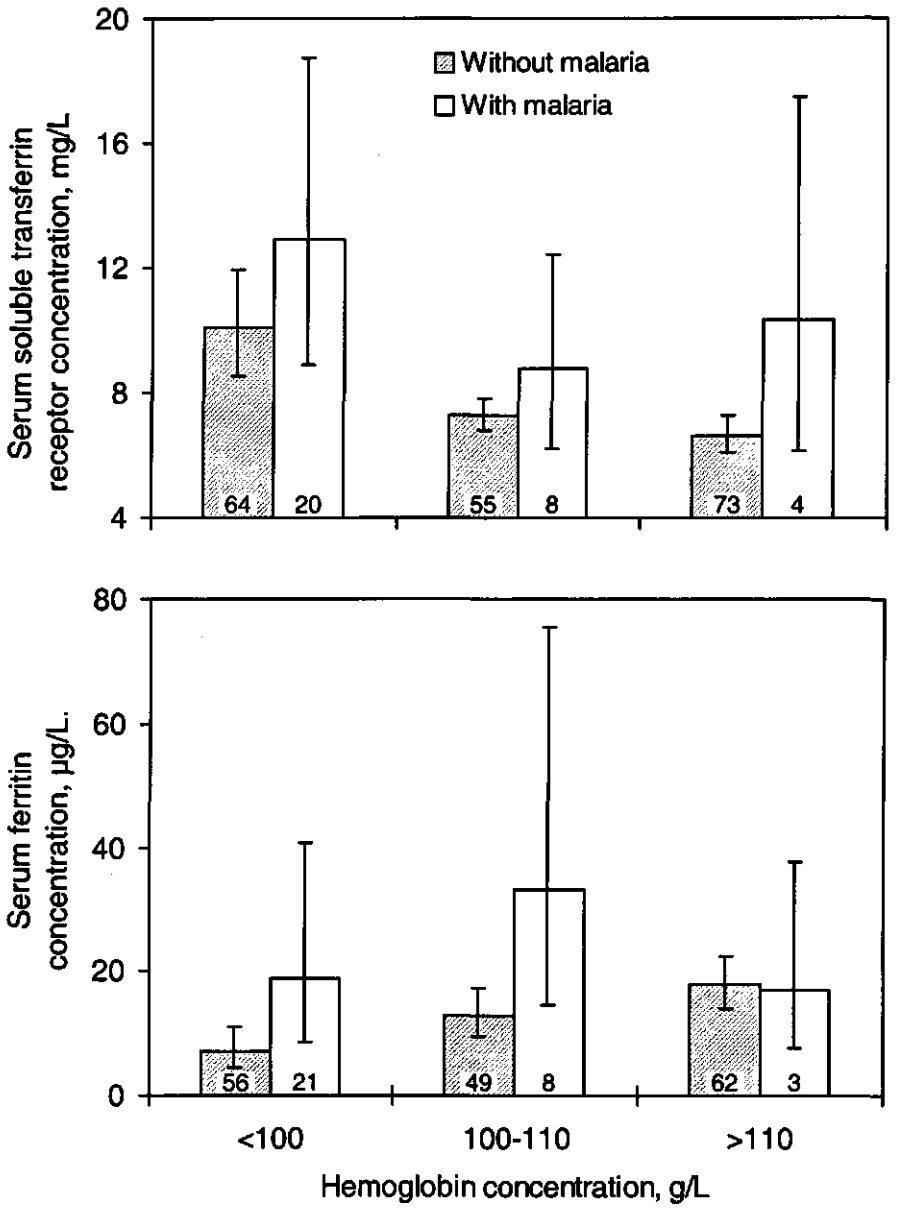

FIGURE 2. Geometric mean serum concentrations of transferrin receptor (top) and ferritin (bottom) in association with malaria and hemoglobin concentrations

Numbers in the graph indicate sample sizes for each category; line bars indicate 95% confidence intervals.

TABLE 3. Estimated geometric mean serum concentrations of soluble transferrin receptor (n=224) and ferritin (n=196), based on the multiple regression models reported in table 4.

Hemoglobin concentration, g/L	Serum soluble transferrin receptor concentration, mg/L *			Serum ferritin concentration, µg/L †		
	Without malaria	With malaria	Difference	Without malaria	With malaria	Difference
<100	8.9	13.3	4.4	5.5	11.6	6.2
100-110	6.8	10.2	3.4	7.1	15.2	8.1
≥110	6	8.9	3	8.1	17.3	9.1

* For children with serum C-reactive protein concentrations <4.7, 4.7-6.3 , 6.3-8.4, and ≥8.4 mg/L, these values have to be multiplied by 1.00, 1.10, 1.04 and 1.00, respectively; † for children with serum C-reactive protein concentrations <4.7, 4.7-6.3 , 6.3-8.4, and ≥8.4 mg/L, these values have to be multiplied by 1.00, 2.37, 2.13 and 5.54, respectively; for children aged 2-11, 12-23 and ≥24 mo these values have also to be multiplied by 1.00, 0.48 and 0.72, respectively (see table 4).

Lack of association between serum C-reactive protein concentration and sTfR concentration, also after adjustment for potential confounding by malarial infection, suggests that the inflammatory response does not influence sTfR concentrations. It can therefore be safely assumed that sTfR concentrations measure both the rate of erythropoiesis or the deficit in functional iron in the erythron.

Sickle cell trait is not substantially associated with hemoglobin concentration (Rana et al. 1993) and occurs rarely in the Akamba tribe (Foy 1954). Urinary schistosomiasis is seldom reported by local medical personnel, and other helminth infections were uncommon. None of these factors is likely to have substantially confounded or modified the relationships observed. The prevalence of anemia (69%) was high but typical for east African preschool children (DeMaeyer and Adiels-Tegman 1985). Hemoglobin concentration and its relationship with malaria were not found to be different between those children for whom data on sTfR concentration were or were not available (not shown); hence, there was no evidence for bias due to missing values. We expect similar results to be found in preschool children asymptomatic for malaria in other areas of unstable malaria.

In malarious areas, it is often difficult to detect iron deficiency in individual children. Serum ferritin concentrations <12 μg/L are highly predictive of depleted iron stores (Skikne et al. 1990), regardless whether infection or inflammation is present or absent. Serum ferritin concentrations increase rapidly during malarial infection (Oppenheimer et al. 1984, Phillips et al. 1986, Das et al. 1997), probably as part of a host immune response (Kent 1994). This was confirmed by our results. Hence, when coupled with anemia, normal or high serum ferritin concentrations indicate infection, but this may mask concomitant iron deficiency. In these cases, contrary to other infections (Ferguson et al. 1992), sTfR concentration is not useful in detecting iron deficiency, because malaria-associated hemolysis may also increase sTfR concentration. In the absence of malaria, serum ferritin concentrations <12 μg/L often fail to detect existing iron deficiency, as indicated by higher estimated prevalences of iron deficiency in children with serum C-reactive protein concentrations <8 mg/L than in children without malaria (**table 3**). This may be due to serum ferritin concentrations being increased by asymptomatic infections other than malaria, and suggests that iron deficiency can only be accurately diagnosed in children with low serum C-reactive protein concentrations. Reports by Stoltzfus et al. (1997, 2000) suggest that malaria leads to increased serum ferritin concentrations in young children, but not in older children in areas with highly endemic malaria. The latter group may have developed partial protective immunity that prevents asymptomatic malaria to affect hemoglobin concentrations or serum ferritin concentrations.

In children without malaria or inflammation, low hemoglobin concentrations were associated with increased sTfR concentrations and decreased serum ferritin concentrations (**table 4**). The latter probably occurred because low hemoglobin concentrations in these children indicate iron deficiency, which develops in several stages. When iron stores are still present, iron depletion results in reduced serum ferritin concentration and a marginal increase in sTfR concentration. Beyond this stage, iron depletion results in a marginal reduction of serum ferritin concentration and substantial increases in sTfR concentration (Skikne et al. 1990). Concentrations of sTfR then indicate the iron deficit in functional

compartments in the body (including hemoglobin). In addition, sTfR concentrations in these children are likely to have increased due to an increased erythropoietic activity in response to the anemia (Beguin et al. 1993).

Malaria was associated with sTfR concentrations that were as high as or higher than expected for the degree of anemia (**figure 2, top; tables 4 and 5**). Brabin (1992) speculated that malaria may induce iron deficiency through iron loss in urine following hemolysis, reduced iron absorption, or iron sequestration in macrophages of the mononuclear phagocyte system. Iron loss associated with hematuria may occur following severe hemolysis, but in mild hemolysis, free heme iron or hemoglobin are probably recycled for use in normal body functions. In our study, children were asymptomatic or perhaps convalescing from recent malaria attacks, so that none or very few were likely to suffer from severe hemolysis. Decreased iron absorption reportedly plays a minor role in the anemia of inflammation (Jurado 1997). The shift of iron distribution that has been observed in inflammatory diseases and infections has long been believed to be a cause of reduced erythropoiesis, but this is not accompanied by an increase in sTfR concentration (Ferguson et al. 1992, Feelders et al. 1999). The observed increase in sTfR concentration is therefore unlikely to be due to iron deficiency. Together with our finding that malaria is associated with decreased hemoglobin concentrations (**table 2**) and anemia (**table 3**), our observations are consistent with the notion that malaria-induced hemolysis leads to lower hemoglobin concentrations, which in turn results in increased erythropoiesis that is appropriate for the degree of anemia.

It cannot be ruled out that, in addition, sTfR concentration is elevated in malaria due to ineffective erythropoiesis, whereby developing erythroblasts are prematurely phagocytosed in the marrow by macrophages without producing mature red cells. Morphological evidence of expanded erythroid mass with ineffective erythropoiesis has been described in studies of bone marrows of patients with severe anemia and chronic falciparum malaria (Abdalla et al. 1980), uncomplicated falciparum malaria (Phillips et al. 1986) and febrile attacks of vivax malaria (Wickramasinghe et al. 1989). These studies found abnormalities in developing erythroblasts and evidence of increased phagocytosis of erythroblasts at various stages of degradation. Results of studies on cobalamin deficiency support the notion that ineffective erythropoiesis may be associated with increased sTfR concentrations (Carmel et al. 1992, Rees et al. 1998). Indicators of cobalamin deficiency were not routinely studied by us, but no megalocytosis was observed. It also cannot be ruled out that sTfR concentrations are increased in malaria because of iron sequestration in hemazoin. This is a waste product found in circulating or phagocytosed red cells and results from hemoglobin degradation by malaria parasites.

Several other studies give support to increased sTfR concentration in asymptomatic malaria (Mockenhaupt et al. 1999, Stoltzfus et al. 2000). Mockenhaupt et al. (1999) found increased sTfR concentrations in asymptomatic and mildly symptomatic falciparum malaria when adjusted for hemoglobin concentration. Stoltzfus et al. (2000) found that sTfR concentrations increased with parasite density in children with asymptomatic malaria but found no evidence for an effect on sTfR concentrations when adjusting for hemoglobin concentration. In pregnant women attending antenatal clinics in Malawi, Huddle et al.

(1999) found that malaria was associated with marginally and not significantly increased sTfR concentrations, despite hemoglobin concentrations being considerably lower. Williams et al. (1999) found no difference in sTfR concentration and hemoglobin concentration between children in Vanuatu with asymptomatic malaria and children with no malaria.

A decrease in sTfR concentrations was found in symptomatic malaria in two studies (Williams et al. 1999, Beesley et al. 2000) but there was no association in two others (Kuvibidila et al. 1995, 1999), possibly because of the small sample size in these studies. Williams et al. (1999) interpreted the decrease in sTfR concentrations as evidence for decreased erythropoiesis in symptomatic malaria, either as a result from acute erythropoietin deficiency (Burgmann et al. 1996) or suppression of marrow response to erythropoietin (Miller et al. 1989, Kurtzhals et al. 1997). However, some studies reported erythropoiesis in symptomatic malaria to be increased, albeit ineffective and lower than expected for the degree of anemia (Abdalla et al. 1980, Phillips et al. 1986, Wickramasinghe et al. 1989), whereas others reported marrow hypoplasia and decreased red cell production (Srichaikul et al. 1969, Phillips and Pasvol 1992). Available evidence also indicates erythropoietin concentrations in malaria to be increased (Burchard et al. 1995, Burgmann et al. 1996, El Hassan et al. 1997, Kurtzhals et al. 1997, Vedovato et al. 1999) albeit lower than expected for the degree of anemia (Burgmann et al. 1996, El Hassan et al. 1997, Vedovato et al. 1999).

In summary, asymptomatic malaria was associated with lower hemoglobin concentrations and elevated sTfR concentrations, which is consistent with the notion that malaria-induced hemolysis leads to increased erythropoiesis. Reported discrepancies about the association between malaria and sTfR concentration are likely to remain unclear until further reports become available on changes in sTfR concentration following treatment of patients with various presentations of malaria. Individuals with high concentrations of serum C-reactive protein or similar acute phase reactants should be excluded when serum ferritin concentrations <12 µg/L are used for measuring the prevalence of iron deficiency in malarious areas.

Contributors

Hans Verhoef, Clive West and Frans Kok were responsible for study design and interpretation of results. In addition, Hans Verhoef was responsible for data collection and analysis. Paul Ndeto assisted in detailed planning of the field work and data collection. Jan Burema advised on statistical analyses. Yves Beguin carried out biochemical analyses and assisted in interpretation of those results.

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Stunting may determine the severity of malaria-associated anemia in African children

Hans Verhoef, Clive E West, Jacobien Veenemans, Yves Beguin, Frans J Kok

ABSTRACT

Objective: Evidence from previous studies that malnourished children are protected against malaria is controversial. Our aim was to evaluate whether observational data support the hypothesis that stunting modifies the associations between malaria and hematologic indicators such as hemoglobin concentration, and serum concentrations of soluble transferrin receptor and C-reactive protein.

Methodology: Community-based cluster survey among Kenyan children aged 2-36 months asymptomatic for malaria or anemia (n=318).

Results: When adjusted for age and wasting, the malaria-associated decrease in mean hemoglobin concentration was 8.5 g/L and 15.8 g/L in nonstunted and stunted children, respectively (p-value of test for difference: 0.08). The malaria-associated increase in geometric mean serum concentrations of soluble transferrin receptor was 1.1-fold and 1.8-fold in nonstunted and stunted children, respectively (p-value of test for difference: 0.05). The malaria-associated increase in geometric mean serum concentrations of C-reactive protein was 1.4-fold and 2.3-fold in nonstunted and stunted children, respectively (p-value of test for difference: 0.05). Thus, children with malaria and who were stunted suffered from more severe anemia, and had higher serum concentrations of C-reactive protein and soluble transferrin receptor than would be expected from the combined effect of the two working independently.

Conclusions: Our results are consistent with the notion that the nutritional inadequacies causing stunting also impair host immunity, thus increasing the degree to which malaria is associated with decreased concentrations of hemoglobin, with increased inflammation, and with increased iron demand in developing erythroblasts. Increased intake of micronutrients may not only reduce stunting and nutritional anemia, but also reduce malaria-associated anemia.

Pediatrics (conditionally accepted)

Children in developing countries generally fail to achieve their genetically determined potential height because of poor diet and infection (Ulijaszek 1994, Waterlow 1994). It is plausible although unproven so far that the nutritional inadequacies that cause stunting also impair host immunity, thereby increasing the incidence, severity, and duration of many infectious diseases (Tomkins 1988).

Individuals with repeated exposure to malaria are protected against severe disease and death before they develop the ability to regulate fever, parasite density, and ultimately infection in itself. This has led to the hypothesis that immunity develops first against severe disease, then against fever, and lastly against parasites (Snow and Marsh 1998). If true, this would suggest that reduced immune function such as may exist in stunted children exacerbates the severity of malarial signs and symptoms, rather than the occurrence of parasitemia. On the other hand, several reports suggest that malnourished children are protected to some degree against malaria (Hendrickse et al. 1971, McGregor 1982, Murray et al. 1975).

The concentration of soluble transferrin receptor (sTfR) in serum is a new indicator of iron demand by the erythroid precursor mass. It provides a measure of both the rate of erythropoiesis and the deficit of functional iron in the erythron (Feelders et al. 1999), and is influenced little or not at all by the inflammatory response to infection. We recently reported increased serum concentrations of sTfR and C-reactive protein in children with asymptomatic malaria (Verhoef et al., accepted). These increased serum sTfR concentrations may be explained, at least in part, by increased erythropoiesis to compensate for malaria-induced hemolysis (Verhoef et al., accepted).

We carried out a cross-sectional study in children aged 2 to 36 months without symptoms of malaria or anemia living in an area with seasonal malaria transmission. The aim of our study was to evaluate whether data from this survey give support to the hypothesis that the relationship between malarial parasitemia and stunting is synergistic, i.e., that the presence of asymptomatic malaria and stunting results in a lower concentration of hemoglobin, and higher serum concentrations of sTfR and C-reactive protein, than would be expected from the combined effect of the two working independently.

Subjects and methods

Study area

The study was conducted in the first annual rainy season (April to June) of 1997 in an area of seasonal malaria in Eastern Province, Kenya. The study area comprises approximately 720 km^2 at an altitude of 800 to 900 m above sea level, located halfway between Nairobi and Mombasa on the road and rail link. Malaria infection reported at the clinical facilities in the area are due exclusively to *Plasmodium falciparum*. No malaria control program is active in the area, and no epidemiologic or entomologic studies on malaria have been carried out previously.

Study population and sampling procedures
The target population comprised children living in the study area aged 2 to 36 mo, with no manifestations reported by mothers or caregivers that were compatible with malaria or anemia. Few of the children studied suffer from intestinal worm infections or schistosomiasis (Verhoef et al., accepted). Sampling procedures have been described in detail previously (Verhoef et al., accepted). In brief, children were selected using a 2-stage cluster sampling procedure. At the first sampling stage, 45 of 79 communities in the study area were sampled systematically, with a sampling probability proportional to size as measured by the number of households. At the second sampling stage, 12 households were randomly selected within each selected community, and all resident children with no symptoms of malaria or anemia and within the desired age range were selected for the study.

Field procedures
Informed consent was sought and obtained from community leaders, locally active auxiliary health workers, and parents of eligible children. Children were treated as deemed necessary upon completion of the survey. The study was approved by the African Medical and Research Foundation and the Kenya Medical Research Foundation, whose ethical standards were followed.

Field staff were trained in anthropometric techniques, and measurements were standardized (Anonymous 1986, Lohman et al. 1988) prior to data collection. Recumbent length (< 2 y) or standing height (≥ 2 y), body weight (Seca 720 baby scale, allowing for measurements to be read within 10 g), mid-upper arm circumference and triceps skinfold thickness (Harpenden calipers, CMS Weighing Equipment, London, UK) were recorded as the average of duplicate measurements, respectively.

The procedures for collection, handling and analysis of capillary blood samples has been described elsewhere (Verhoef et al., accepted). Hemoglobin concentrations were measured using a field meter (HemoCue, Ängelholm, Sweden) and malaria was detected by microscopic examination of Giemsa-stained blood slides. Concentrations of sTfR were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis MN, USA). C-reactive protein concentrations were interpreted as indicators of the activity of infection and measured by a standard turbidimetric method. Intra- and interassay coefficients of variation for these biochemical tests are reported elsewhere (Verhoef et al., accepted).

Response and missing values
The study included 318 children. Of 35 children who did not participate or fully participate, 14 were replaced by random selection, and weighting was used to maintain the validity of assuming an equal probability sample (Bennett et al. 1991, Brogan et al. 1994). Sample sizes reported below 318 are due to missing values.

Statistical analysis
Height-for age and weight-for-height z-scores were calculated using Epi Info v6.04 (Dean et al. 1994). Being stunted or wasted was defined by height-for-age or weight-for-height

z-scores < -2 SD of the median of the NCHS reference population, respectively (WHO 1983). Data were explored using SPSS (v7.5 for Windows; SPSS Inc. Chicago Ill., USA) and analyzed using SUDAAN (stand-alone software v7.5.2a for Windows; Research Triangle Institute, Research Triangle Park NC, USA), assuming two-stage cluster sampling with replacement at the first sampling stage. The variance estimates under this assumption do not take into account that clusters were sampled from a finite population. Thus the standard error values and confidence intervals reported here are overestimates, and statistical tests are conservative in detecting existing associations.

As a first analytic step, the relationships between malaria and height-for-age z-score and between malaria and weight-for-height z-score were assessed by logistic regression. Serum concentrations of sTfR and C-reactive protein were normalized by decimal logarithm transformations, and their relationship with stunting or wasting were assessed by linear regression analysis. Associations between height-for-age z-score and age class (2-11 mo, 12-23 mo, >24 mo) or continuous variables (weight-for-height z-score, hemoglobin concentration) were assessed by analysis of variance and linear regression analysis, respectively.

Multiple linear regression was used to compare stunted and nonstunted children regarding their associations between malaria and hemoglobin concentration, or between malaria and log transformed serum concentrations of sTfR or C-reactive protein, respectively. Possible interaction was tested directly by multivariate linear regression analysis. Adjustment for age class and sex led to similar conclusions and effect estimates, so that these variables were excluded from the final model.

Results

The prevalence of children who were stunted or wasted was 38.7% (95% CI: [32.0-45.4] and 4.7% (95% CI: [1.6-7.8], respectively. The prevalence of malaria was 17.6% (95% CI: [11.3-23.8]; n=318): for age classes 2-11 mo, 12-23 months and >24 mo, these figures were 12.6% (n=92), 18.8% (n=116) and 20.4% (n=111), respectively, and for girls and boys they were 16.1% (n=145) and 18.8% (n=173), respectively. Other characteristics of the study population, broken down by age class and sex, are given in **table 1**. Stunting was most pronounced in children aged 12-23 mo, whose height-for-age z-score was 0.75 below the value of children aged 2-11 mo.

An increase of one unit z-score of height-for-age was associated with an odds ratio of malaria of 0.87 (95% CI: 0.69-1.09; p=0.23), a weight-for-height increase of 0.16 z-scores (95% CI: 0.04-0.27; p=0.009), and a hemoglobin concentration increase of 2.0 g/L (95% CI: 0.7-3.4 g/L; p=0.004). Each increase of one unit z-score of weight-for-height was associated with an odds ratio of malaria of 0.78 (95% CI: 0.58-1.05; p=0.10) and an increase in hemoglobin concentration of 2.3 g/L (95% CI: 1.0-3.6 g/L; p=0.001), respectively. When adjusted for wasting (weight-for-height z-score quartiles), an increase of one unit z-score of height-for-age corresponded to an odds ratio of malaria of 0.93 (95% CI: 0.73-1.17; p=0.52). When analysed by univariate linear regression techniques,

TABLE 1. Characteristics indicating nutritional status of the population studied, by age class and sex

		Height-for-age z-score		Weight-for-height z-score		Mid-upper arm circumference, * cm		Triceps skinfold thickness,* mm	
Age class	2-11mo	-1.29 ± 1.56	(92)	-0.03 ± 0.96	(92)	14.5 [13.2-15.1]	(92)	7.51 [5.76-7.89]	(91)
	12-23 mo	-2.04 ± 0.91	(113)	-0.85 ± 0.74	(113)	14.7 [13.7-15.4]	(116)	6.89 [6.00-7.85]	(114)
	>24 mo	-1.93 ± 0.90	(107)	-0.88 ± 0.57	(107)	14.7 [14.1-15.4]	(108)	6.97 [6.25-7.98]	(107)
Sex	Male	-1.90 ± 1.23	(169)	-0.59 ± 0.55	(169)	14.8 [14.0-15.4]	(146)	7.05 [5.95-7.94]	(143)
	Female	-1.65 ± 0.66	(142)	-0.65 ± 0.54	(142)	14.5 [13.5-15.2]	(135)	6.93 [5.96-7.92]	(134)
All		-1.79 ± 0.81	(311)	-0.62 ± 0.42	(311)	14.6 [13.8-15.3]	(316)	6.97 [5.95-7.95]	(311)

Mean ± SD or * median [interquartile range] (n). Figures were derived from weighted analysis and were rounded off to integer values.

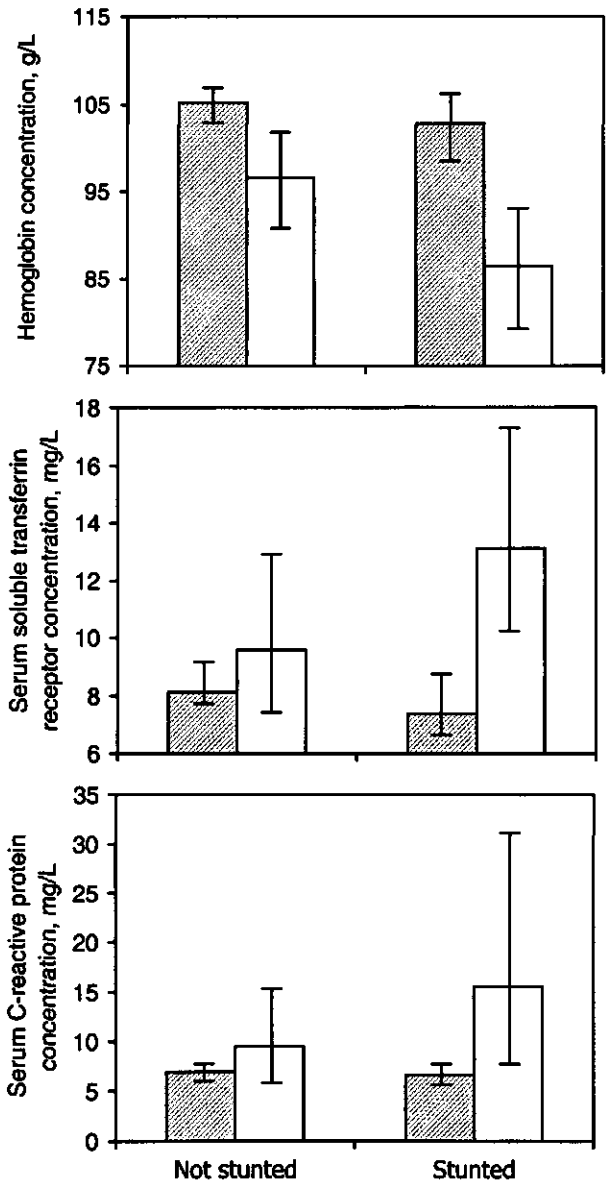

FIGURE 1. Modification by stunting of the relation between malaria and mean hemoglobin concentration (top; n=311), and geometric mean serum concentrations of transferrin receptor (center; n=222) and C-reactive protein (bottom; n=90).

When adjusted for age and wasting, p-values indicating significance of the malaria-associated differences in these hematologic indicators were 0.08, 0.05, and 0.05, respectively (see text). Absence and presence of malaria are indicated by black and white columns respectively; error bars indicate 95% confidence intervals.

there was no evidence of a biologically or statistically significant relationship between stunting and log transformed serum concentrations of sTfR or C-reactive protein, or between wasting and log transformed serum concentrations of sTfR or C-reactive protein (data not shown).

Univariate analysis showed that children with malaria had lower mean hemoglobin concentrations than children without malaria (92.7 g/L compared with 104.1 g/L; difference 11.3 g/L; 95% CI: 6.4-16.3 g/L; p=0.0001). Children with malaria also had higher geometric mean serum concentrations of sTfR (11.4 mg/L compared with 7.8 mg/L; ratio 1.4; 95% CI: 1.1-1.9; p=0.005) and C-reactive protein (12.5 mg/L compared with 6.8 mg/L; ratio 1.8; 95% CI: 1.2-2.7; p=0.004).

Figure 1 compares stunted and nonstunted children regarding their association between malaria and hematological indicators. There was no evidence for substantial effects of stunting on these indicators in the absence of malaria. The malaria-associated decrease in mean hemoglobin concentration was 8.6 g/L (95% CI: 2.6-14.6 g/L) in nonstunted children, and 16.4 g/L (95% CI: 9.3-23.5 g/L) in stunted children (**figure 1, top**). In nonstunted children, geometric mean serum concentrations of sTfR were 1.2-fold (95% CI: 0.9-1.6) higher in children with malaria than those without malaria (**figure 1, center**); in stunted children, this ratio was 1.8 (95% CI: 1.3-2.5). Among nonstunted children, geometric mean serum concentrations of C-reactive protein were 1.4-fold (95% CI: 0.8-2.3) higher in children with malaria than those without malaria (**figure 1, bottom**); among stunted children, this ratio was 2.3 (95% CI: 1.2-4.7).

These relationships were further assessed by multivariate regression analysis, which was used to test the significance of product terms directly and to adjust for possible confounding by age class and wasting (entered into the model as weight-for-height z-score quartiles). When so analysed, the malaria-associated decrease in mean hemoglobin concentration was 8.5 g/L and 15.8 g/L in nonstunted and stunted children, respectively (p-value of test for difference: 0.08). The malaria-associated increase in geometric mean serum sTfR concentrations was 1.1-fold and 1.8-fold in nonstunted and stunted children, respectively (p-value of test for difference = 0.05). The malaria-associated increase in geometric mean serum concentrations of C-reactive protein was 1.4-fold and 2.3-fold in nonstunted and stunted children respectively (p-value of test for difference: 0.05). These effect estimates are similar for non-adjusted estimates (see preceeding paragraph), indicating little or no confounding by age or wasting. There was no evidence that the relationship between malaria and these hematologic indicators was modified by age (data not shown).

Missing data were mostly due to insufficient serum being available to determine serum concentrations of sTfR and C-reactive protein. Children with missing data (n=96), and who were therefore excluded from the multiple regression analysis, did not have substantially different hemoglobin concentrations or height-for-age z-scores from those who were included (not shown), although fewer of them sufferered from malaria (14% compared with 25%). Selection bias would have occurred if the observed relationships were different in children with missing values.

Discussion

Our findings support the hypothesis that in stunted children, malaria is associated with lower hemoglobin concentrations and higher serum concentrations of sTfR and C-reactive protein than in their nonstunted counterparts. There is no clear evidence that stunting is associated with an increased prevalence of malarial infection.

The prevalences of stunted and wasted children were 39% and 5% respectively. The mean hemoglobin concentration was 102 g/L, and we showed earlier that the prevalence of anemia in this population was 71% (Verhoef et al., accepted). These values are typical for this age group in Kenya and other countries in Africa (DeMaeyer and Adiels-Tegman 1985, Keller 1988, Victora 1992, Anonymous 1996). Our study confirmed that stunting was most pronounced in children aged 12-23 mo. This corroborates findings in most studies that stunting in developing countries peaks at the age of 24 months and stabilizes thereafter (WHO Working Group 1986). Missing values occurred mainly because of insufficient volume of serum being available for biochemical analysis: there is no reason to assume that this led to selection bias in the observed relationships. Our conclusions may therefore apply to African preschool children who are asymptomatic for anemia and malaria and living in areas of seasonal malaria.

Serum sTfR concentrations are closely correlated to the number of receptors expressed on the surface of erythroblasts, where they transport transferrin-bound iron into the cell. An expansion of the erythroid mass results in increased serum sTfR concentrations, and increased transferrin receptor expression takes place on the surface of iron-deficient erythroblasts. Serum sTfR concentrations thus reflect both the rate of erythropoeisis and the degree of iron deficiency in the erythron (Feelders et al. 1999). Serum sTfR concentrations are not or only marginally influenced by the inflammatory response to infection (Feelders et al. 1999, Weiss 1999), and no infections investigated so far except for malaria have been shown to be associated with serum sTfR concentrations. We proposed earlier that the observed increase in serum sTfR concentration in malaria is due in part to hemolysis, which occurs in malaria is not a common feature in other infections (Verhoef et al., accepted). Malaria-induced hemolysis leads to lower hemoglobin concentrations, which in turn results in increased erythropoeisis under influence of increased production of erythropoeitin. Red cell destruction may occur as a result of direct action by malaria parasites, or because a malaria-induced immune response leads to lysis of parasitized and non-parasitized red cells (Menendez et al. 2000). It cannot be ruled out that, in addition, serum sTfR concentration is increased in malaria due to ineffective erythropoeisis, whereby developing erythroblasts die prematurely in the marrow without producing mature red cells (Verhoef et al., accepted).

Our findings indicate that malaria-associated anemia, iron demand and inflammation are greater in stunted than in nonstunted children. Although it is conceivable that malaria causes stunting, we consider it more likely that stunting exacerbates malaria, given that malarial infection is a transient condition, and stunting becomes manifest only after a prolonged period of slowing in skeletal growth. Nutritional inadequacies that cause stunting – such as deficiencies in zinc (Umeta et al. 2000), iron (Angeles et al. 1993, Adish

et al. 1999) and possibly vitamin A (Muhilal et al. 1988, Hadi et al. 2000) – are also known or suspected to impair host immunity (Dalmann 1987, Brock 1994, Semba 1998, Salgeiro et al. 2000, Shankar et al. 1998). As reviewed by Scrimshaw et al. (1968), there is compelling evidence that reduced immunity due to nutritional deficiencies reduce host capacity to resist the consequences of a wide range of infections. This most likely explains the modification by stunting of the effects of malaria as observed in our study.

Before 1950 it was widely accepted though little supported by epidemiologic evidence, that malnutrition is associated with greater frequency and severity of malaria (Shankar 2000). A number of influential studies subsequently appeared that in fact suggested that malnourished children are to some degree protected against malaria. However, Shankar (2000) in a review concluded that these studies suffered from methodologic shortcomings and that the available evidence indicates that malnutrition is associated with increased occurrence of infection and symptomatic malaria, and considerably higher likelihood of malaria mortality in humans. This is in line with our findings, and implies that nutritional interventions using micronutrients may not only reduce stunting and nutritional anemia, but also reduce malaria-associated anemia. Famine or starvation may be a notable exception, because there is strong evidence that refeeding of starved individuals with latent infections carries an increased risk of symptomatic malaria. This may suggest that parasite proliferation following refeeding temporarily outpaces development of protective immunity (Shankar 2000). We could not demonstrate an influence of wasting on malaria or malaria-associated anemia (data not shown), perhaps because few of the children studied were wasted.

In summary, our results give observational support to the proposed hypothesis that the nutritional inadequacies that cause stunting also impair host immunity, thus increasing the degree to which malaria is associated with decreased concentrations of hemoglobin, with increased inflammation and with increased iron required for developing erythroblasts. Increased intake of micronutrients may not only reduce stunting and nutritional anemia, but also reduce malaria-associated anemia. However, longitudinal studies are required to confirm causality.

Contributions

Hans Verhoef, Clive West, and Frans Kok were responsible for study design and interpretation of results. Hans Verhoef was responsible for data collection and analysis. Jacobien Veenemans and Yves Beguin assisted in interpretation of results, and Yves Beguin carried out the biochemical analyses. Yves Beguin is a Research Director of the National Fund for Scientific Research (FNRS, Belgium).

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5

Intermittent administration of iron and sulfadoxine-pyrimethamine to control anaemia in Kenyan children: a randomized controlled trial

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SUMMARY

Background: Iron supplementation is recommended for children at high risk of anaemia, but its benefits may not outweigh the associated risk of malaria in areas of seasonal transmission. We determined the efficacy in improving haemoglobin concentrations of intermittent administration of iron supplements and sulfadoxine-pyrimethamine (SP) in asymptomatic children under intense health surveillance.

Methods: In a trial with a 2×2 factorial design, 328 anaemic Kenyan children were randomly assigned to receive either iron or placebo, and SP or placebo. Outcomes considered were change in haematological indicators of iron status and inflammation, and occurrence of malaria attacks. Morbidity surveillance comprised 4-weekly medical examinations, continuous passive case-detection, and twice-weekly visits.

Findings: Intermittent administration of iron and SP resulted in substantially improved haemoglobin concentrations (12.5 g/L, 95% CI: 8.5 to 16.4 g/L; adjusted for prognostic factors at baseline) compared with the placebo group. It also reduced the estimated prevalence of anaemia from 100% at baseline to 36% at 12 w, and of iron deficiency from 66% at baseline to 8% at 12 w. Survival analysis indicated no evidence of substantially increased risk of malaria following iron supplementation. Intermittent administration of SP, either alone or in addition to iron supplementation, gave no or little haematological response.

Interpretation: Iron supplementation gives substantial health benefits, which may outweigh possible inherent risks caused by malaria. Further studies should determine the benefits and risks of intermittent administration of SP in reducing the incidence of malaria attacks in areas of seasonal malaria transmission.

Submitted for publication

Approximately three quarters of children <5 y of age in eastern Africa suffer from anaemia, defined by haemoglobin concentrations below 110 g/L (DeMaeyer and Adiels-Tegman 1985). Iron deficiency, when sufficiently severe to cause anaemia, has been associated with impaired physical growth (Chwang et al. 1988, Latham et al. 1990, Lawless et al. 1994) and mental development (Grantham-McGregor and Ani 2001), decreased appetite (Lawless et al. 1994), reduced physical activity (Haas and Brownlie 2001), and possibly with increased incidence of some infections (Oppenheimer 2001).

Routine iron supplementation of children living in communities with a high prevalence of anaemia has been advocated (UNICEF/WHO 1994, INACG/WHO/UNICEF 1998) but in certain, unknown conditions may lead to increased risks of malaria (INACG Consensus Statement 1999, Shankar 2000, Oppenheimer 2001). Oral iron supplementation (2 mg/kg) given daily to infants aged 8-24 mo in an area of high, perennial transmission did not increase the incidence of malaria (Menendez et al. 1997). In areas of seasonal malaria or in somewhat older age groups, children are likely to have less protective immunity, and the risk of malaria or malaria-related outcomes associated with iron supplementation may accordingly be higher. Comparison of evidence from trials that were conducted in infants and preschool children in areas of marked seasonal malaria (Smith 1989, Adam 1997) and highly endemic areas (Chippaux et al. 1991, Menendez et al. 1997, Stoltzfus et al. 1998) would appear to give some support to this hypothesis. Thus, the benefits of iron supplementation may not outweigh the risk of adverse effects in populations with low immunity against malaria.

Although mild-to-moderate anaemia often occurs in asymptomatic malaria (Brabin 1992, McElroy et al. 2000), little is known about its functional importance. These infections are probably associated with increased risk of malaria attacks (Bouvier et al. 1997) but are needed to gain protective immunity against severe disease and subsequent death, which develops much faster than against fever and parasite density (Snow and Marsh 1998, Gupta et al. 1999). Thus, the effect of treatment on all-cause mortality remains unknown. Chemoprophylaxis has been shown to reduce malaria morbidity and all-cause mortality (Bradley-Moore et al. 1985, Greenwood et al. 1988, Menon et al. 1990, Menendez et al. 1997) but is no longer advocated in endemic areas (Greenwood 1991, WHO 1993), mainly for fear of accelerated spread of resistance, and of increased risk of malaria attacks following cessation of the intervention (Greenwood et al. 1995, Menendez et al. 1997).

The potential gains in haemoglobin concentrations of iron supplementation and malaria control in children in seasonal malaria remain uncertain. Hence, with the aim of maximising the beneficial effects of iron supplementation and chemoprophylaxis whilst minimizing possible adverse risks, we determined the efficacy in improving iron status of intermittent administration of iron and sulfadoxine-pyrimethamine (SP), when given either alone or in combination, to Kenyan children in conditions of seasonal malaria. In addition, we explored the effects of interventions on the time until first occurrence of a malaria attack.

Subjects and methods

Area and population

The study was conducted in Mtito Andei Division, Eastern Province, Kenya. This area is described in more detail elsewhere (Verhoef, 1999; accepted a). An earlier survey among children aged 2-36 mo in this area had shown prevalences of malaria, anaemia, *Ascaris lumbricoides*, and *Trichuris trichiura* of 31%, 72%, 3% and 5%, respectively (Verhoef, unpublished results). Reported malaria infections were exclusively due to *Plasmodium falciparum*. No hookworm or schistosomiasis was found. A 14-day *in vivo* drug sensitivity study was carried out in 1997 among children (mean age 4.3 years) in the out-patients department of a nearby health centre. Out of 36 studied children treated with SP, 32 (89%; 95% confidence interval, CI: 79-99%) were found to be sensitive to treatment, three showed R1 resistance, and one showed R2 resistance (Piet Kager, University of Amsterdam, personal communication).

The present study was facilitated by community health workers who had been trained to provide health education and promotion, but who are not routinely involved in treatment of illnesses. A research clinic was established in the area and children were recruited from neighbouring communities in three groups, each at the start of a rainy season in the period 1998-2000. The study received ethical approval from AMREF and the Kenya Medical Research Institute. Informed consent was obtained from community leaders and local government officials, and from parents of participating children. Children included in the study and their siblings received free medical care for common childhood illnesses.

Study type and interventions

The study consisted of a trial with a 2×2 factorial design with an intervention period of 12 w. Children were randomly assigned to either iron supplement or placebo, and either sulfadoxine-pyrimethamine or placebo, and were all under intense health surveillance. Both placebos and active compounds were administered as suspensions that were indistinguishable by taste and appearance, and were prepared under supervision by an experienced pharmacologist (G van der Meer, Gelderse Vallei Hospital, Ede, The Netherlands). Bottles were colour-coded, but none of the field investigators was aware of the code until after crude analysis and a plan for further analysis had been prepared.

Iron was administered by community health workers twice weekly as ferrous fumarate in a 6.25 g/L suspension at a target dose of 6 mg elemental iron kg body weight $^{-1}$ wk $^{-1}$. Compared to ferrous sulphate tablets, the use of ferrous fumarate suspension has the advantages of increased dosing accuracy that can be achieved, a longer shelf-life (up to 6 wk), it may cause fewer side effects, while it is absorbed at a similar rate (Brise et al. 1962). No suspension was used beyond 6 w after opening of bottles.

Sulfadoxine-pyrimethamine was administered by project staff every 4 w at therapeutic doses (25 mg and 1.25 mg kg^{-1} body weight, respectively). When given intermittently to infants alongside routine vaccinations against childhood diseases, SP may substantially reduce the frequencies of occurrence of malaria attacks and severe anaemia (Schellenberg et al. 2001).

Eligibility and withdrawal criteria
Eligibility criteria were: haemoglobin concentrations 60-110 g/L; age 2-36 mo; axillary temperature <37.50 °C; absence of symptoms suggestive of malaria or anaemia, or of any systemic illness occurring in combination with a blood dipstick test indicating current or recent malarial infection; parents reported their intention to stay in the study area during the intervention period; parental consent given; absence reported of allergy to sulfa drugs; and no sulfa drugs used in the previous 3 w.

Children were withdrawn and treated as appropriate if haemoglobin concentrations were <50 g/L, if they met one or more criteria of severe and complicated malaria (Warrel et al. 1990), or if they had manifestations of other severe disease.

Sampling procedures and treatment allocations
Malaria, anaemia and other factors known or suspected to be geographically or temporally clustered (Verhoef et al., see chapter 2) might be prognostic for the effects of treatments. Balanced block randomization was expected to give an even distribution over the experimental treatment groups of these factors. Thus, children were sampled from an up-to-date census, and recruitment continued until eight children had been randomly selected per community. Interventions were randomly allocated in duplicate within 41 of these blocks, using tables with randomised permutations, and only after eligibility for study had been established. The study was designed to measure a difference between groups in change in haemoglobin concentration within 5 g/L of the true value (α=0.05).

Field procedures
Children were medically examined at baseline, and after 4, 8 and 12 w. At each of these visits, samples of capillary blood from each child were tested by malaria dipstick, and haemoglobin concentration was measured. For each day of the recruitment period, the community health worker and mothers from one community were asked to bring invited children to the research clinic. A questionnaire was administered to collect vital information. Anthropometric measurements were taken at baseline and after 12 w as described previously (Verhoef et al., accepted b). Children were then photographed for future identification, and their mothers and community health workers received a treatment schedule and an identification card. The distance between the homestead of each child and the research clinic was measured in duplicate using a global positioning system (Model 12, Garmin International Inc., Olathe KS, USA).

Parents were asked to report immediately to the clinic with their child when suspecting fever or other illnesses. Community health workers each supervised one block of eight children within their own community through meetings twice per week with each child. At these meetings, they administered iron or iron placebo using syringes on which amounts of suspension, colour code for each suspension and the name of the child had been pre-indicated. They also recorded axillary temperature on a one-page questionnaire, together with information about whether or not the child had been sick on previous days, and if adverse drug reactions had occurred. Questionnaires were handed in and perused within one day after completion. Compliance of the work of community health workers was also

checked unseen to them by inspecting whether residue of suspension was present in syringe tips.

Sick children

Children reported sick were medically examined, and blood samples were collected and assayed by malaria dipstick test, and to determine haemoglobin concentration. Children with malaria attacks, defined by the occurrence of both fever confirmed by thermometer and a positive dipstick test result, were treated with amodiaquine or, if this failed, with halofantrin under supervision. Afebrile children with a reported history of recent fever were tested repeatedly by thermometer and dipstick assay at intervals of several hours until positive or a diagnosis of malaria could be excluded. Febrile children with a negative dipstick test had their blood slide examined within 24 h at the nearest facility, at 50 km distance. Children were always promptly treated for common illnesses, or referred if needed. During the intervention period, treatment with sulfa antibiotics was avoided. Those with anaemia at the end of the intervention period, or with a positive dipstick test result for malaria, were treated with iron, and with amodiaquine and sulfadoxine-pyrimethamine, respectively.

Laboratory measurements

Axillary temperature was measured by electronic thermometer (Model HP 5316; Philips, Groningen, The Netherlands). Haemoglobin concentrations were determined by photometer (HemoCue, Ängelholm, Sweden), and dipstick tests (Model ML02; AMRAD/ICT, Sydney, Australia) were used for rapid detection in blood of antigens specific to *Plasmodium falciparum* (Anonymous 2000). In patients with manifestations of malaria, the sensitivity and specificity this test is estimated to be >95% in detecting parasitaemia as determined by microscopy (Garcia et al. 1996, Kumar et al. 1996, Kilian et al. 1997, Durrheim et al. 1998). A negative test result is highly predictive for the absence of malarial infection, whilst a positive test result probably has less predictive value for the presence of infection (Eisen and Saul 2000). Resources were lacking for routine microscopic examination of blood smears.

Procedures for collection and handling of blood samples have been described elsewhere (Verhoef et al., accepted a). Ferritin concentrations were determined using commercial reagent kits according to instructions from the manufacturer (Diagnostic Products Corporation, Los Angeles CA, USA). C-reactive protein concentration was determined as an indicator of inflammation in an assay (Orion Diagnostics, Espoo, Finland) adapted to allow measurement in low volumes of ranges from zero to highly elevated values. This method correlated excellently with standard methods (Beckman Coulter, Brea CA, USA; Roche Diagnostics, Mannheim, Germany).

Statistical analysis

Anthropometric indices were calculated by Epi Info (v6.04b; CDC, Atlanta, GA, USA). Data cleaning and standard analysis was undertaken using SPSS (v7.5.2; SPSS Inc., Chicago, Ill, USA) on an intention-to-treat basis. Iron deficiency was defined as serum ferritin concentrations <12 µg/L, measured in children who concurrently had both negative results when their blood was assayed by malaria dipstick test and serum C-reactive protein

concentrations ≤15 mg/L. Lower cut-off values of serum C-reactive protein concentrations did not result in appreciably different prevalence estimates.

Change in haemoglobin concentration was calculated as the difference between values before and after intervention. Effects of interventions were measured as the difference in change between treatment groups and the placebo group, adjusted for block effects to take the study design into account, and with adjustment for imbalances in prognostic factors at baseline. Interaction effects were assessed directly by multiple regression. Intervention groups were also compared regarding their prevalence at the end of the intervention period of anaemia and iron deficiency, or having a positive dipstick test result.

Kaplan-Meier curves were constructed to examine the effect of the interventions on time until first occurrence of a malaria attack, assessed both directly and when stratified by malaria dipstick test result at baseline. Logistic regression was used to assess the effects on the occurrence of a malaria attack over the 12 w intervention period of interventions and malaria dipstick test result at baseline. Adjustments were made for block effects and age class.

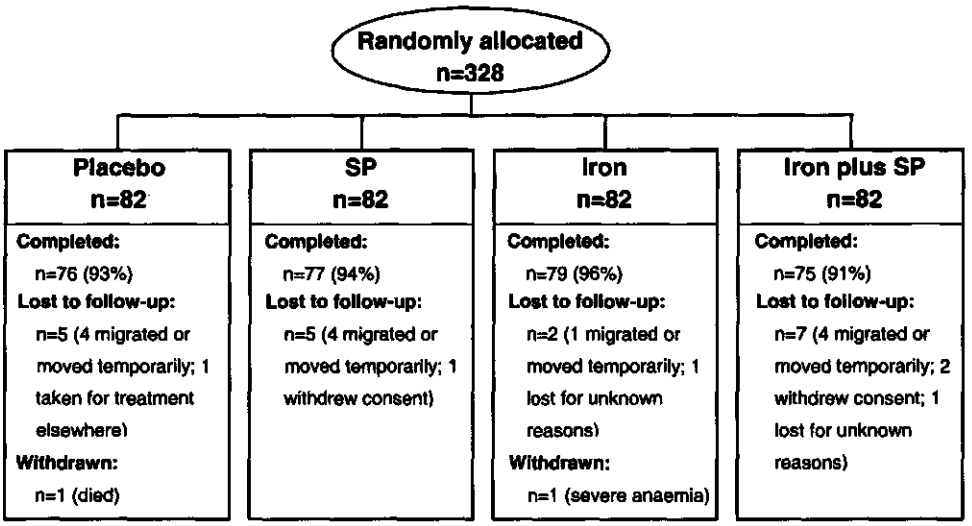
Chronic inflammation unrelated to malaria may be an important cause of anaemia. This was examined by measuring, in children who received placebo and whose blood sample tested negative when assasyed by malaria dipstick tests at all surveys (baseline, 4 w, 8 w, and 12 w), the proportion who had serum C-reactive protein concentrations ≥10 mg/L both at baseline and at 12 w.

Results

Of 328 children who underwent randomisation (**figure 1**), 307 (94%) completed the trial, and 21 (6%) did not, for the following reasons: migrated or moved temporarily from the study area (13), parents withdrew consent (3), withdrawn due to occurrence of severe anaemia (1), died (1), developed malaria but parents took the child for treatment elsewhere (1), unknown reasons (2). Of all iron or iron placebo doses scheduled until the time of censoring, 99% were administered. This estimate was the same whether censoring was due to completion of the trial, or due to being either withdrawn from the study or being lost to follow-up. All SP or SP placebo doses scheduled until the time of censoring were administered. No severe skin reactions were reported in 3824 child-weeks of follow-up.

The intervention groups were similar regarding their distribution of baseline characteristics (**table 1**). The effect over the entire intervention period on change in haemoglobin concentration of combined iron supplementation and intermittent administration of SP was 12.2 g/L (**table 2**; adjusted for block effects). Similarly measured, the effects of either iron supplementation alone or intermittent administration of SP alone were 13.1 g/L and 5.0 g/L. Additional adjustment for differences in prognostic factors at baseline led to similar effect estimates (12.5 g/L, 12.7 g/L, and 4.3 g/L, respectively; **table 2**). Thus, the effect

FIGURE 1. Trial profile



Placebo: iron placebo and sulfadoxine/pyrimethamine placebo; SP: iron placebo and sulfadoxine/pyrimethamine; Iron: iron and sulfadoxine/pyrimethamine placebo; Iron plus SP: iron and sulfadoxine/pyrimethamine. Children who completed an intervention were allocated and completed that intervention, and were included in the analysis.

of combined iron supplementation and intermittent administration of SP appeared less than the summed effects of the individual interventions (difference: -4.6 g/L; 95% confidence interval, CI: -10.2 g/L to 1.1 g/L; p=0.11). There was no evidence that the effect of treatment on change in haemoglobin concentration depended on age class or haemoglobin concentration at baseline (not shown). The interventions also led to small increases in height-for-age z-scores (**table 2**).

Results from the repeated surveys suggested that iron supplementation, when administered either alone or in combination with SP, continued to increase haemoglobin concentrations over the entire intervention period of 12 w (**figure 2**). Despite these gains, anaemia had not been resolved at the end of the intervention in 51% of children who had received iron alone, and in 36% of children who had received iron plus SP. The corresponding prevalences of anaemia in children who received SP only or placebo were 73% and 71%, respectively.

Iron supplementation, when given either alone or with SP, resulted in a marked reduction in the prevalence of iron deficiency (**table 3**). SP did not substantially affect this prevalence.

TABLE 1. Baseline characteristics of intervention groups

Variable	Intervention group			
	Placebo	SP *	Iron	Iron plus SP *
Sex ratio, boys % / girls %	50 / 50 (82)	58 / 42 (82)	56 / 44 (82)	55 / 45 (82)
Age, mo	19.6 ± 9.3 (82)	17.8 ± 9.5 (82)	17.3 ± 9.8 (82)	18.4 ± 9.7 (82)
No of siblings	1.9 ± 1.5 (81)	1.8 ± 1.4 (82)	2.2 ± 1.7 (82)	2.1 ± 1.8 (82)
Distance between homestead of the child and the research clinic, km	3.8 ± 2.8 (82)	3.8 ± 2.7 (82)	4 ± 2.9 (82)	4 ± 3.0 (82)
Haemoglobin concentration, g/L	97.6 ± 9.3 (82)	94.4 ± 11.8 (82)	93 ± 11.1 (82)	95.4 ± 10.9 (82)
Serum C-reactive protein concentration, mg/L	17 [14-20] (78)	17 [14-22] (80)	18 [13-25] (78)	17 [14-21] (79)
Serum ferritin concentration, µg/L	16 [5.8-33.5] (76)	12.1 [5.3-49.2] (76)	15.1 [4.8-37.7] (77)	15.8 [5.4-43.7] (75)
Prevalence of malaria	31.7 (82)	28 (82)	31.7 (82)	30.5 (82)
Height-for-age, z-score	-1.47 ± 1.27 (82)	-1.56 ± 1.11 (82)	-1.70 ± 1.09 (82)	-1.64 ± 1.10 (82)
Weight-for-height, z-score	-0.25 ± 1.03 (81)	-0.09 ± 1.01 (77)	-0.18 ± 1.14 (77)	-0.28 ± 1.00 (80)
Weight-for-age, z-score	-1.09 ± 1.29 (81)	-1.10 ± 1.11 (77)	-1.22 ± 1.24 (77)	-1.28 ± 1.24 (80)
Mid upper-arm circumference, cm	14.95 ± 1.10 (82)	14.82 ± 1.27 (82)	14.73 ± 1.18 (82)	14.54 ± 1.10 (82)

Mean ± standard deviation, SD, or median [25th - 75th percentiles] (n)

* SP: sulfadoxine-pyrimethamine

TABLE 2. Effects of interventions on change in haemoglobin concentration, and stunting as indicated by height- for-age z-score

Response indicator	n	Intervention					
		SP *		Iron		Iron plus SP *	
Change, adjusted for block effects							
Haemoglobin concentration, g/L	307	5	(0.9 to 9.1)	13.1	(9.0 to 17.3)	12.2	(8.0 to 16.3)
Height-for-age, z-score	305	0.09	(-0.05 to 0.22)	0.13	(0.00 to 0.26)	0.1	(-0.04 to 0.26)
Change, adjusted for block effects and prognostic factors at baseline †							
Haemoglobin concentration, g/L	296	4.3	(0.3 to 8.3)	12.7	(8.7 to 16.7)	12.5	(8.5 to 16.4)
Height-for-age, z-score	294	0.08	(-0.06 to 0.22)	0.11	(-0.03 to 0.24)	0.07	(-0.06 to 0.21)

Mean change (95% confidence interval) relative to the placebo group

* SP: sulfadoxine- pyrimethamine

† Age class, sex, current or recent malarial infection as indicated by dipstick test, and z- scores for both height-for-age and weight-for-height

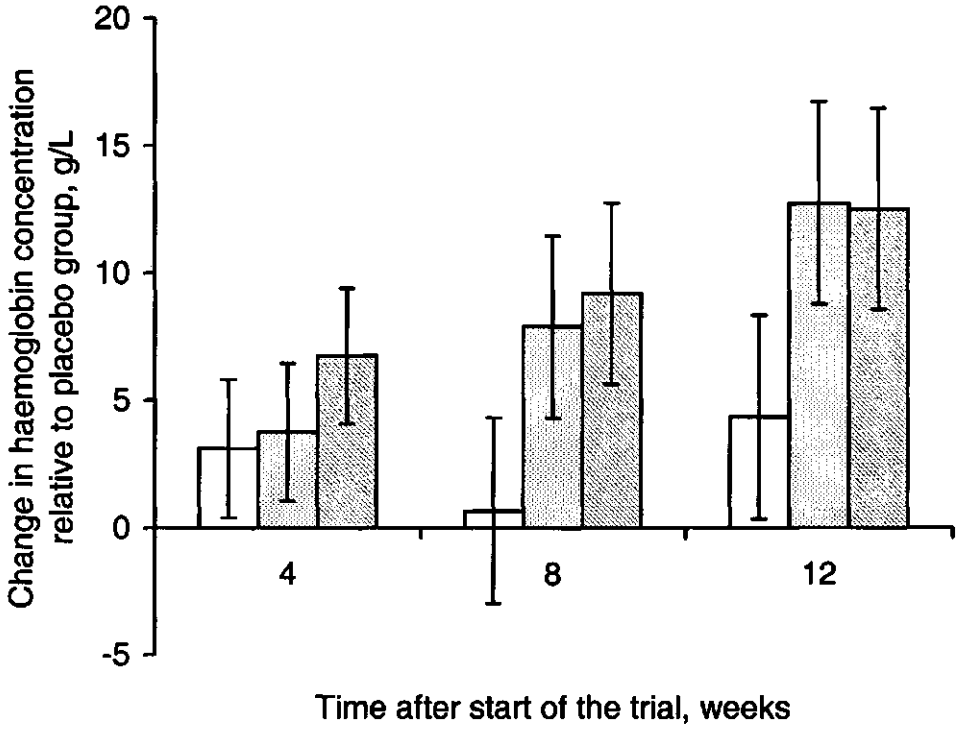


FIGURE 2. Haemoglobin response to interventions in the course of the trial

White bars: SP; light grey bars: Iron; dark grey bars: Iron + SP. Change in haemoglobin concentration was measured as the difference between values after and before intervention. Treatment response was measured as the difference in change between treatment groups and the placebo group, adjusted for block effects to take study design into account, and for prognostic factors at baseline (age class, sex, current or recent malarial infection as indicated by dipstick test, and z-scores for height-for-age and weight-for-height).

TABLE 3. Effect of the interventions on the prevalence of iron deficiency, defined by serum ferritin <12 µg/L *

Experimental treatment group	Prevalence of iron deficiency, % (95 confidence interval)	
	At baseline	At 12 w
Placebo	61 (41-81)	77 (53-94)
SP †	70 (53-88)	60 (42-78)
Iron	67 (48-86)	10 (0-24)
Iron plus SP †	66 (48-83)	8 (0-19)

* Measured in children with both a negative result when their blood was assayed by malaria dipstick test and serum C-reactive protein concentration ≤15 mg/L (see text); † SP: sulfadoxine-pyrimethamine.

TABLE 4. Prognostic factors for the development of malaria, defined by axillary temperature ≥37.50 ºC plus a positive malaria dipstick test result; multivariate analysis

Variable	Odds ratio *	(95% CI) †	p
SP ‡	0.5	(0.2-1.2)	0.12
Iron supplementation	1.3	(0.5-3.0)	0.59
Iron plus SP	1.0	(0.4-2.4)	0.99
Malaria dipstick test result at baseline	5.6	(2.6-12.4)	<0.001

* Adjusted for design (block effects) and age class; † CI: confidence interval; ‡ SP: sulfadoxine/pyrimethamine

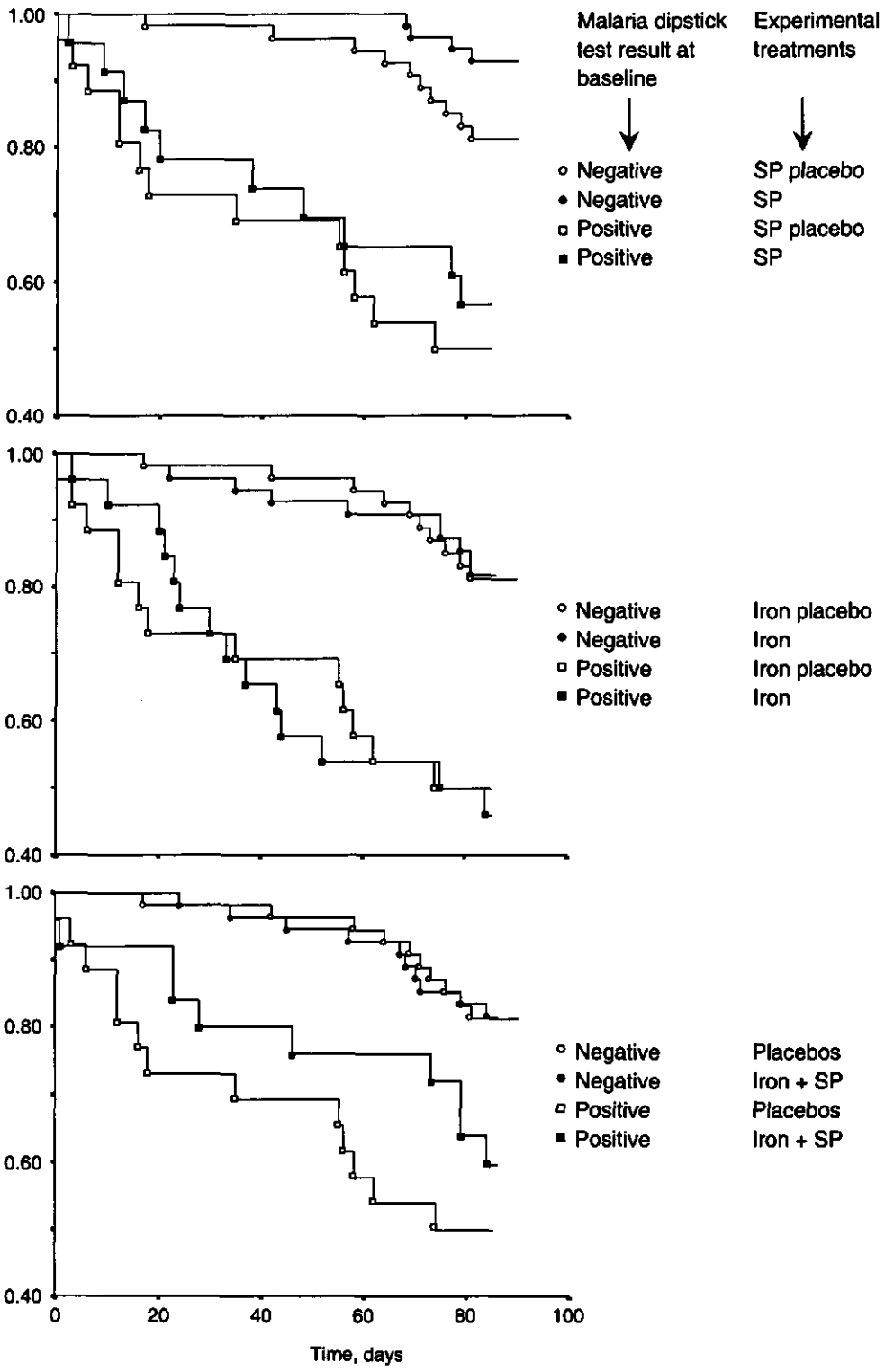
In those who completed the trial, estimates for the cumulative incidence of malaria attacks were 0.29, 0.30, 0.17, and 0.25 for children receiving placebo, iron alone, SP alone, and iron plus SP, respectively. Survival analysis indicated a protective effect of SP (p=0.08; log-rank test) on the time until first occurrence of a malaria attack, and a smaller, statistically insignificant effect of SP when given in combination with iron (not shown). Iron, when given alone or in combination with SP, did not appear to have an effect when examined by inspection of Kaplan-Meier curves or significance testing (not shown). In addition, we found no evidence that iron supplementation led to changed serum C-reactive protein concentrations (not shown).

Stratification by malaria dipstick test result at baseline led to the same conclusions drawn in the preceding paragraph whether children tested positive or negative (**figure 3**), and provided strong evidence that a positive test result at baseline markedly reduced the time until first occurrence of a malaria attack. This was confirmed by logistic regression (**table 4**). Children who received iron supplementation had a marginal and statistically not-significant increase in the odds of developing a malaria attack (**table 4**).

Of 35 children who received placebo and whose blood tested negative for malaria at all surveys (baseline, 4 w, 8 w, 12 w), 30 (86%; 95% CI: 74%-97%) had serum C-reactive protein concentrations ≥10 mg/L both at baseline and at 12 w.

FIGURE 3 (NEXT PAGE). Kaplan-Meier curves for the intervention groups, stratified by malaria dipstick test result at baseline (y-axis: proportion of children free from malaria attacks; circles: test result negative; squares: test result positive)

Top: Analysis restricted to children receiving intermittent sulfadoxine-pyrimethamine (SP; closed circles or squares) or SP placebo (open circles or squares), thus excluding children receiving intermitted iron supplementation. **Centre:** Analysis restricted to children receiving iron (closed circles or squares) or iron placebo (open circles or squares), thus excluding children receiving SP. **Bottom:** Analysis restricted to children receiving iron plus SP (closed circles or squares), or their corresponding placebos (open circles or squares), thus excluding children receiving either iron only or SP only.


Malaria dipstick test result at baseline
Experimental treatments
1.00
0.80
0.60
0.40
Negative SP placebo
Negative SP
Positive SP placebo
Positive SP
Negative Iron placebo
Negative Iron
Positive Iron placebo
Positive Iron
Negative Placebos
Negative Iron + SP
Positive Placebos
Positive Iron + SP
0
20
40
60
80
100
Time, days

Discussion

Our findings show that iron supplementation results in a marked improvement of haemoglobin concentrations, and substantially reduces the prevalence of both iron deficiency and anaemia. There was no evidence that it was accompanied by a substantially increased risk of malaria. Intermittent administration of SP alone yielded a marginal increase in haemoglobin concentrations but protects substantially against malaria. Administration of SP in addition to iron supplementation did not result in further gains of haemoglobin concentrations.

The intervention groups had similar characteristics at baseline, and the number of children lost to follow-up in the course of the study was low. Hence, these factors are unlikely to have produced substantial bias. Compliance to iron supplementation was excellent, and there were no indications that children missed a substantial number of scheduled doses. Similar haematological responses may be expected when these interventions are implemented in children aged 2-36 mo with mild to moderate anaemia living in areas of seasonal malaria.

Malaria dipstick tests are highly sensitive in detecting parasitaemia, but may have low specificity because positive results may be obtained for some time after clearance of parasitaemia (Eisen and Saul 2000). As a consequence, the effects on reported survival time until first occurrence of a malaria attack (**figure 3**, squares) may have been underestimated in children with a positive test result at baseline. In their counterparts with a negative test result at baseline (**figure 3**, circles), however, the reported survival curves validly indicate no or little risk of a malaria attack following iron supplementation. Our study was probably limited in statistical power to detect such an effect. Serum C-reactive protein concentrations remained unaffected by iron supplementation, indicating that it did not lead to increased inflammation.

We showed earlier that worm infections are relatively rare in similarly-aged children in our study area (Verhoef et al, accepted a). Iron status should therefore be improved by increasing the intake of absorbable iron. As indicated by the prevalence of anaemia at 12 w, our interventions were not as efficacious as anticipated. This might possibly be improved by using longer intervention periods than the one we maintained, or by increasing the frequency of iron administration (Beaton and McCabe 1999). Other possible causes of anaemia should also be considered, including deficiencies in vitamin A and other micronutrients. We found that the majority (86%) of children who received placebo and who remained free of malaria throughout the trial had elevated serum C-reactive protein concentrations both at baseline and at 12 w. This indicates that chronic disease due to infections other than malaria might be an additional cause of anaemia (Sears 1992, Jurado 1997, Spivak 2000).

The age group studied probably represents the range at highest risk of anaemia due to either iron deficiency or malaria. Hence, the interventions might have been less efficacious in older children. Only one case of severe anaemia was observed. Hence, intermittent administration of SP is unlikely to have a future role in public health programmes to control

anaemia in areas of seasonal malaria. It may possibly be used, however, to reduce the incidence of malaria attacks or – in areas with high, perennial transmission – to prevent severe anaemia (Schellenberg et al. 2001).

We conclude that, when giving iron twice weekly at 6 mg kg^{-1} body weight wk^{-1}, iron supplementation gives substantial health benefits that may outweigh the associated risk of adverse effects caused by malaria. Intermittent administration of SP in addition to iron supplementation does not result in further gains of haemoglobin concentrations; further studies are warranted, however, to determine its possible benefits in reducing the incidence of malaria attacks. Future research is needed to evaluate the benefits and risks of more frequent administration of iron over longer intervention periods.

Contributions

Hans Verhoef, Clive West, and Frans Kok were responsible for study design and interpretation of results. Hans Verhoef carried out the data analysis with assistance by Stefan de Vogel and Rikkert van der Valk. Hans Verhoef, Silas Nzyuko, Stefan de Vogel, Rikkert van der Valk, Mike Wanga, Anneleen Kuijsten, Jacobien Veenemans, collected the data.

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6

Malarial anaemia leads to adequately increased erythropoiesis in asymptomatic Kenyan children

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SUMMARY

Background: Malarial anaemia is associated with a shift in iron distribution from functional to storage compartments. This suggests a relative deficit in erythropoietin production or action similar to that observed in other infections. Our study aimed at investigating whether malaria causes increased erythropoiesis in Kenyan children with asymptomatic malaria, and whether the erythropoietic response appeared appropriate for the degree of resulting anaemia.

Methods: Longitudinal and baseline data were used from a trial with a 2×2 factorial design, in which 328 anaemic Kenyan children were randomly assigned to receive either iron or placebo, and SP or placebo. Erythropoiesis was evaluated by serum concentrations of erythropoietin and soluble transferrin receptor.

Findings: Prospectively collected data showed that malarial infection resulted in decreased haemoglobin concentrations, and increased serum concentrations of erythropoietin and transferrin receptor. Conversely, disappearance of malarial antigenaemia resulted in increased haemoglobin concentrations, and decreased concentrations of these serum indicators. In addition, our baseline data showed that current or recent malarial infection is associated with increased serum concentrations of erythropoietin and transferrin receptor, and that these were as high as or perhaps even higher than the values of children without malaria and without inflammation.

Interpretation: Our findings indicate that the erythropoietic response is adequate for the degree of anaemia, and that inflammation probably plays no or a minor role in the pathogenesis of anaemia associated with asymptomatic malaria. Further research is needed to demonstrate the role of deficient erythropoietin production or action in the pathogenesis of the anaemia of acute, symptomatic malaria.

Submitted for publication

Impaired erythropoiesis is believed to play an important role in the pathogenesis of malarial anaemia, and may exacerbate anaemia due to malaria-induced haemolysis (Menendez et al. 2000). Several mechanisms might explain this erythropoietic suppression. Malarial anaemia is associated with a shift of iron distribution from functional towards storage compartments (Das et al. 1997). This suggests a relative deficit in erythropoietin production or decreased marrow responsiveness to erythropoietin in malaria, similar to that observed in other infections (Means and Krantz 1992, Spivak 2000).

Reduced erythropoietin production or action might possibly explain reports (Abdalla 1990a) of reduced numbers of red cell precursors in acute malaria. Evidence for erythroid hypoplasia is supported by ferrokinetic studies showing reduced incorporation of iron into red cells during acute malaria (Srichaikul et al. 1969), and showing decreased serum soluble transferrin receptor (sTfR) concentrations during episodes of febrile malaria (Williams et al. 1999, Beesley et al. 2000). Concentrations of sTfR measure both erythropoietic activity and the deficit in the erythron of iron; they are not influenced by the inflammatory response to infections (Cook et al. 1993, 1996; Feelders et al. 1999).

Studies from Zaire, however, failed to show an effect on sTfR concentrations of malaria (Kuvibidila et al. 1995, 1999), while reports from cross-sectional studies among non-hospitalised Africans give support to increased sTfR concentration in malaria (Mockenhaupt et al. 1999, Stoltzfus et al. 2000, Verhoef et al., accepted). Erythroid hyperplasia with dyserythropoiesis may also occur in malaria, and appears more common in patients with severe anaemia and low grade parasitaemia than in those with acute malaria (Abdalla 1990a).

We used longitudinal data to investigate whether malaria and iron deficiency independently cause increased erythropoiesis in Kenyan children with asymptomatic malaria. Erythropoiesis was evaluated by serum concentrations of erythropoietin and sTfR (Barosi 1994, Beguin et al. 1993). The data used were collected as part of a randomised controlled trial to determine the efficacy in improving iron status of intermittent administration of iron and sulfadoxine-pyrimethamine (SP). In addition, we used the baseline data from this trial to evaluate whether observational data indicated that the erythropoietic response to malaria was appropriate for the degree of resulting anaemia. Detailed descriptions of study design and other results will be published elsewhere.

Subjects and methods

Area and population

The study was conducted in Mtito Andei Division, Eastern Province, Kenya, which is described in more detail elsewhere (Verhoef et al., in press). An earlier survey among children aged 2-36 mo in this area had shown prevalences of malaria, anaemia, and *Ascaris lumbricoides*, and *Trichuris trichiura* of 31%, 72%, 3% and 5%, respectively (Verhoef, unpublished results). Reported malaria infections were exclusively due to *Plasmodium falciparum*. No hookworm was found.

A research clinic was established in the area and children were recruited from neighbouring communities at the start of rainy seasons in the period 1998-2000. The study received ethical approval from AMREF and the Kenya Medical Research Institute. Informed consent was obtained from community leaders and local government officials, and from parents of participating children. Children included in the study and their siblings received free medical care for common childhood illnesses.

Design and procedures

The study consisted of a double-blind trial with a 2x2 factorial design with an intervention period of 12 w (n=328). Children randomly selected from communities in the study area were randomly allocated to receive either iron supplement or placebo, and either sulfadoxine-pyrimethamine or placebo. Iron was administered twice weekly as ferrous fumarate in a 6.25 g/L suspension at a target dose of 6 mg elemental iron kg^{-1} body weight wk^{-1}. Sulfadoxine-pyrimethamine was administered every 4 w at therapeutic doses (25 mg and 1.25 mg kg^{-1} body weight, respectively).

Eligibility criteria were: haemoglobin concentrations 60-110 g/L; age 2-36 mo; axillary temperature <37.50 °C; absence of symptoms suggestive of malaria or anaemia, or of any systemic illness occurring in combination with a dipstick test result indicating current or recent malarial infection; parents reported their intention to stay in the study area during the intervention period; parental consent given; absence reported of allergy to sulfa drugs; and no sulfa drugs used in the previous 3 w.

Children were withdrawn and treated as appropriate if haemoglobin concentrations were <50 g/L, if they met one or more criteria of severe and complicated malaria (Warrell et al. 1990), or if they had manifestations of other severe disease.

Children were medically examined at baseline and at 12 w. At both visits, samples of capillary blood from each child were assayed by dipstick test to detect current or recent malarial infection, and haemoglobin concentration was measured. Children were always promptly treated for common illnesses, or referred if needed; details will be described elsewhere.

Laboratory measurements

Haemoglobin concentrations were determined by photometer (HemoCue, Ängelholm, Sweden), and dipstick tests (Model ML02; AMRAD/ICT, Sydney, Australia) were used for rapid detection in blood of antigens specific to *Plasmodium falciparum* (Anonymous 2000). In patients with manifestations of malaria, this test has sensitivity and specificity estimates >95% in detecting parasitaemia as determined by microscopy (Durrheim et al. 1998, Garcia et al. 1996, Kilian et al. 1997, Kumar et al. 1996). A negative test result is highly predictive for the absence of malarial infection, whilst a positive test result probably has less predictive value for the presence of infection (Eisen and Saul 2000). Resources were lacking for routine microscopic examination of blood smears.

Procedures for collection and handling of blood samples have been described elsewhere (Verhoef et al., accepted). Ferritin concentrations were measured using commercial

reagent kits according to instructions from the manufacturer (Diagnostic Products Corporation, Los Angeles, CA, USA). C-reactive protein concentration was determined as an indicator of inflammation in an assay (Orion Diagnostics, Espoo, Finland) adapted to allow measurement in low volumes of ranges from zero to highly elevated values. This method correlated excellently with standard methods (Beckman Coulter, Brea, CA, USA; Roche Diagnostics, Mannheim Germany). Insufficient blood was available for determination of all haematological indicators in all blood samples: as a result, the total number of children included in the subgroups was sometimes less than the total number of children investigated (n=328).

Statistical analysis

Data cleaning and standard analysis was undertaken using SPSS (v7.5.2; SPSS Inc., Chicago, Ill, USA. Cross-sectional data from the baseline were used to compare blood samples testing positive and negative when assayed by malaria dipstick test with regard to haemoglobin concentration, and serum concentrations of ferritin, erythropoietin and sTfR. Values of serum indicators were normalised before analysis by log-transformation.

The cross-sectional data were subsequently used to assess serum concentrations of ferritin, erythropoietin and sTfR in children with a positive malaria dipstick test in comparison with what might be expected for the resulting degrees of anaemia and inflammation (Barosi 1994, Beguin et al. 1993). For this purpose, children were divided into three groups. Group 1 comprised children whose blood tests indicated the absence of both malarial infection and inflammation (serum C-reactive protein concentration ≤10 mg/L). Group 2 comprised children without malarial infection as indicated by a negative dipstick test result, and with inflammation (serum C-reactive protein concentration >10 mg/L). Group 3 comprised children with current or recent malarial infection as indicated by a positive dipstick test result. Haemoglobin classes were defined using cut-off values that were selected to balance the number of children in categories formed by cross-tabulation with the groups into which children had been divided. Serum concentrations of ferritin, erythropoietin and sTfR from group 3 were subsequently compared with those from groups 1 and 2, assuming independent t-distributions of log-transformed values.

In a subsequent analysis, change in haematological indicators was calculated as the difference in values after and before intervention. For serum indicators, these changes were either positively or negatively skewed within intervention groups and could not be normalised. Thus, we could not use linear regression models to evaluate the response of these indicators to malaria or iron supplementation. The effects on haematological indicators of iron supplementation were assessed in children without malarial infection at both baseline and at 12 w, thus excluding children with a positive dipstick test result at either of these points in time. In addition, we excluded children receiving SP in this analysis, regardless of whether it was given alone or in combination with iron. Significance of differences in the distributions was determined by t-test in the case of change in haemoglobin concentrations, and by Mann-Whitney U-test in the remaining indicators.

TABLE 1. Effect of malaria on various haematological indicators; cross-sectional data from the baseline

Indicator	No malarial infection (negative dipstick test result)		Current or recent malarial infection (positive dipstick test result)		Difference (95% CI *) or factor †	
	n	Mean	n	Mean		
Haemoglobin concentration ‡, g/L	228	97.3	100	89.9	7.4	(5.0-9.9)
Serum erythropoietin concentration, IU/L	216	21.4	92	49.6	2.3 †	(1.9-2.9)
Serum transferrin receptor concentration, mg/L	219	3.1	94	4.7	1.5 †	(1.4-1.7)
Serum ferritin concentration, µg/L	213	11.2	91	28.1	2.5 †	(1.8-3.4)
Serum C-reactive protein concentration, mg/L	219	14.7	96	23.9	1.6 †	(1.4-1.8)

Group estimates are geometric or ‡ arithmetic means

* CI: confidence interval; estimated effects were all highly significant upon testing ($p<0.001$).

† Factors indicate the proportional change in values measured in children with current or recent infection relative to those in their counterparts with no infection

Finally, we examined the effects of malaria in children receiving either SP or placebo, thus excluding children receiving iron. These children were divided into three groups. Group A comprised children without infection at either baseline or at 12 w as assessed by dipstick test. Group B contained children who became infected during the trial (dipstick test result negative at baseline, and positive at 12 w), whilst group C comprised children with a positive dipstick test at baseline, and a negative result at 12 w. The results from the groups B and C were both compared with those from group A, and significance of differences in the distributions were determined by Mann-Whitney U-test. Data from children with a positive dipstick test both at baseline and at 12 w were not considered because parasite density might not have been the same at the two time points. Thus, it would be difficult to interpret the meaning of any differences in the parameters between these time points.

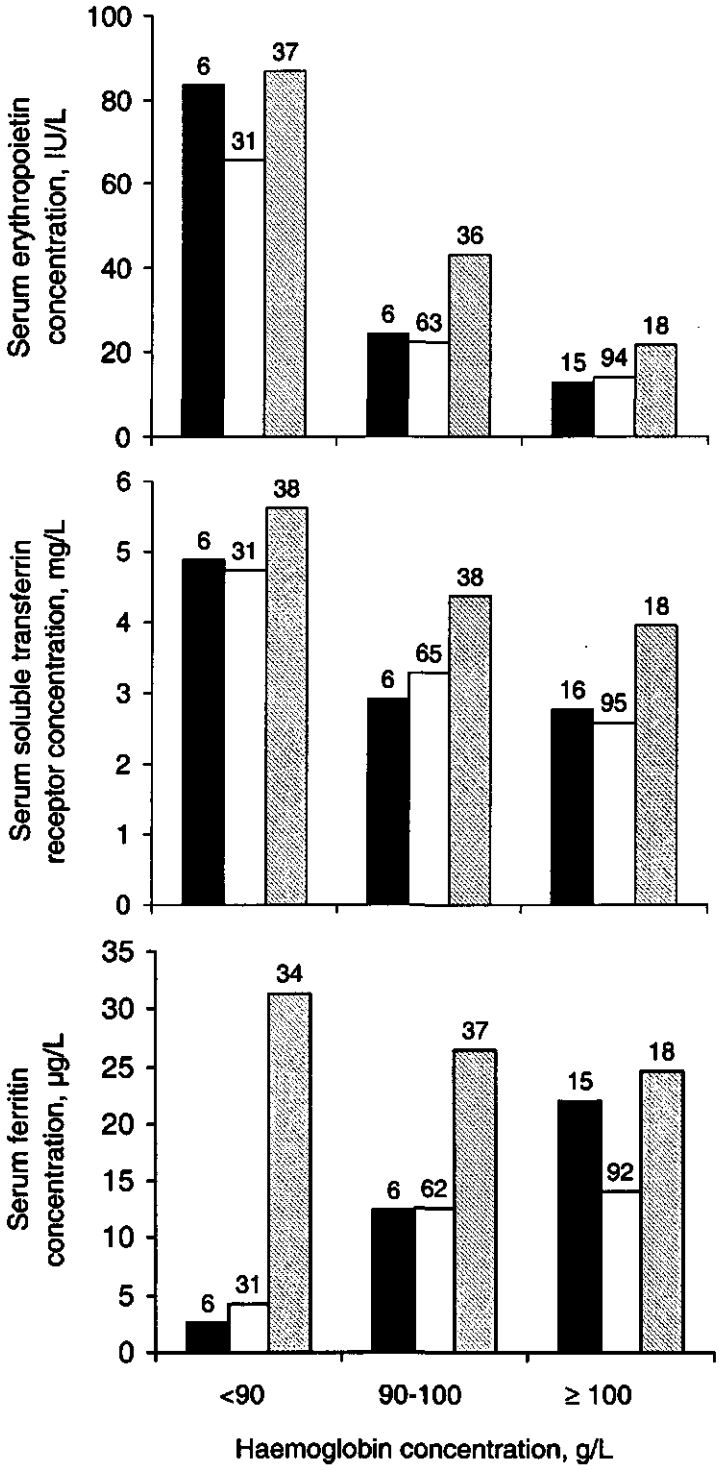
Results

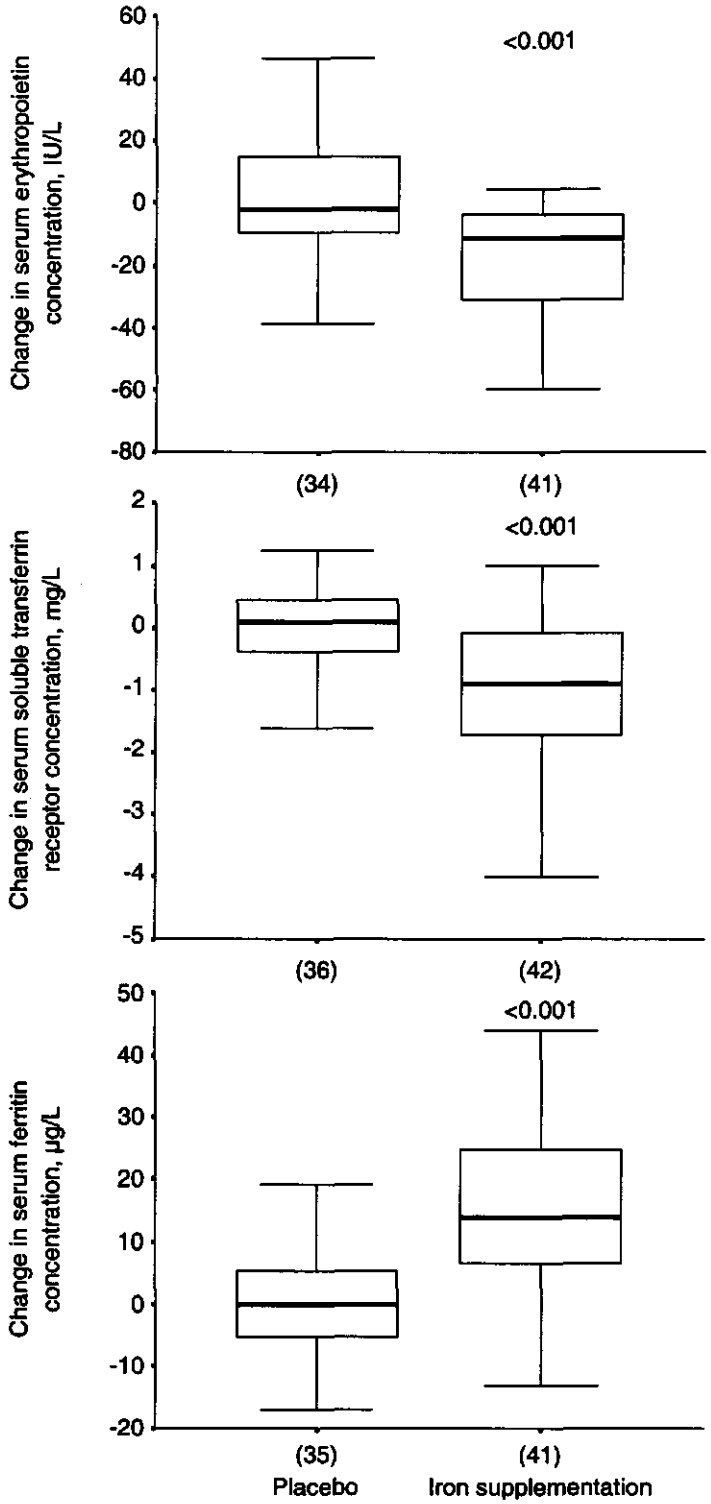
Findings from the cross-sectional data of the effect of malaria on haematological indicators are shown in **table 1**. Malaria was associated with a mild reduction of haemoglobin concentrations, mild inflammation as indicated by serum C-reactive protein concentration, and increased serum concentrations of erythropoietin, sTfR and ferritin.

Serum concentrations of erythropoietin and sTfR from children with current or recent malarial infection as indicated by dipstick test were as high as or perhaps even beyond what the values of children without malaria, regardless of whether serum C-reactive protein concentration values from children in groups 1 or 2 indicated the presence or absence of inflammation (**figure 1**). Blood samples from children in group 3 had somewhat higher serum C-reactive protein concentrations than their counterparts in group 2 (not shown).

FIGURE 1 (OPPOSITE PAGE). Serum indicators (geometric means) in relation to haemoglobin concentration, for three groups of children

Group 1 (black bars): children whose blood tests indicated the absence of both malarial infection and inflammation; group 2 (open bars): children without malarial infection, and with inflammation; group 3 (shaded bars): children with current or recent malarial infection (positive dipstick test result). Numbers above bars indicate group sample sizes. See text for further explanation.


Serum erythropoietin concentration, IU/L
100
80
60
40
20
0
6
31
37
6
63
36
15
94
18
Serum soluble transferrin receptor concentration, mg/L
6
5
4
3
2
1
0
6
31
38
6
65
38
16
95
18
Serum ferritin concentration, µg/L
35
30
25
20
15
10
5
0
6
31
34
6
62
37
15
92
18
<90
90-100
≥ 100
Haemoglobin concentration, g/L


Change in serum erythropoietin concentration, IU/L
60
40
20
0
-20
-40
-60
-80
<0.001
(34)
(41)
Change in serum soluble transferrin receptor concentration, mg/L
2
1
0
-1
-2
-3
-4
-5
<0.001
(36)
(42)
Change in serum ferritin concentration, µg/L
50
40
30
20
10
0
-10
-20
<0.001
(35)
Placebo
(41)
Iron supplementation

Haemoglobin concentrations increased more in children who received iron than in their counterparts who received iron placebo (16.0 g/L versus 4.1 g/L; difference: 11.9 g/L, 95% confidence interval: 7.9-15.9 g/L). The corresponding changes in concentrations of serum indicators are shown in **figure 2**. Iron supplementation led to decreased serum concentrations of erythropoietin and sTfR, and increased serum ferritin concentrations. There was no evidence of an effect of iron on serum C-reactive protein concentrations (not shown), or of differences between the groups compared regarding baseline characteristics (not shown).

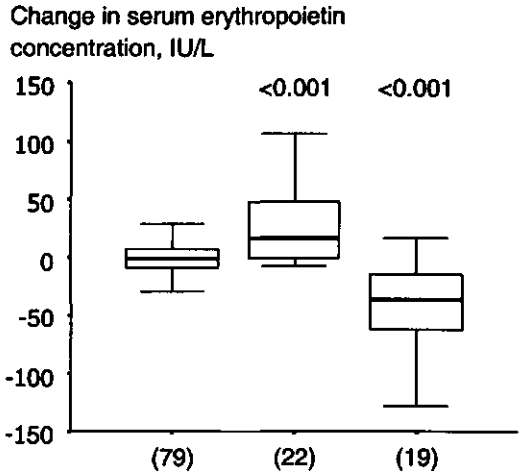
Children who became infected by malaria in the course of the study had decreased haemoglobin concentrations (**table 2**), and increased serum concentrations of erythropoietin, sTfR, C-reactive protein and ferritin at 12 w when compared to baseline (**figure 3**). Conversely, those with a positive and negative dipstick test results at baseline and at 12 w, respectively, had increased haemoglobin concentrations (**table 2**), and decreased serum concentrations of erythropoietin, sTfR, C-reactive protein and ferritin at 12 w when compared to baseline (**figure 3**). Exclusion of children who received SP did not lead to different conclusions (not shown).

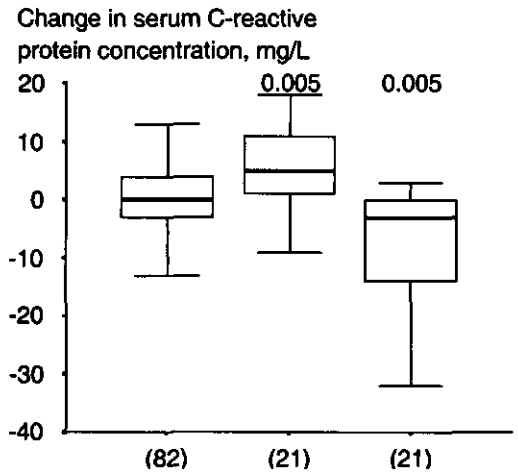
FIGURE 2 (OPPOSITE PAGE). Effect of iron supplementation on change in serum indicators

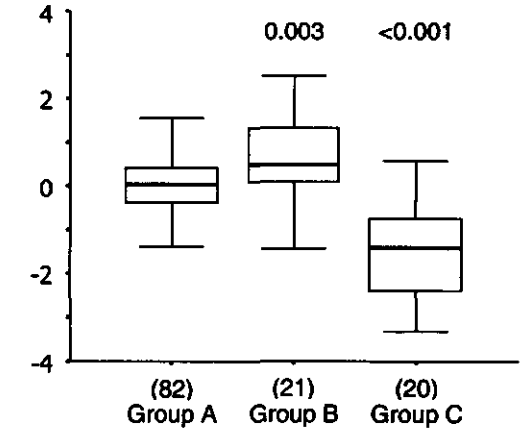
Box plots indicate 25th and 75th percentiles (box) and the median (thick line across the box). Lines extending from each box indicate highest and lowest values, excluding outliers (five largest and five smallest values). Values between brackets indicate group sample size; fractions indicate the level of significance when testing for differences in distributions of sampled populations of groups 2 and 3 relative to group 1 (Mann-Whitney U-test). See text for further explanation.

FIGURE 3 (NEXT PAGE). Effect of malaria on change in serum indicators

Group A: children without infection at either baseline or at 12 w; group B: children who became infected during the trial; group C: children with a positive dipstick test at baseline, and a negative result at 12 w. Fractions indicate the level of significance when testing for differences in distributions of sampled populations of groups B and C relative to group A (Mann-Whitney U-test). See caption figure 2 and text for further explanation.


Change in serum erythropoietin concentration, IU/L
150
100
50
0
-50
-100
-150
<0.001
<0.001
(79)
(22)
(19)


Change in serum C-reactive protein concentration, mg/L
20
10
0
-10
-20
-30
-40
0.005
0.005
(82)
(21)
(21)


Change in serum soluble transferritin receptor concentration, mg/L
4
2
0
-2
-4
0.003
<0.001
(82)
Group A
(21)
Group B
(20)
Group C

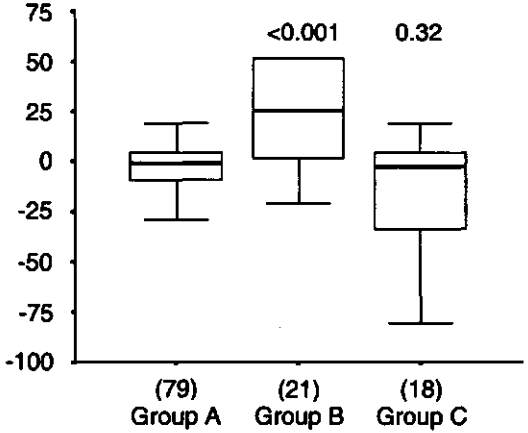

Change in serum ferritin concentration, ug/L
75
50
25
0
-25
-50
-75
-100
<0.001
0.32
(79)
Group A
(21)
Group B
(18)
Group C

TABLE 2. Effect of malaria on haemoglobin concentrations; longitudinal data

Group	Dipstick test result		n	Change in haemoglobin concentration, g/L	
	At baseline	At 12 w		Difference in values after and before intervention	Compared with Group 1 *
1	Negative	Negative	83	5.4 ± 8.8	–
2	Negative	Positive	22	-7.8 ± 19.2	-13.3 (-4.6 to 22.0)
3	Positive	Negative	21	20.4 ± 11.5	15.0 (10.4 to 19.5)

Mean ± standard deviation (95% confidence interval)

* Calculated as the change in haemoglobin concentration in either group 2 or 3 minus the corresponding change in group 1

Discussion

Our findings showed prospectively that the development of malaria resulted in decreased haemoglobin concentrations, and increased serum concentrations of erythropoietin and transferrin receptor. Conversely, the disappearance of malarial antigenaemia resulted in increased haemoglobin concentrations, and decreased concentrations of these serum indicators. In addition, our baseline data showed that current or recent malarial infection is associated with increased serum concentrations of erythropoietin and transferrin receptor, and that these were as high as or perhaps even higher than the values of children without malaria and without inflammation.

Iron supplementation led to decreased serum concentrations of erythropoietin and transferrin receptor. This is in agreement with reports that iron deficiency leads to increased serum concentrations of erythropoietin and hypoproliferative erythropoiesis (Baynes 1994).

Malaria causes a shift of iron distribution from functional towards storage compartments (Das et al. 1997) which has also been observed in a range of other infections (Sears 1992, Means and Krantz 1992, Konijn 1994, Jurado 1997, Spivak 2000). Thus in malaria, stainable macrophage iron stores are often present in bone marrow (Abdalla 1990b, Phillips et al. 1986) while serum ferritin concentrations are increased (Das et al. 1997, Oppenheimer et al. 1984, Phillips et al. 1986). Ferritin is an iron storage protein, but its serum concentration also increases in infection, probably as part of a host immune response (Kent et al. 1994). This was confirmed by our results. Serum ferritin concentrations <12 μg/L are highly predictive of depleted iron stores (Skikne et al. 1990), whilst values above this range, when coupled with anaemia, indicate inflammation but may mask coexisting iron deficiency (Verhoef et al., accepted).

Several authors have raised the possibility that malaria leads to iron sequestration in the mononuclear phagocyte system, thus resulting in iron-limited erythropoiesis (Phillips et al. 1986, Phillips and Pasvol 1992, Weatherall et al. 1983). There are several reasons to believe that this is not the case. First, the observed presence of iron in bone marrow corroborates experimental evidence (Spivak 2000) that iron therapy in patients with inflammatory diseases does not result in correction of anaemia. Second, contrary to a state of iron deficiency, iron absorption is normal or somewhat decreased in the anaemia of chronic disease (Jurado 1997), and absorbed iron is effectively incorporated in the erythron (Pollack 1992). Third, instead of being incorporated into haemoglobin, it appears that iron taken up in developing red cells is stored in ferritin and haemosiderin (Pollack 1992). Fourth, expression of transferrin receptors on individual erythroblasts is increased in iron deficiency but decreased in anaemia of chronic disease (Feelders et al. 1993, Kuiper-Kramer et al. 1997, 1998).

The anaemia of chronic disease is primarily due to reduced erythropoietin production and reduced responsiveness of erythroid progenitor cells to erythropoietin (Means and Krantz 1992, Spivak 2000) under influence of proinflammatory cytokines such as tumour necrosis factor and interleukin-1. Therapy using recombinant erythropoietin can correct the anaemia of chronic disease but not of iron deficiency (Mean and Krantz 1992). Thus, the distinctive abnormalities in body iron distribution appear to be a by-product of these mechanisms (Walter et al. 1997). It cannot be ruled out, however, that some iron sequestration occurs in malaria in haemazoin, a product from haemoglobin degradation by malaria parasites that is found in circulating or phagocytosed red cells.

Contrary to what is observed in the anaemia of chronic disease (Means and Krantz 1992, Spivak 2000), we found no evidence that erythropoietin production was impaired in malaria (**figure 1**). In addition, serum transferrin receptor concentrations in children with current or recent malaria appeared appropriate for the degree of anaemia (**figure 1**). In a study among asymptomatic children, Mockenhaupt et al. (1999) also reported increased serum transferrin receptor concentrations when adjusting for haemoglobin concentration. These findings provide evidence that the erythropoietic response of asymptomatic malaria is adequate for the degree of anaemia, and that inflammation probably plays no or only a minor role in the pathogenesis. This indicates that the malaria-associated anaemia observed in the present study is primarily due to haemolysis.

We found that a positive dipstick test result was associated with a minor increase in serum C-reactive protein concentration, indicating that malaria was associated with a minor degree of inflammation. Acute malaria is probably associated with higher parasite densities and thus more intense inflammation. Thus, it seems likely that the decrease in sTfR concentrations observed in acute malaria (Williams et al. 1999, Beesley et al. 2000) might be due, in addition to haemolysis, to deficient erythropoietin production or suppressed marrow response to erythropoietin.

In summary, our findings indicate that in asymptomatic malaria, the erythropoietic response is adequate for the degree of resulting anaemia. Inflammation and the pathogenic mechanisms involved in the anaemia of chronic disease probably play no or a

minor role. Further research is needed to demonstrate the role of deficient erythropoietin production or action in the pathogenesis of the anaemia of acute, symptomatic malaria.

Contributions

Hans Verhoef, Clive West, and Frans Kok were responsible for study design and interpretation of results. Rob Kraaijenhagen was responsible for biochemical analyses and assisted in the interpretation of their results. Hans Verhoef carried out the data analysis. Hans Verhoef, Silas Nzyuko, Rose King, Mary M Mbandi, Susanne van Laatum, Roos Hogervorst, and Carla Schep, collected the data.

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7

Assessment of a simple test to detect anaemia in developing countries

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SUMMARY

Background: A new and inexpensive colour scale may facilitate anaemia diagnosis in developing countries. Previous studies to assess its use had methodological shortcomings. We used data collected in various populations to determine sensitivity and specificity of the scale given haemoglobin concentration of any individual. These characteristics were used to predict test performance in two populations with different distributions of haemoglobin concentration.

Methods: Blood was collected from populations in Kenya, Malawi and Tanzania, and assessed by colour scale and photometer. Logistic regression was used to estimate test characteristics of the scale in detecting anaemia or severe anaemia as a continuous function of haemoglobin concentration. These findings were applied to predict characteristics and performance of the scale in two representative samples of children aged 6-24 mo in areas with stable and seasonal malaria, respectively.

Findings: Scores of the colour scale ≤10 indicated anaemia (haemoglobin concentration <110 g/L) and severe anaemia (haemoglobin concentration <70 g/L) with sensitivities >0.68 and >0.99, respectively. When used in populations with a heavy burden of anaemia, sensitivity estimates were higher, and the scale could provide valid estimates of the prevalence of anaemia.

Interpretation: The colour scale is satisfactory and for many purposes superior to all other methods for detection of anaemia at primary care level. Low scores of the scale may give unsatisfactory results when used to detect patients with severe anaemia and to decide on their management. The methodology presented in this report may facilitate development of strategies for its use in various target groups.

Submitted for publication

Few primary care facilities in developing countries can afford photometric equipment to measure blood haemoglobin concentrations accurately. A newly introduced colour scale (Lewis et al. 1998, Montresor et al. 2000) is inexpensive and does not require chemical reagents or electric power. It is used to compare the colour of a person's blood absorbed onto filter paper with a series of standard colours mounted on a rigid card. The version presently available comprises six shades of red (indicated by scores 4, 6, 8, 10, 12 and 14), corresponding to six haemoglobin concentrations between 40 and 140 g/L in incremental steps of 20 g/L. Previous studies to assess the scale have suffered from methodological shortcomings, and failed to take into account that test characteristics (sensitivity and specificity) and performance (predictive values and ability to estimate prevalence of anaemia) depend on the distribution of haemoglobin concentration in the population considered.

We used data collected in various populations to determine sensitivity or specificity of the scale given haemoglobin concentration in any individual. We then showed how these findings can be used in clinical practice, or extrapolated to predict performance of the scale in populations with different distributions of haemoglobin concentration.

Subjects and methods

An inventory was made of all studies that been carried out up to December 2000 to evaluate the utility of the colour scale. For this purpose, a literature search was undertaken in the Medline and Current Contents databases, and one of the persons who developed the colour scale (SM Lewis, Imperial College School of Medicine, Hammersmith Hospital, London, UK) was asked to identify additional, unpublished studies. Of the studies thus identified and for which we managed to obtain data, we selected those in which blood samples were collected from patients, thereby excluding those in which test blood samples were prepared in a laboratory. To standardise the methods employed in various studies, we also excluded studies in which haematocrit was used as the gold standard to measure the degree of anaemia. This yielded published and unpublished data collected from three groups of children and pregnant women.

The first group comprised children aged 2-36 mo who were studied by two of us (HV and RH) during a survey (n=178) and when subsequently reporting sick (n=31) in the course of a period of 12 w to a rural research clinic in Eastern Province, Kenya. Children were selected by stratified random sampling from 18 rural communities surrounding the research clinic. Not all children thus selected were included in the survey, but the distribution of haemoglobin concentration in this sample is thought to be representative for the population studied.

The second group comprised patients in Tanzania and Malawi. These included pregnant women attending antenatal clinics (n=1086), various other patient groups in outpatient and inpatient departments of hospitals (n=1646) and samples of rural preschool children (n=510). Some but not all of the findings from these groups were reported elsewhere (Van den Broek et al. 1999, Montresor et al. 2000). In addition to these samples, haemoglobin

concentrations in other blood samples were diluted with homologous plasma to obtain a range of 33-181 g/L as measured by photometer. These aliquots were assessed by various observers during a training course in Malawi (n=636).

The third and last group comprised children aged 6-24 mo selected through simple random sampling or cluster sampling from rural populations in Nyanza Province (n=927) and Eastern Province (n=185), Kenya, respectively. Whereas malaria is highly endemic in Nyanza Province, it is less endemic and seasonal in Eastern Province.

In the first group, blood collected by finger puncture was used to determine haemoglobin concentration by colour scale and photometer (HemoCue Inc., Ängelholm, Sweden). Instructions given by manufacturers were followed, persons reporting colour scale scores were blinded to photometer readings, and the photometer's accuracy was checked every 4 h by control cuvette. In the second group, haemoglobin concentrations were likewise measured, while in the third group, haemoglobin concentrations were only assessed by photometer. The photometers used in these two groups were either HemoCue or Coulter equipment (Beckman Coulter, Fullerton CA, USA).

Data were analysed using SPSS (v7.5.2 for Windows; SPSS Inc., Chicago Ill., USA). Measurements of haemoglobin concentrations by photometer were considered as the gold standard. In the first group, these concentrations were calculated as the mean of at least two measurements. Anaemia and severe anaemia were defined as haemoglobin concentrations <110 g/L and <70 g/L, respectively, as determined by photometer. On the colour scale, scores ≤10 and ≤6 were used to indicate anaemia and severe anaemia, respectively. Confidence intervals of proportions were computed assuming a normal approximation of the binomial distribution.

Logistic regression analysis was used to estimate the probability of detecting anaemia by colour scale, P, as a continuous function of haemoglobin concentration (see annex). This probability was estimated for cut-off values of the score of the colour scale ≤10 and ≤6, which might be used to indicate anaemia and severe anaemia, respectively. In ranges of haemoglobin concentration <110 g/L and ≥110 g/L, values of P and $(1 - P)$ were interpreted as sensitivity and specificity estimates of detecting anaemia by colour scale, respectively. In ranges of haemoglobin concentration <70 g/L and ≥70 g/L, values of P and $(1 - P)$ were interpreted as sensitivity and specificity estimates of detecting severe anaemia by colour scale, respectively.

In the graph thus obtained, it was considered that the slope of the inverted S-curve and its relative position along the X-axis might vary between individual observers. Thus, the role of interobserver variation was assessed by visual inspection and comparing of graphs obtained by univariate analysis for individual observers in the first two groups. Data from two observers were subsequently excluded because their test characteristics in detecting both anaemia and severe anaemia were strikingly inferior to those from other observers. The observations of the remaining observers were pooled for further analysis.

The findings from this pooled analysis were applied to calculate expected characteristics and performance of the scale in detecting anaemia and severe anaemia in children from the third group. Thus, the probability of detecting anaemia by colour scale was calculated for each child given his or her haemoglobin concentration. The averaged values of these probabilities in ranges of haemoglobin concentrations <110 g/L or ≥110 g/L were interpreted as the predicted sensitivity and specificity, respectively, of detecting anaemia by colour scale in the populations studied. These values were used to calculate predictive values or reporting anaemia by colour scale, and to calculate the prevalence of anaemia as estimated by colour scale. Similar procedures were followed to predict test characteristics and performance in detecting severe anaemia.

Results

Test characteristics and performance of the colour scale in blood samples from the first study group are summarised in **table 1** (first row). Scores of the colour scale ≤10 correctly identified children with or without anaemia with probabilities of 0.76 and 0.44, respectively. Thus assessed, proportions of children correctly reported with or without anaemia were 0.67 and 0.56, respectively.

The logistic function for one single observer in group 1 is shown in **figure 1**. As predicted from this regression curve, scores of the colour scale ≤10 would have correctly identified children with haemoglobin concentrations <120 g/L, <110 g/L or <90 g/L with probabilities >0.53, >0.72 and >0.93, respectively.

Similar test characteristics were found when observations from the first two groups were pooled (**figure 2, top**, right curve). As calculated from this function, scores of the colour scale ≤10 would have correctly identified children with haemoglobin concentrations <110 g/L, <100 g/L or <70 g/L with probabilities >0.68, >0.84 and >0.99, respectively. Scores of the colour scale ≤6 would have correctly identified children with haemoglobin concentrations <70 g/L, <60 g/L or <50 g/L with probabilities >0.57, >0.83 and >0.95, respectively (**figure 2, top**, left curve).

These logistic functions were applied to predict performance of the colour scale in children surveyed in the third group. The cumulative frequency distributions of haemoglobin concentration in these populations are shown in **figure 2 (bottom)**. Those in Nyanza Province generally suffered from more severe anaemia than their similarly aged counterparts in Eastern Province (see also **table 2, top**). If used in these surveys, scores of the colour scale ≤10 would have been more sensitive in detecting children with anaemia in Nyanza Province than those in Eastern Province (0.94 versus 0.85; **table 2**). Specificity estimates were similar for these two areas, and the scale would have given no or negligible bias in estimating the prevalence of anaemia as determined by photometer. These data also show that scores of the colour scale ≤10 would have detected virtually all children (>99%) with severe anaemia in both surveys.

TABLE 1. Test characteristics and performance of the colour scale in detecting anaemia

Reference	n	Estimates by photometer		Test characteristics and performance of scores of the colour scale ≤10				
		Haemoglobin concentration, g/L*	Prevalence of anaemia	Sensitivity	Specificity	PPV †	NPV ‡	Prevalence of anaemia
Population-based studies								
1	178	106.6 ± 10.7	0.60	0.76 (0.68-0.84)	0.44 (0.33-0.56)	0.67 (0.59-0.75)	0.56 (0.43-0.69)	0.68
2	535	98.6 ± 15.1	0.79	0.85 (0.82-0.89)	0.77 (0.70-0.85)	0.93 (0.91-0.96)	0.59 (0.51-0.67)	0.72
3	1066	106.6 ± 14.6	0.58	0.75 (0.72-0.79)	0.47 (0.43-0.52)	0.66 (0.63-0.70)	0.58 (0.53-0.63)	0.66
Laboratory-based studies ¶								
4 §	1213	–	0.67	0.86 (0.83-0.88)	0.91 (0.88-0.94)	0.95 (0.93-0.97)	0.76 (0.72-0.80)	0.61
5 §	–	–	–	0.89 ‖	0.94 ‖	0.97 ‖	0.86 ‖	–

(-) indicates the absence of values

References: [1] Verhoef et al. (present report); [2] Montresor et al. (2000); [3] Van den Broek et al. (1999); [4] Lewis et al. (1998); [5] Muenster et al. (1997)

* Mean ± SD; figures between brackets indicate 95% confidence intervals

† PPV and ‡ NPV: positive and negative predictive value, respectively

Anaemia was defined as haemoglobin concentrations <110 g/L or else <120 g/L §

¶ Observations were made in a series of pre-standardised blood samples made in a reference laboratory

‖ Confidence intervals could not be calculated using the data reported.

If, in addition, scores of the colour scale ≤6 would have been used to refer those with severe anaemia, then 82% and 87% of those with this condition would have been referred in Nyanza and Eastern Provinces, respectively. Of those referred, however, 38% and 65% would not be severely anaemic, respectively. Substantial bias would have occurred in both populations if the colour scale would have been used to estimate the prevalence of severe anaemia, but more so in Eastern Province (estimated versus true prevalences of 33% versus 25% in Nyanza Province, and 8% versus 3% in Eastern Province).

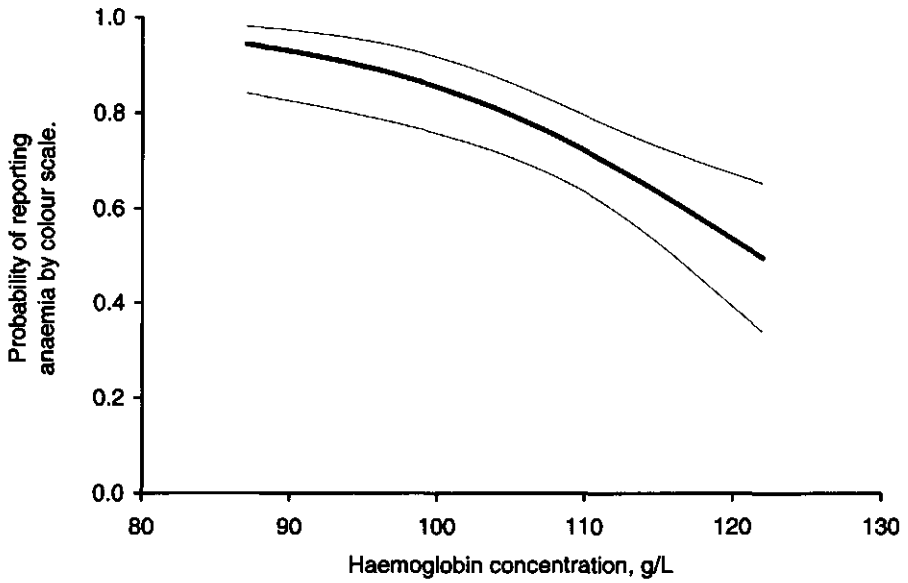

FIGURE 1. Estimated probability of reporting anaemia by colour scale (scores ≤10), *P*, as a logistic function of haemoglobin concentration for one observer (n=140).

The equation for this line is given by $e^{(\alpha + \beta Hb)} / [1 + e^{(\alpha + \beta Hb)}]$, where α and β indicate regression coefficients with estimated values of 9.86 and -0.08, respectively. Thin lines indicate confidence bands. The curves are only indicated within the 95% range of observed haemoglobin concentrations. Within ranges of haemoglobin concentrations <110 g/L and ≥110 g/L, *P* and (1 – *P*) indicate sensitivity and specificity, respectively (see text)

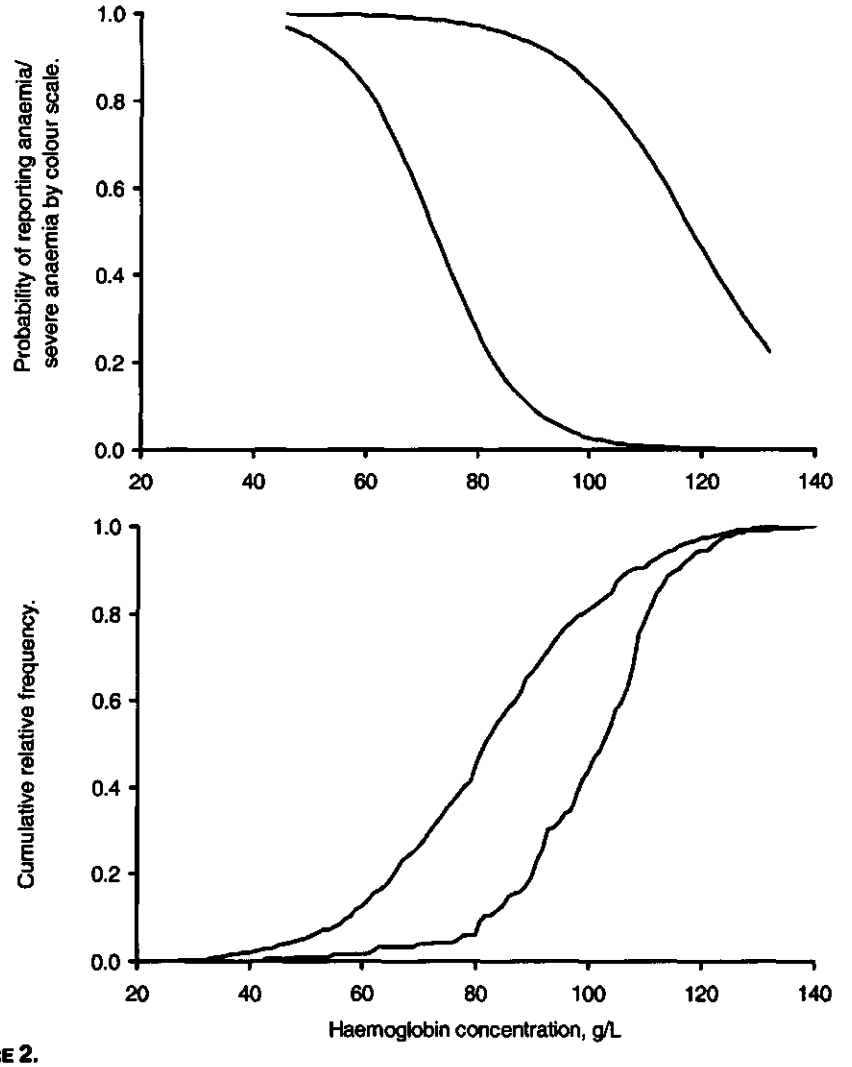

FIGURE 2.

TOP: Estimated probabilities of reporting anaemia (right curve) by scores of colour scale ≤10, or of reporting severe anaemia (left curve) by scores of colour scale ≤6, as logistic functions of haemoglobin concentration (pooled observations; n=4087).

Estimated values of the regression coefficients α and β were 10.695 and -0.091, respectively, for the right curve, and 9.285 and –0.129, respectively, for the left curve (see caption figure 1 for details).

BOTTOM: Cumulative frequency distribution of haemoglobin concentration in representative samples of rural children 6-24 mo of age in Nyanza Province (left curve; n=927) and Eastern Province, Kenya (right curve; n=185), areas with stable malaria and perennial transmission, and with unstable malaria and seasonal transmission, respectively

TABLE 2. Comparison of estimated diagnostic characteristics and performance of the colour scale in two populations of Kenyan preschool children (6-24 mo of age) with different distributions of haemoglobin concentration

	Estimated parameter	Location of the survey *	
		Nyanza Province	Eastern Province †
Measurements by photometer	Haemoglobin concentration, g/L ‡	82.3 ± 20.1 (927)	100.8 ± 14.5 (185)
	Prevalence of anaemia §	0.90	0.75
	Prevalence of severe anaemia ¶	0.25	0.03
Report scores of colour scale ≤10	Sensitivity of detecting anaemia	0.94	0.85
	Specificity of detecting anaemia	0.49	0.46
	Positive predictive value	0.95	0.83
	Negative predictive value	0.47	0.50
	Estimated prevalence	0.90	0.77
	Sensitivity of detecting severe anaemia	0.99	1.00
Report scores of colour scale ≤6	Sensitivity of detecting severe anaemia	0.82	0.87
	Specificity of detecting severe anaemia	0.83	0.95
	Positive predictive value	0.62	0.35
	Negative predictive value	0.93	1.00
	Estimated prevalence	0.33	0.08

* Nyanza Province: stable malaria with perennial transmission; Eastern Province: unstable malaria with seasonal transmission

† These data were included in a previous report describing a broader age range (Verhoef et al., accepted)

‡ *Mean ± SD (n)*

§ ¶ Anaemia and severe anaemia were defined as haemoglobin concentrations <110 g/L and <70 g/L, respectively.

TABLE 3. Diagnostic characteristics and performance of conjunctival pallor in detecting anaemia and severe anaemia

Population studied *	Prevalence of anaemia†	Test characteristics and performance in diagnosing anaemia †			Prevalence of severe anaemia‡	Test characteristics and performance in diagnosing severe anaemia ‡		
		Sensitivity	Specificity	PPV		Sensitivity	Specificity	PPV
Urban survey §, Nigeria [1]	0.30	0.25	0.89	0.49	–	–	–	–
Urban survey, Pakistan [2]	0.78	0.74	0.83	0.90	–	–	–	–
Pregnant women attending antenatal clinics, Malawi [3]	0.59	0.33	0.84	0.75	0.04	0.62	0.76	0.11
Outpatient department of hospital, Bangladesh [4]	0.81	0.45	0.71	0.87	0.19	0.79	0.67	0.35
Outpatient department of hospital, Malawi [5]	0.82	0.24	0.95	0.96	0.35	0.37	0.89	0.64
Outpatient department of hospital, Kenya; assessed by health workers [6]	–	–	–	–	–	0.68	0.33	0.85
Outpatient department of hospital, Kenya; Assessed by physician [6]	–	–	–	–	0.41	0.81	0.65	0.61
Inpatient department of hospital, Kenya [6]	–	–	–	–	0.56	0.74	0.76	0.8
School children, ¶ Tanzania [7]	0.32	–	–	–	0.01	0.48	0.98	0.12
Postpartum women attending antenatal clinics, ¶ Nepal [7]	0.54	–	–	–	0.03	0.81	0.93	0.26
Pregnant women attending antenatal clinics, ¶ Nepal [7]	0.71	–	–	–	0.04	0.63	0.97	0.45
Preschool children, ¶ Tanzania [7]	0.78	–	–	–	0.03	0.17	0.96	0.12
Preschool children, ¶ Tanzania [7]	0.95	–	–	–	0.15	0.2	0.93	0.33

(-) indicates that values were not studied or reported, or could not be calculated from the published reports because data were either missing or internally inconsistent;

* References: [1] Ekunwe 1997; [2] Thaver et al. 1994; [3] Van den Broek et al. 1999; [4] Kalter et al. 1997; [5] Luby et al. 1995; [6] Zucker et al. 1997; [7] Stoltzfus et al. 1999;

† Anaemia was defined as haemoglobin concentrations <110 g/L or else § <100 g/L, while ‡ severe anaemia was defined as haemoglobin concentrations <80 g/L or else ¶ <70 g/L

Discussion

Our findings indicate that scores of the colour scale ≤10 have good sensitivity in detecting anaemia among young children in areas where this condition is highly prevalent. When used in this way and for this purpose, the scale can also provide valid estimates of the prevalence of anaemia, and it has excellent sensitivity in detecting persons with severe anaemia. The colour scale might thus be useful for excluding severely anaemic individuals during the initial screening of possible donors for blood transfusion.

The distribution of haemoglobin concentrations in the first group is probably representative for similarly aged children in many parts of Africa with seasonal malaria transmission. Thus, our findings from this group (**table 1**) may broadly apply to these populations, but not to older children, who are more likely to suffer from less severe anaemia. They may also not apply to similarly aged children in areas where malaria is highly endemic, and who are more likely to suffer from more severe degrees of anaemia.

When assuming that test characteristics of the colour scale exclusively depend on haemoglobin concentration, the results presented in **figure 2 (top)** may be used to predict sensitivity or specificity of the scale given the haemoglobin concentration of any individual or group of individuals with the same haemoglobin concentration. For example, our model **(figure 2, top)** predicts that colour scale scores ≤10 have a sensitivity of 0.99 in anaemia in an imaginary, selected population in which all individuals have haemoglobin concentrations of 70 g/L. Similarly, such scores have a sensitivity of 0.84 of detecting anaemia in a population exclusively comprising individuals with haemoglobin concentrations of 100 g/L. When applied to populations, diagnostic characteristics and performance of the scale vary according to the distribution of haemoglobin concentration in the population considered. This principle is illustrated by our findings in **table 2.**

Low scores of the scale might give unsatisfactory results to screen and refer patients with severe anaemia. Such use has recently been recommended by an Expert Panel convened by the International Nutritional Anemia Consultative Group (INACG) in primary care settings where determination of haemoglobin concentrations or haematocrit is not feasible (Stoltzfus and Dreyfuss 1998). In the primary care setting that we studied, this would have resulted in a large proportion of patients with severe anaemia being detected (>0.80), but among those referred, a large proportion would not suffer from severe anaemia (**figure 2** and **table 2**). On theoretical grounds, and as illustrated by the positive predictive values in **table 1** (population-based studies) and **table 2**, this proportion is inversely correlated with the burden of anaemia.

The INACG Panel also recommended universal iron supplementation for children aged 6-12 mo, and for children aged 12-24 mo in areas where the prevalence of anaemia in children aged 6-24 mo is ≥40% (Stolztfus and Dreyfuss 1998). The question might arise how accurate the colour scale could estimate this parameter. As indicated by the findings in **table 2**, this accuracy is probably sufficient for large parts of rural Africa, but it may provide gross overestimates when used in populations with a low prevalence of anaemia (not shown).

Initial investigations to assess the colour scale were mostly exploratory; whilst they demonstrated its potential diagnostic utility, the reports have several shortcomings. These include poor descriptions of populations studied or of sampling procedures (Stott et al. 1995, Van den Broek et al. 1999, Montresor et al. 2000), selection of samples that are not representative for any defined population (Stott and Lewis 1995, Lewis et al. 1998), lack of precision estimates of test characteristics (Stott and Lewis 1995, Van den Broek et al. 1999), inappropriate use of statistical techniques (Stott and Lewis 1995, Lewis et al. 1998, Van den Broek et al. 1999, Ingram and Lewis 2000, Montresor et al. 2000), and insufficient effort to evaluate test characteristics and performance in the light of previously published evidence (Stott and Lewis 1995, Montresor et al. 2000).

Linear regression and correlation analysis have been used in most reports (Stott and Lewis 1995, Lewis et al. 1998, Van den Broek et al. 1999, Ingram and Lewis 2000, Montresor et al. 2000). These techniques are inappropriate because they presume a continuous and normally distributed dependent variable (Kleinbaum et al. 1998), whereas the outcome in this case – the score of the scale – concerns a categorical variable with six classes. The problem is compounded because correlation coefficients have been interpreted as measures of agreement between paired observations made when using two methods of determining haemoglobin concentration, whereas they measure the strength of their association (Feinstein 1985, Bland and Altman 1986). To overcome the limitations of comparing a continuous variable (haemoglobin concentration determined by photometer) with a categorical outcome variable, several workers inappropriately pooled observations by individual observers for analysis in a Bland-Altman plot (Coetzee et al. 2000, Ingram and Lewis 2000).

The methodology presented in this report overcomes many of these shortcomings. Our logistic regression analysis provides a powerful tool to extrapolate previous findings, to reconcile their discrepancies, and to judge the usefulness of the scale in other settings or for different purposes. For example, the logistic function may be used to predict the sensitivity of detecting severe anaemia, which is relatively rare and therefore difficult to study in populations with a low to moderate burden of anaemia. Alternatively, if the distribution of haemoglobin concentration among women attending antenatal clinics were known from previous surveys, then a decision whether or not to use the scale for future screening for anaemia in these women might be supported by application of our logistic function to predict its performance. In some cases, it might be useful to construct logistic regression curves for individual health workers to assess and standardise their performance using the scale. Lastly, our techniques allow for meta-analysis of data collected in various studies.

To detect anaemia, health workers commonly rely on pallor assessed in one or various anatomical sites. Comparison of our findings with other reports consistently indicates that screening for anaemia by colour scale has higher sensitivity in detecting anaemia and severe anaemia (**table 2**) than assessment for pallor of conjunctivae (**table 3**; see also discussion by Van der Broek et al. 1999), of hand palms or pallor at any of several sites (Luby et al. 1995, Zucker et al. 1997, Stoltzfus et al. 1999). Hand palm pallor is considered

a slightly better sign for detection of severe anaemia and is included in current guidelines for clinical management of sick children in developing countries (Anonymous 1997, 2000). Available reports also indicate considerable variability between observers – including experienced physicians (Gjørup et al. 1986, Nardone et al. 1990) – and between studies (Anonymous 1997) in the sensitivity of signs to diagnose anaemia. By providing an external reference, this problem may be reduced when using the colour scale, but this remains to be confirmed. Several studies have indicated that the relative performance of signs may be more consistent when pallor is assessed at multiple anatomical sites (Luby et al. 1995, Zucker et al. 1997, Stoltzfus et al. 1999, Kalter et al. 1997) but the performance of such an approach still appears to be less (sensitivity of detecting haemoglobin concentration <110 g/L: 0.11-0.60) than that of the colour scale. Recent changes in the instructions accompanying the scale may assist in avoiding errors in its use and thereby improve its accuracy (Muenster et al. 1997). Contrary to other available laboratory methods for estimating haemoglobin concentration (Stone et al. 1984), the colour scale satisfies the criteria of accuracy, ease of use, low-cost and freedom of reliance on regular supplies.

In summary, the colour scale is satisfactory and for many purposes superior to all other available methods for detection and treatment of anaemia at the primary care level. Its diagnostic characteristics and performance vary according to the aims of its intended use and the distribution of haemoglobin concentration in the population considered. Low scores of the scale may give unsatisfactory results when used to detect patients with severe anaemia and to decide on their management. The methodology presented in this report may facilitate development of strategies for its use in various target groups. Further research is needed to show its performance in diagnosing severe anaemia when used in combination with signs or symptoms, and when used to estimate the prevalence of anaemia in populations with a relatively low burden of anaemia.

Contributions

Hans Verhoef, Clive West and Frans Kok were responsible for study design of the studies in Eastern Kenya. Hans Verhoef carried out the data analysis with assistance from Roos Hogervorst. All four were responsible for interpretation of data and writing of the report. Nynke van den Broek provided data from Malawi.

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Annex

Logistic regression was used to estimate the ln-odds of reporting anaemia by colour scale as a function of haemoglobin concentration. The ln-odds parameter was assumed to be normally distributed, and corresponding confidence intervals were calculated from its standard error, SE. The latter was estimated (Snedecor and Cochran 1989) as:

$$\sqrt{n^{-1}\,\hat{\sigma}_{(\ln\text{-odds})}^{2}} = \sqrt{n^{-1}\,[\hat{\sigma}_{(\alpha+\beta\ Hb)}^{2}]} = \sqrt{n^{-1}\,(\hat{\sigma}_{\alpha}^{2} + Hb^{2}\,\hat{\sigma}_{\beta}^{2} + 2\ Hb\ \hat{cov}_{\alpha,\beta})}$$

where n indicates sample size, the variable *Hb* indicates haemoglobin concentration, α and β indicate regression coefficients, and $\hat{cov}$ indicates their estimated covariance. The latter was calculated as the product of the standard deviation estimates of the regression coefficients and the estimate of the correlation coefficient, which is provided in the covariance matrix reported by SPSS. Values for ln-odds thus obtained were exponentiated and converted to P, the probability of detecting anaemia by colour scale and its corresponding confidence interval.

8

Diagnosis of fever in Africa

Hans Verhoef, Elsa Hodgins, Clive E West, Jane Y Carter, Frans J Kok

Lancet 1998; 351: 372-73.

Einterz and Bates (1997) report a striking unreliability in the assessment of fever by patients or their carers attending an outpatient service in Cameroon, and ask whether patients know when they are hot. Their data suggest they do. Recalculation of their data shows that a high proportion of respondents correctly identified fever, particularly in children (sensitivity; **table 1**). The proportion of patients correctly identified without fever (specificity) was low but constant over the age groups. The proportion of patients who correctly reported fever (positive predictive value) varied with the prevalence of fever as measured by axillary temperature. Hence, the reported low positive predictive values are largely explained by the low prevalences of fever, and to some degree by a low specificity.

Can fever be diagnosed with an acceptable level of accuracy without using a thermometer? We recently carried out a community-based cluster sample survey among children aged 2.5 - 37 months in Mtito Andei Division, which is an area of seasonal malaria transmission in Kenya. A trained interviewer demonstrated to mothers (or carers) how to assess fever by feeling the child's forehead and comparing this temperature with that of their own forehead. The mother was then asked in her own language if the child suffered from a raised body temperature, the expression of which differs from that for malaria or malaria-like illnesses. The prevalence of fever, defined by concurrent measurement of an axillary temperature ≥37.5°C by electronic thermometer (Philips HP5316), was 0.06 (19/317 examined children). The sensitivity of this test was similar (0.89; 17 of 19 febrile children) to the value reported by Einterz and Bates[1], but the specificity was much higher (0.80; 238 of 298 non-febrile children). The proportion of mothers who correctly reported the absence of fever (negative predictive value) of this test was 0.99. The positive predictive value was 0.22 (17 of 77 children) but would be expected to be higher in a clinical setting, where self-selection for fever before presentation would result in a higher prevalence of fever than at the community level. For example, using the prevalence data observed by Einterz and Bates (1997), the predictive values shown in the above table are obtained.

Our findings suggest that mothers can accurately diagnose the absence of fever without a thermometer. The use of reported fever alone will necessarily result in an unacceptably high proportion of false positives, especially at peripheral levels of health care. Patients or carers should refer to health workers to confirm diagnosis of fever by thermometer before initiating antimalarial treatment, although this should not result in a delay of treatment. For this purpose, we believe that thermometers should be made available at the lowest level of health care – for example community health workers. We strongly support the suggestion by Einterz and Bates (1997) that in the absence of fever on examination, history of fever should be supported by other clinical signs before antimalarial treatment is prescribed.

Reference

Einterz EM, Bates ME. Fever in Africa: do patients know when they are hot? Lancet 1997; 350: 781.

TABLE 1. Predictive values of reports of fever

Age, y *	Prevalence * †	Fever reported upon asking *				Fever reported upon feeling ‡	
		Sensitivity	Specificity	PPV	NPV	PPV	NPV
<1	0.26	0.90	0.41	0.35	0.92	0.61	0.95
1-5	0.42	0.94	0.48	0.56	0.91	0.76	0.91
5-15	0.33	0.76	0.55	0.45	0.82	0.68	0.94
≥15	0.1	0.74	0.45	0.13	0.94	0.33	0.99
All	0.2	0.84	0.46	0.28	0.92	0.53	0.97

PPV = positive predictive value: proportion of patients or carers who correctly reported fever; NPV = negative predictive value: proportion of patients or carers who correctly reported absence of fever

* Data from Einterz and Bates (1997) or ‡ calculated using prevalence data from Einterz and Bates1, with sensitivity and specificity values of 0.89 and 0.80 respectively (see text)

† Prevalence of axillary temperature ≥37.50 ºC

9

Maternal screening for fever in African children without thermometer

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SUMMARY

Diagnostic performance of African mothers in detecting fever in children without thermometer was assessed in a randomised trial. Mothers were questioned whether or not their child had fever, either directly or after they had palpated the child's forehead. Palpation had similar sensitivity (>90%) but better specificity in sick children (50.0% versus 33.8%; p=0.05). Fever is more prevalent in children reported sick to a health facility than at community level, where early detection takes place. Mothers' reports of fever are therefore more reliable in sick children. Regardless of actual body temperature, mothers are more likely to report fever in sick children.

Submitted for publication

Early diagnosis and prompt treatment of febrile tropical diseases such as malaria, acute respiratory diseases and measles may reduce morbidity and risk of mortality. The diagnostic process in children often starts when mothers, who commonly lack thermometers, suspect fever. Whereas undetected fever results in diagnostic delay, chemotherapy following incorrect diagnosis of current or recent fever may unnecessarily expose children to adverse drug effects, and lead to selection and spread of drug-resistant parasite strains.

Because prevalence of fever at household level is low, diagnosis without thermometer unavoidably results in a high proportion of children falsely reported with fever (Verhoef et al. 1998). Thus, mothers' reports of fever ideally require confirmation by thermometer. In practice, however, antimalarial drugs or analgesics are commonly administered at household level (Verhoef et al. 1999), without children being presented to health workers. Moreover, health workers are often faced with the dilemma of whether to prescribe antimalarials to afebrile children with a history of fever (Einterz and Bates 1998). Thus, practical means are needed to improve both sensitivity and specificity of detecting fever by mothers. We demonstrated how mothers should assess fever by palpating their child's forehead with the back of the fingers and comparing this temperature with that of their own forehead (Verhoef et al. 1998). Comparison of our results with those from another study (Einterz and Bates 1998) suggests that such maternal assessment of fever has similar sensitivity, and perhaps higher specificity, than directly questioning mothers whether their child has fever. The present study was undertaken to assess this hypothesis directly in a randomised trial. In addition, we evaluated whether the proportion of mothers who correctly report fever (positive predictive value) is higher in clinical settings, where self-selection for fever before presentation presumably results in a higher prevalence of fever than at community level (Verhoef et al. 1998).

The study was conducted during the rainy seasons of 1998-2000. It was based at a rural community-based research clinic in Mtito Andei Division, and at Kibwezi Hospital, Eastern Province, Kenya, which represents a higher level of referral than the research clinic. Children were eligible for study when aged 2-36 mo and if accompanied by their mother. Sick children were only included if having current or recent symptoms or signs of systemic disease. The study comprised three groups of children. Children studied at the research clinic were either randomly selected from each of 22 surrounding communities (first group), or presented sick in the course of the study (second group). The third group comprised sick children attending the outpatient department of Kibwezi Hospital.

Within the first and third groups, method of fever assessment – questioning after palpation or direct questioning – was randomly allocated to mothers after eligibility of the child for study had been ascertained. Axillary temperature was measured by electronic thermometer (model HP5316, Philips, Groningen, Netherlands) immediately following the mother's judgement. In the second group, fever was assessed during repeated episodes of illness by thermometer only. Fever was defined as axillary temperature $\geq$37.50°C. Duplicate measurements in 311 children showed high correlation between thermometer recordings (r=0.90).

TABLE. Comparison of methods of fever assessment without thermometer, in various study populations

Population studied	Method of questioning	n	Axillary temperature, * ºC	Prevalence of fever †	Measurement by mothers				
					Sensitivity	Specificity	PPV ‡	NPV §	Prevalence
Community survey	Direct	246	36.78	7.7 (19/246)	52.6 (10/19)	91.2 (207/227)	33.3 (10/30)	95.8 (207/216)	12.2 (30/246)
	After palpation	240	36.81	5.8 (14/240)	78.6 (11/14)	91.6 (207/226)	36.7 (11/30)	98.6 (207/210)	12.5 (30/240)
Community clinic, sick		187	37.36	45.5 (85/187)	–	–	–	–	–
Hospital, sick	Direct	148	37.57	52.0 (77)	92.2 (71/77)	33.8 (24/71)	60.2 (71/118)	80.0 (24/30)	79.7 (118/148)
	After palpation	114	37.59	52.6 (60)	93.3 (56/60)	50.0 (27/54)	67.5 (56/83)	87.1 (27/31)	72.8 (83/114)

Prevalence or * median
† Axillary temperature ≥37.50 °C
‡ PPV: positive predictive value; § NPV: negative predictive value

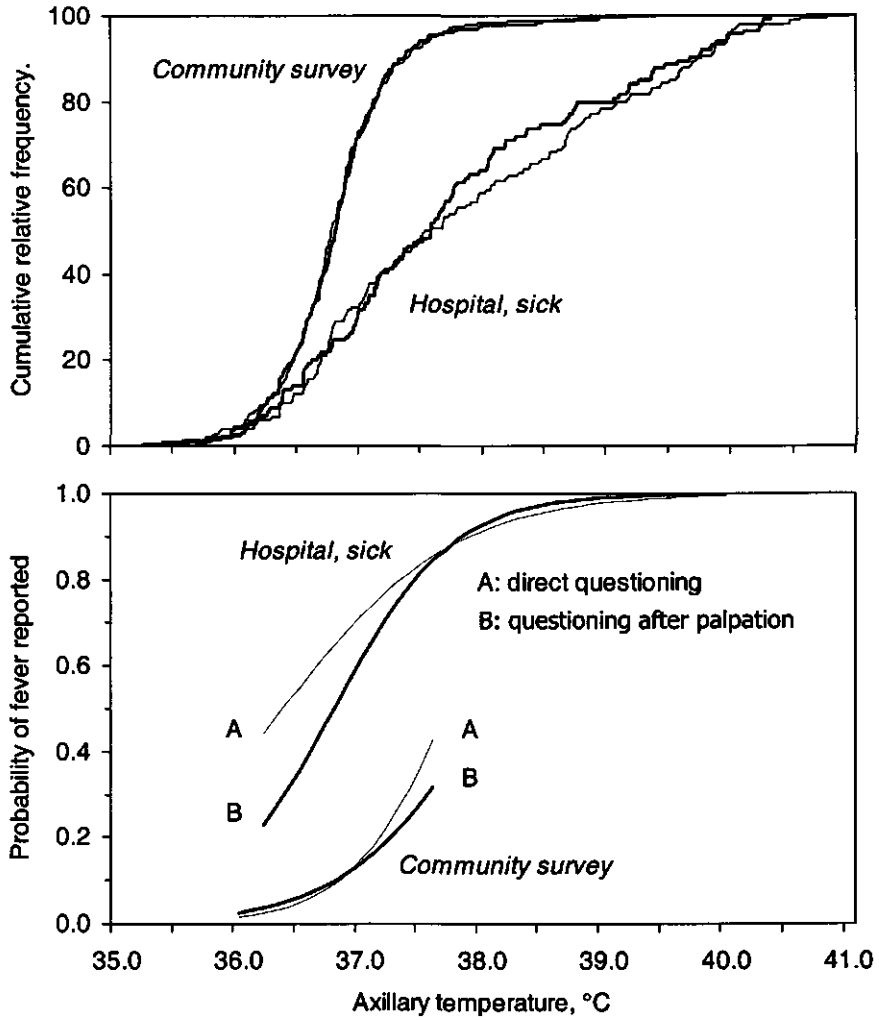

FIGURE.

TOP: Cumulative frequency distribution of temperature in children randomly selected in a community survey (two virtually overlapping lines at left) and sick children attending Kibwezi hospital (two overlapping lines at right). Fever was assessed by mothers who were questioned after palpating their child's forehead (thick lines) or by direct asking (thin lines).

BOTTOM: Probability of fever, *P*, in relation to body temperature in the study groups above (top two lines: children studied at Kibwezi Hospital; bottom two lines: children studied in the community survey). When axillary temperatures were ≥37.5 °C, *P* was interpreted as an estimator of sensitivity, whereas below this range, (1 – *P*) was interpreted as an estimator of specificity (see text for further explanation). Lines are only indicated within the 95% temperature range for each group. When measured across all temperatures <37.5°C, specificity in sick children was higher when fever was assessed by questioning after palpation than by direct questioning (p=0.05; see text).

Specificity of fever detection in the community survey was similar when reported after palpation or directly (**table**). There were few fever cases in this population. Sensitivity appeared higher after palpation but the observed difference may have been due to chance (difference 26.0%; p=0.16; exact test). In sick children, sensitivity was >90% and almost identical when assessed after palpation or by direct questioning; specificity was higher when fever was assessed after palpation than by direct questioning (difference 16.2%; 95% confidence interval: -1.1% to 33.5%; p=0.05).

Sensitivity estimates appear higher, and specificity estimates much lower, in sick children than in those in the community survey (**table**). This may indicate that mothers are more likely to report fever in sick children, regardless of a child's body temperature, but may also be due to different temperature distributions between the two populations (figure, top). The first hypothesis was further explored by logistic regression techniques, which were used to estimate the probability of fever being reported, P, as a function of body temperature (figure, bottom). When axillary temperatures were ≥37.5°C, P was interpreted as an estimator of sensitivity, whereas below this range, $(1 - P)$ was interpreted as an estimator of specificity. The results confirm that specificity was much lower when fever was assessed in sick children than in their counterparts in the community survey, and that this was independent of body temperature. Although there were few children with fever, extrapolation from the graph shows that – at least within body temperature ranges indicating mild fever – sensitivity is much higher in the former group, even when differences due to body temperature were taken into account. The results also confirm that in sick children, questioning after palpation provided similar sensitivity but higher specificity than direct questioning.

Although these and other reports (Einterz and Bates 1998, Verhoef et al. 1998) show that African mothers are remarkably good at detecting fever, their reports of fever are not reliable when assessed in populations with low prevalence of fever. Fever is more prevalent in sick children brought to health facilities, so that a mother's report of fever is more reliable. Our results show, however, that mothers are much more likely to report fever in sick children, regardless of the actual body temperature of their child. This leads to improved sensitivity, but much lower specificity of detecting fever as compared to children who are not perceived to be sick. Teaching mothers to palpate their child's forehead cannot improve the sensitivity of detecting fever in sick children, but is likely to increase specificity, thus avoiding unnecessary treatment for febrile diseases.

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10

Anti-malarial drug use among preschool children in an area of seasonal malaria transmission in Kenya

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SUMMARY

The aims of this study were to estimate the proportion of asymptomatic Kenyan preschool children using antimalarial drugs; to identify factors associated with chloroquine use; and to assess the validity of frequency of febrile episodes and drug use reported by mothers or carers. Of 318 children studied, 38% (95% CI: 30-47%) tested positive for chloroquine or sulfadoxine. Of chloroquine-positive children, 15% had concentrations exceeding the estimated minimum therapeutically effective values. Among those testing negative for sulfadoxine, chloroquine-positive children were more frequently parasitemic (odds ratio = 2.6, 95% CI: 1.3-5.2), and had lower mean hemoglobin concentrations (6.1 g/L, 95% CI: 2.1-10.1), than chloroquine-negative children. Mothers overreported the frequency of malaria or fever episodes as usually defined in medical studies, and underreported antimalarial drug use. We conclude that antimalarials are frequently given for treatment of malaria or malaria-associated illness, rather than prophylactically or for symptoms unrelated to malaria. Questionnaire surveys cannot replace biochemical markers to obtain information on antimalarial drug use.

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In developing countries, drugs are often used without prescription. They may be purchased from local shops, markets or street vendors, obtained by sharing with other users or used when left over from previous treatments. Home treatment or prophylaxis of illnesses with anti-malarials is more often the rule than the exception in many endemic countries (Foster 1991a,b, 1995). Little is known about the administration of anti-malarial drugs to children by their parents. Whilst early diagnosis and prompt treatment is one of the elements of the global strategy for malaria control (WHO 1993), overuse may lead unnecessarily to the intensification and spread of drug-resistance and to increased risk of adverse effects associated with these drugs.

Studies in eastern Africa up to now exclusively used interviews to obtain information on antimalarial use. In one such study in Western Kenya, where malaria is holoendemic, Ruebush et al. (1995) estimated that antimalarial drugs, mostly chloroquine, were used for 67% of incident febrile episodes self-diagnosed as malaria, and for 28% of those diagnosed otherwise. On the Kenyan coast, where malaria endemicity is much lower, Snow et al. (1992) conducted a community survey to study treatment and prevention of childhood malaria by mothers. When asked what they would do in a hypothetical situation where their child had fever, 72% of mothers reported that they would purchase antimalarials at a shops for self-treatment, while 67% of these claimed they would buy chloroquine-based drugs. In the same area, Mwenesi et al. (1995) found that of 118 mothers who had diagnosed their child as having malaria currently or in the two weeks prior to questioning, 26% had given anti-malarials, 27% had given antipyretics or other medications, 23% said they had taken the child to a health facility, 6% had given a home remedy, and 18% had not given any treatment or had done nothing about treatment.

Little is known about the validity of questionnaires surveys in studying reported self-medication with antimalarial drugs. We studied antimalarial drug use by blood test and by questionnaire among asymptomatic preschool children living in an area of seasonal transmission in Kenya. The aims of the study were to estimate the proportion of children testing positive for antimalarial drugs in their blood, to identify factors associated with chloroquine use, and to assess the validity of the frequency of febrile episodes and drug use reported by mothers or carers. The study was conducted as part of a larger cross-sectional study on the prevalence and risk factors for anemia.

Subjects and methods

Study area

Data were collected in the first annual rainy season (April to June) of 1997 in three administrative areas (Kathekani, Muthingiini and Mangelete) in Mtito Andei Division, Makueni District, Kenya. The area is located halfway along the road between Nairobi and Mombasa. Taken together, the study area comprises approximately 720 km^2 with more than 40,000 inhabitants living in scattered homesteads. No entomological or epidemiological data on malaria had been collected previously in the area. The study was conducted before the harvest, and after a wet season (November-December 1996) that failed to produce rain. As a result, many people were probably short of cash. Muthingiini and Mangelete each have a dispensary staffed with a nurse, and there are a government clinic and a pharmacy in Mtito Andei town, located within 30 km of each homestead in the study population. The nearest health center with microscopic facilities for malaria diagnosis is in Kibwezi town, located about 40 km west of Mtito Andei. Malarial infections reported at

this facility are exclusively due to *Plasmodium falciparum*. Through a project implemented in the study area by the African Medical and Research Foundation over the last few decades, hundreds of community health workers and traditional birth attendants have been trained in basic health issues. Most villages have one or more of these auxiliary health workers, but they have not been trained in malaria diagnosis and treatment, and they are not usually involved in dispensing antimalarial drugs. Many communities in the study area have shops or kiosks selling drugs, including chloroquine and analgesics. Amodiaquine and combination drugs containing a sulfa compound and pyrimethamine are only available in a few larger communities. No mosquito net distribution program is in place, but nets are for sale in Mtito Andei.

Sampling procedures

A cluster sampling procedure (Henderson and Sundaresan 1982), incorporating modifications proposed by Bennett et al. (1991) and Brogan et al. (1994), was used to draw a sample representative of all asymptomatic children living in the study area and born between 15 April 1994 and 15 February 1997. The sample was drawn in two successive stages (**figure 1**). At the first stage, a systematic sample of 45 communities was drawn from a north to south ordered list of all 79 communities ('clusters') in the study area, with probability proportional to size, and excluding urban centers (Mtito Andei town). At the second sampling stage, 12 households were randomly drawn with replacement from each of the selected communities. For each of these households, the resident children were listed and their dates of birth were recorded from the child health card. All resident children thus identified who were asymptomatic (without illness reported by the mother) and within the desired age range were selected for the study (n=302). Some children migrated with only part of their household between the time of the census and the time of examination. These households were considered not to have eligible children. Where possible, children who migrated with all of their household, were still missing after repeated visits, or had parents who refused consent, were replaced by children from randomly selected households within the same community.

Field procedures

An inventory of over-the-counter antimalarial drugs and analgesics in the area was made by visiting the only pharmacy in Kibwezi town plus five shops that were well-stocked with these drugs. Visits to other shops did not yield additional brands or types of drugs. All brands and types of antimalarial drugs and analgesics in stock were purchased and made into a visual display of drug packaging and contents for use during interviews. Mothers or their primary carers were interviewed separately by lay native speakers in their own language. They were questioned using structured interviews with closed-ended questions without additional prompts. When asked to identify drugs, mothers were presented with the visual display. The distance between the child's residence and the nearest health facility was estimated by asking respondents for the time that would be needed to take the child to the nearest health care facility, using means of transport that would normally be used if the child was sick. Responses were recorded on precoded questionnaire forms, which were designed in English, translated into local language, translated back into English by a different person and compared with the original. All questions contained a precoded category 'do not know, or no answer'. Questionnaires were field-tested by four interviewers on a limited number of mothers attending Kibwezi Rural Health Centre outpatient department, and modified to ensure that respondents understood the questions. Blood samples were taken from children by finger or heel puncture and collected on filter paper, dried out of direct sunlight and kept at ambient temperature until laboratory analysis. Additional blood samples were taken for microscopic examination for

malaria parasites and for immediate determination in the field of hemoglobin concentration using a HemoCue field meter (HemoCue Inc., Mission Viejo CA, USA). Prior consent for the study was obtained from the communities and the parents involved. Children were treated as deemed necessary upon completion of the survey; because such treatment was carried out after all observations were made, this had no influence on the data collected. The study was approved by the African Medical and Research Foundation and the Kenya Medical Research Institute whose ethical standards were followed.

Laboratory procedures
The enzyme-linked immunosorbent assay to detect whole blood chloroquine has been described by Eggelte (1990). The monoclonal antibodies used (F149) show no (< 0.1%), or hardly any cross-reactivity with amodiaquine. The limits of detection for chloroquine and sulfadoxine were 10 µg/L and 10 µg/L, respectively.

Blood slides were stained using Field's stain, and thick films were examined by experienced microscopists and crosschecked independently. At least 100 high power fields were examined before the slide was considered negative.

Response and missing values
A total of 302 children were selected for study of whom 16 were double-selected at the second sampling stage (**figure 1**). Observations for these 16 children were weighted twice. Thus 318 cases were included in the study of which 35 did not participate or fully participate for the following reasons: refused consent (26); not home or temporarily absent (7); hospitalized for burns (2). Of these 35 children, 14 were replaced by random selection, which brought the total number included in the analysis to 297. In the case of non-participating children who were not replaced, weighting was used to maintain the validity of assuming of an equal probability sample. Thus, observations on those who participated within the same cluster were inflated by weighting with a multiplication factor calculated as the number of selected children in that cluster divided by the number of participating children (Bennett et al. 1991, Brogan et al. 1994). This brought the number back to 318 children; sample sizes reported below this value are due to missing values.

Statistical analysis
Data were entered, cleaned and managed on a personal computer using SPSS (v7.5 for Windows; SPSS Inc., Chicago IL, USA). Computations were performed in SUDAAN, stand-alone v7.5.2a for Windows (Research Triangle Institute, Research Triangle Park NC, USA), assuming two-staged cluster sampling with replacement at the first sampling stage. Because the variance estimates under this assumption do not take account that clusters were sampled from a finite population, the standard error values and confidence intervals reported here are overestimates, and the statistical tests are less sensitive in detecting an existing effect.

Results

Interviews
Of the various brands and types of antimalarial drugs available in the stores in Kibwezi, seven contained chloroquine, three contained amodiaquine and five contained combinations of sulfa compounds with pyrimethamine. The brands with sulfa drugs

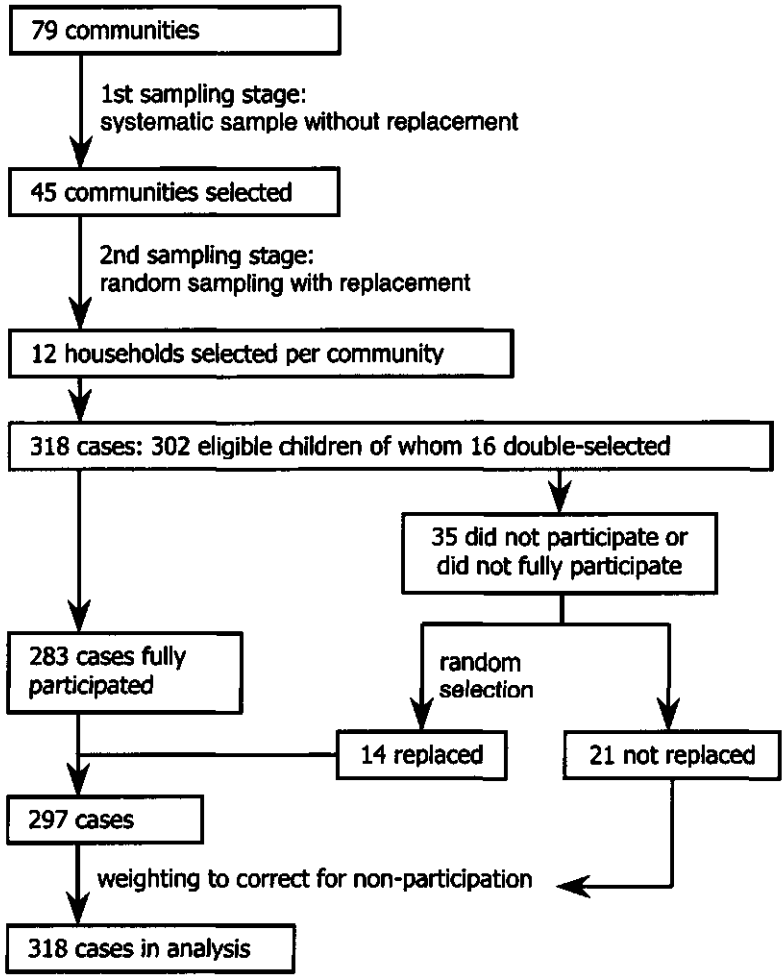

FIGURE 1. Framework for selection and analysis

contained sulfadoxine, except for two brands that contained other sulfa compounds (Medikelfin contains 3-sulphanilamido-6-methoxy-pyridazine, whilst Metakelfin contains 2-sulphanilamido-3-methoxy-pyrazine). Sulfadoxine was exclusively available in combination with pyrimethamine. Out of 317 respondents, 58 (18%) reported that the child had received antimalarials in the two weeks prior to the interview (**table 1**). No child was reported having received a sulfa compound-pyrimethamine drug, and only 2 were reported having received amodiaquine. When asked to indicate what they considered to be the best medicine for malaria, 186 (54%) out of 314 respondents named an analgesic, 97 (31%) a drug containing chloroquine, 2 (1%) a sulfa compound-pyrimethamine drug and 29 (9%) did not know, or named other drugs.

In the local language, Kikamba, there are words or expressions to distinguish malaria or malaria-like illnesses ('ndetema') from the physical phenomenon of an elevated body temperature. When asked if the child had suffered from 'ndetema' in the two weeks prior to the interview, 190 (60%) out of 316 respondents answered positively (**table 2**). Of the same respondents, 226 (71%) answered positively when asked if the child had suffered from an elevated body temperature, and 178 (56%) reported that the child had suffered both from 'ndetema' and an elevated body temperature.

When asked if the respondent had discussed the child's health with anyone, and if so, with whom, 213 out of 316 respondents reported not to have discussed it with anyone. Out of 103 respondents who had consulted someone, 10 had seen a relative, 10 a community health worker, 16 a traditional birth attendant, 40 a government health facility, 7 a non-governmental health facility, 30 a private health facility and 2 had used other sources (respondents could name more than one source).

Antimalarial blood tests and parasitemia

The results of the blood tests for chloroquine and sulfadoxine are summarized in **table 3**. Out of 314 children, 37% tested positive for the presence in blood of chloroquine (95% confidence interval, CI: 29-46%), 4% tested positive for sulfadoxine (95%CI: 2-7%) and 38% tested positive for either chloroquine or sulfadoxine (95%CI: 30-47%). Sulfadoxine was reported to have been taken by 11% children whose blood tested positive for antimalarial drugs, and most of these had taken sulfadoxine in combination with chloroquine. **Figure 2** shows the relative distribution of children in relation to estimated chloroquine concentrations. The median blood concentration of children testing positive was 56 µg/L for chloroquine and 9.9 mg/L for sulfadoxine. To examine the relationships of a positive chloroquine test with malaria or hemoglobin concentration, we excluded children testing positive for sulfadoxine. In the remaining children (**table 4**), the odds ratio of malaria for testing positive to testing negative for chloroquine was 2.6 (95%CI: 1.3-5.2). In the same group, children testing positive for chloroquine had lower mean hemoglobin concentrations than children testing negative for chloroquine (98.3 ± 6.5 (mean ± S.D.) compared with 104.4 ± 3.8 g/L; difference 6.1 g/L , 95%CI: 2.1-10.1). Whether or not children tested positive for chloroquine was not statistically significantly influenced by sex, the presence or absence of the mother, father or grandmother in the household, or whether or not the mother had activities to support her income. Children with a positive blood test for chloroquine were not found to live at a greater distance from the nearest health facility than children with a negative blood test (Chi-square=7.01, df=5, p=0.24; **figure 3**).

Discussion

To our knowledge, this is the first report to assess antimalarial drug use in eastern Africa by blood tests. We found 37% of children to be positive for chloroquine, 4% for sulfadoxine and 38% for either of these drugs. When those testing positive for sulfadoxine were excluded from analysis, children who tested positive for chloroquine had more frequently malaria, and lower mean hemoglobin concentrations, than children testing negative for chloroquine. Although chloroquine resistance is most likely to occur in the area, as in other parts of eastern Africa, this drug remains the most widely used antimalarial as assessed by blood test and as reported by mothers or carers.

TABLE 1. Frequency distribution of antimalarial or analgesic drugs use as reported in the previous 14 days and antimalarial drug use detected by ELISA

Antimalarials reportedly taken	Drug use tests by ELISA					
	S+P	Amodiaquine	Chloroquine, S+P	Chloroquine	Negative	Total
Amodiaquine	0	0	0	0	2	2
Chloroquine	0	0	3	37	16	56
None	3	0	7	71	178	259
Total	3	0	10	108	196	317

S+P: combined sulfa drug plus pyrimethamine; no child was reported to have taken S+P in the last two weeks.

TABLE 2. Frequency distribution of children with reported fever or malaria in the previous two weeks

Child reported to have suffered from 'ndetema' in the last 2 weeks	Child reported to have suffered from a raised body temperature in the last 2 weeks		
	Yes	No	Total
Yes	177	13	190
No	49	78	127
Total	226	91	317

TABLE 3. Frequency distribution of children with positive blood tests for chloroquine or sulfadoxine

Chloroquine in blood	Sulfadoxine in blood		
	Positive	Negative	Total
Positive	10	108	118
Negative	3	197	200
Total	13	305	318

TABLE 4. Frequency distribution of children with malaria or testing positive for chloroquine among children testing negative for sulfadoxine

Malarial parasitemia	Chloroquine in blood		
	Positive	Negative	Total
Positive	28	23	51
Negative	80	170	250
Total	108	193	301

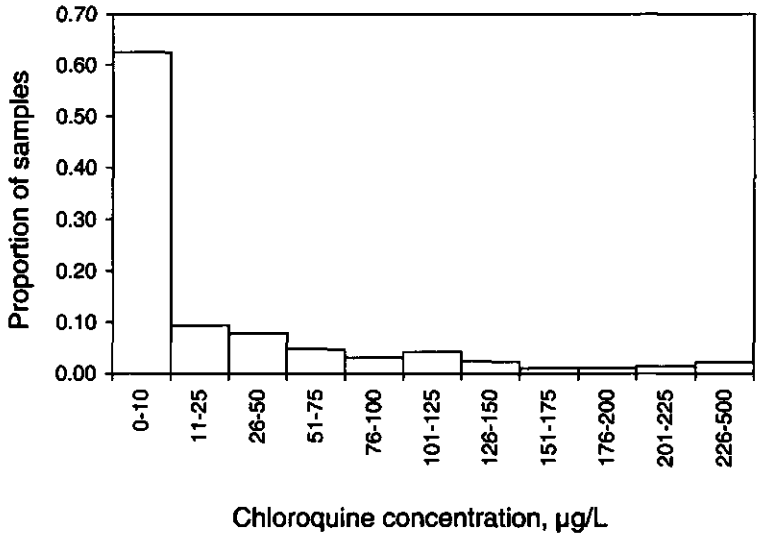

FIGURE 2. Relative frequency distribution of blood samples in relation to chloroquine concentrations

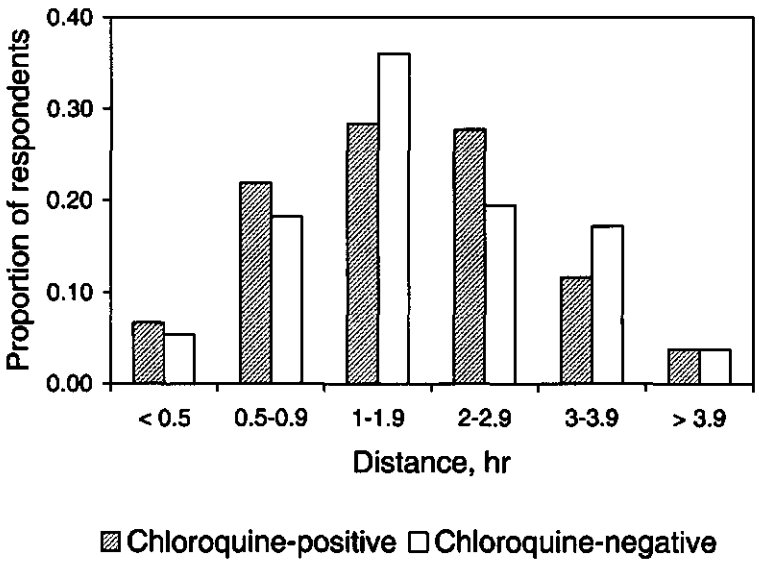

FIGURE 3. Relative frequency distribution of children in relation to the travelling time between their residence and the nearest health facility, as reported by their mothers or caretakers

The study concerned a representative sample of asymptomatic children born between 15 April 1994 and 15 February 1997 (corresponding to age 2-37 months) and resident in the study area. Non-response was 11%, and increased after replacement by random selection of 14 children to 7%. Blood test results were missing for 4 children (1%). We therefore judge the conclusions to be valid for the study population.

Our estimated prevalence of chloroquine use corresponds to a value of 35% for positive chloroquine tests by ELISA in Gambian children aged between 6 months and 5 years (Alonso et al. 1993; non-intervention group). Surveys in rural Zimbabwe (Stein et al. 1988) showed that 8% of urine samples tested positive for 4-aminoquinolines (a group of antimalarial compounds including chloroquine and amodiaquine), whilst 13% of urine samples were positive in Papua New Guinea (Cattani et al. 1986). The Dill-Glazko test that was used in these last two studies has a low sensitivity (Verdier et al. 1985), suggesting that the prevalence values are underestimates. Moreover, Stein et al. (1988) and Cattani et al. (1986) studied populations of all ages, whereas we studied children, in whom antimalarial drug use may be higher because they may be considered at greater risk of malaria or death from malaria.

Why are children given antimalarials? Children testing positive for chloroquine were more frequently parasitemic, and had lower mean hemoglobin concentrations, than children who had negative chloroquine tests. This suggests that antimalarials are frequently given for treatment of malaria or malaria-associated illness, rather than prophylactically or for symptoms unrelated to malaria. Further studies would be needed to validate this hypothesis.

To what extent are the blood chloroquine concentrations found in our study therapeutically meaningful? In a study among asymptomatic Tanzanian schoolchildren, Hellgren et al. 1989) found that chloroquine administration at a WHO-recommended dosage (10, 10 and 5 mg per kg body weight on days 0, 1 and 2, respectively) resulted in peak whole blood concentrations on days 2 and 3 with median values in the order of 500 µg/L. On day 7, median concentrations had receded to around 150-200 µg/L (Hellgren et al. 1989). The latter range corresponds to the therapeutically effective chloroquine concentration, which is in the order of 30 µg/L plasma against drug-sensitive falciparum malaria (Black et al. 1986), or approximately 150 µg/L whole blood (Eggelte, personal communication). The whole blood chloroquine concentrations in our study ranged between 0 and 466 µg/L, with a median value of 56 µg/L. Only 15% of children who tested positive for chloroquine had concentrations exceeding 150 µg/L. These findings do not take into account when chloroquine was taken. They should not be interpreted as providing evidence to support the common supposition by field workers that self-treatment using antimalarial drugs often occurs at subtherapeutic dosages.

Children whose blood tested positive for chloroquine were not found to be living at a greater distance from the nearest health facility than children who tested negative. Physical access to health care facilities did not appear to be an important constraint for chloroquine use, although it may act as a contributory factor for self-treatment and as a barrier for seeking health care.

Additional motivation for self-medication with antimalarial drugs, and their implications for public health and clinical practice, has been discussed by many workers and are well-summarized by Foster (1991a,b, 1995). The remainder of this paper will focus on the

usefulness of proxy reporting for detecting malaria episodes in children in our study and the implications of our findings for future research studies.

Most studies on antimalarial drug use have relied on questionnaire surveys (Kaseje et al. 1987, Mburu et al. 1987, Breman and Campbell 1988, Stein et al. 1988, Snow et al. 1992, Mnyika et al. 1995, Mwenesi et al. 1995, Ruebush et al. 1995, Ahorlu et al. 1997). One concern about the use of interview surveys to estimate morbidity and drug use is their amenability to bias (Kroeger 1985, Hardon et al. 1991). How well does the occurrence of illness episodes and drug use reported in questionnaire surveys correspond to the occurrence of such episodes measured using biomedical criteria? In our study, 60% and 71% children were said to have suffered from 'ndetema' and elevated body temperature, respectively, in the two weeks prior to the interview. A similarly high 14-day cumulative malaria incidence (73%) was noted in children under four years of age in a questionnaire survey in western Kenya (Kaseje et al. 1987). Overreporting of fever is inevitable if body temperature is assessed without a thermometer, particularly at community level (Verhoef et al. 1998). In children aged below five years attending an outpatient service in Cameroon, mothers or carers correctly reported fever in 46% of cases, and the absence of fever in 92% of cases (fever defined as axillary body temperature ≥37.5 °C) (Einterz and Bates 1997). When these predictive values are applied to our study, the two-week cumulative incidence of fever drops from 72% to 35% (calculations not shown).

This adjusted value is still higher than would be expected on the basis of previously conducted prospective studies. In Mali, the 9-day cumulative incidence of fever (oral temperature >38°C) in children aged 1-3 years who were examined daily was 14.2% in the wet season (Bouvier et al. 1997), which, extrapolated to a 14-day period, would have been 22.1%. This estimate is lower than ours, despite being measured during the wet season in an area where malaria was more endemic. Our reported values therefore appear overestimates of the cumulative incidence of fever measured by thermometer.

One explanation for this bias is a desire for drugs by the respondents participating in the study. This may have caused them to exaggerate the frequency of illness and downplay prior use of drugs. The desire for drugs was obvious on various occasions during the survey and became even more apparent in mothers who did not receive drugs upon completion of the survey. Many remained dissatisfied even when explained that their child's condition did not require drug treatment. Antimalarial drug use, which was reported in 18% of children in the past two weeks, may have been further underestimated by the long recall period used in our study. For inconspicuous events, such as the use of drugs, Kroeger (1985) recommended to reduce the length of the recall time to several days. Schulpen and Swinkels (1980), in a study area close to ours, found 60% underreporting of self-medication when a recall period of two weeks was used instead of one day.

To obtain valid information on antimalarial drug use, we recommend that questionnaires should be used in addition to biochemical markers, but not to replace them. Blood tests measure the prevalence of drug use, whereas interviews measure a cumulative incidence. Asking for history of antimalarial drug use as part of the medical examination has limited value. Biochemical markers, however, have the disadvantage of not being able to quantify the incidence or quantity of drug use, or to describe the social or cultural context in which people use drugs.

The relationship between fever and the level of parasitemia is at the core of recent attempts to find suitable case definitions of malaria attacks for use in epidemiological

studies (Armstrong-Schellenberg et al. 1994, Smith et al. 1994, Greenwood 1997). Both antimalarials and antipyretics may influence this relationship. The finding that a large proportion of children may have antimalarials in their blood requires further research to assess if and how the presence of these drugs can be taken into account in case definitions.

We conclude that antimalarials are frequently given for treatment of malaria or malaria-associated illness, rather than prophylactically or for symptoms unrelated to malaria. Questionnaire surveys overestimate the occurrence of febrile episodes. They should be used in addition to biochemical markers, but not to replace them.

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Discussion

The path to be travelled towards better public health programmes is illuminated by well-conducted, internally valid epidemiological studies. Although laboratory studies or observations made in clinical or public health practice may serve to predict health benefits or risks of interventions, such effects must ultimately be estimated in the target population. The findings presented in this thesis shed light on the benefits and risks of measures to alleviate anaemia in children aged 2-36 mo in an area of seasonal malaria.

In a strict sense, however, health policies are always formulated for populations that have never been studied, or that have been inadequately studied. For example, the findings presented in this thesis may assist in formulating policies for children who are older, more exposed to malaria or other infections, or who may differ in other respects from the children studied. Even future generations of children growing up in the area studied may experience different levels of exposure to malaria (for example, during droughts or abundant rainfall), despite having the same distribution of characteristics such as age, sex, and so on. In addition, epidemiological studies are often limited by their tendency to consider study populations as homogenous. Later experience may show that there were sub-groups in the study population for which the conclusions were considered too broadly or do not apply.

Thus, the ability to generalise the findings to other populations ultimately and necessarily depends on our understanding of the underlying biological mechanisms. This is particularly the case when considering diseases with such demonstrated variability in occurrence and effects as malaria. Evidence from internally valid studies forms the point of departure from which generalisations can be made. Understanding of biological mechanisms is provided not only by epidemiological field studies, but also by evidence accumulated from clinical experience and laboratory studies, both in humans and animals. This chapter aims to assist policy-makers in public health in the interpretation and extrapolation of the main findings, and to assist researchers in identifying and prioritising areas of future work. This discussion will be presented under three headings (lessons learnt and conclusions; implications for public health; and future research), and in the light of the four objectives presented in the introduction of this thesis:

1. to measure the efficacy in improving haemoglobin concentrations of intermittent iron supplementation and intermittent administration of sulfadoxine-pyrimethamine (SP);
2. to develop and evaluate survey methods for rapid assessment at community level of the burden anaemia and its risk factors;
3. to contribute to improved methods for diagnosis of anaemia, iron deficiency, and malaria;
4. to evaluate the role of impaired erythropoiesis in the pathogenesis of malarial anaemia.

Administration of iron and sulfadoxine-pyrimethamine (SP)

Lessons learnt and conclusions

When judged by the effect on haemoglobin concentration, intermittent administration of SP resulted in marginal improvements when given alone, and in no gains when given in addition to iron supplementation (chapter 5). Iron supplementation markedly improved haemoglobin concentrations, and substantially reduced the prevalence of both iron deficiency and anaemia. There was no evidence that iron supplementation resulted in a substantially increased risk of malaria at the regimens and doses used. These findings

indicate that, when using the regimen employed, the benefits of iron supplementation may outweigh the associated risk of adverse effects caused by malaria.

Implications for public health

The findings from this study give little support for intermittent administration of SP. The observed protection against malarial attacks, however, corroborates findings from a recent study in Tanzania showing that SP, when given intermittently to infants alongside routine vaccinations against childhood diseases, may substantially reduce the frequencies of occurrence of symptomatic malaria and severe anaemia (Schellenberg et al. 2001). Although this could be further developed as a tool for malaria control, the switch of African countries to SP for first-line treatment of uncomplicated malaria justifies concerns that parasite resistance against SP may rapidly develop to levels where this intervention is no longer efficacious.

The effectiveness of iron supplementation (i.e. how well the benefits can be achieved under operational conditions of public programmes) was not considered in this study, and neither was its cost-effectiveness. Both are key elements to judge the feasibility of intervention programmes (Yip 1997).

Current guidelines for mass programmes recommend daily iron supplementation at preventive doses for anaemia (2 mg iron d^{-1} kg body $weight^{-1}$) for all infants and young children in areas where anaemia is common (Stoltzfus and Dreyfuss 1998, UNICEF/UNU/WHO/MI 1999). The rationale for administering iron at less frequent intervals but at therapeutic doses (twice weekly at 3 mg iron d^{-1} kg body $weight^{-1}$) was controversial. When designing the trial, experience accumulated from many countries in pregnant women and preschool children had shown that iron supplementation programmes were not effective in improving iron status (ACC/SCN 1991). Reasons cited for this poor effectiveness included, among others, the failure of health care systems to deliver an adequate supply of tablets at the community level, and poor compliance by women for fear of adverse effects of iron (ACC/SCN 1991, Galloway and McGuire 1994). The occurrence and severity of side effects were generally accepted as major determinants of poor compliance in developed countries, and were known to be related to dose of the supplement (Beaton and McCabe 1999).

Intermittent iron supplementation appeared as a possible alternative strategy that might improve the effectiveness of programmes. This hope was founded in controversial ideas that weekly supplementation could reduce side effects and increase compliance when compared to daily dosing while maintaining net iron absorption at similar or even higher levels (Stephenson 1995, Beaton and McCabe 1999). When designing the trial described in this thesis, it was considered that the published reports showed marginally but consistently higher efficacy when iron was supplemented daily instead of weekly. It was also judged that possible effects of iron supplementation on malaria were likely to depend on the dose and regimen in which iron was administered. Thus, a regimen using twice-weekly iron supplementation at therapeutic doses was a compromise to maximise the beneficial effects of iron supplementation whilst minimising possible adverse risks of malaria.

In a recent analysis of the empirical evidence from 22 completed trials in pregnant women, adolescents and school children, and preschool children (Beaton and McCabe 1999), it was concluded that weekly supplementation is likely to be less effective than daily administration except in situations where supervision is feasible with weekly regimens and

not with daily supplementation. It was also concluded that weekly supplementation may be particularly disadvantageous in situations where the prevalence of anaemia is very high.

Future research
In the last decade, organisations such as the World Health Organization and UN Children's Fund have endorsed iron supplementation in preschool children (Stoltzfus and Dreyfuss 1998, UNICEF/WHO 1994, UNICEF/UNU/WHO/MI 1999), whilst 22 countries have adopted public health policies calling for iron supplementation of preschool children (UNICEF/UNU/WHO/MI 1999). This growing commitment occurs despite the lack of documented instances of large-scale voluntary iron supplementation programmes significantly reducing levels of iron deficiency anaemia in a population (UNICEF/UNU/WHO/MI 1999). As a matter of urgency, ways should be explored by which iron can be effectively delivered to populations in need.

Such assessments should include methods outside the conventional health care system. For example, supplements might be distributed at subsidised prices through commercial networks, with education being provided by health workers, shopkeepers and community volunteers. Compliance might be enhanced by screening for anaemia using the colour scale (chapter 6) and selective supplementation. Such an approach may be feasible judging the encouraging results achieved by social marketing programmes of insecticide-treated mosquito nets and net treatment for malaria control in rural Tanzania (Schellenberg et al. 1999), and by the success of a programme to improve home treatment of childhood fevers through training of shopkeepers in rural Kenya (Marsh et al. 1999).

Beaton and McCabe (1999) recommended that a regimen of daily iron supplementation should continue to be used rather than weekly supplementation (see above). A further placebo-controlled trial might be considered to investigate whether the benefits of a daily regimen outweigh the risks of all-cause mortality in children with low levels of protective immunity in areas of seasonal malaria. This requires a large sample size, however, and the number of sites where such studies can be carried out is limited in Africa.

The results do not preclude an increased risk of malaria or other infections from iron supplementation in some individuals (for example, children with HIV/AIDS – see Delanghe et al. 1998, De Monye et al. 1999, Savarino et al. 1999), or when iron is given at higher frequency or for longer periods of time. Priorities for additional research concern the safety of iron supplementation in low-birth-weight infants, and during refeeding of severely wasted children. In these cases, randomised controlled trials seem justified.

Rapid community assessment of the burden of anaemia and its risk factors

Lessons learnt and conclusions
Cluster sample surveys are ideally suited for rapid assessment of the burden of anaemia and its risk factors. The methodology can be used in national surveys, with stratification as required to obtain precise estimates for individual provinces or districts (Bennett et al. 1991). Although these surveys were originally designed to provide descriptive statistics, chapter 2 provides methods to expand their use to assess associations between exposures and health outcomes at a single point in time.

Implications for public health

Both anaemia and malaria were geographically clustered between communities in the study area (chapter 2). When such clustering is ignored in design and analysis, surveys may produce biased estimates. An important consideration concerns the choice of indicators and measurement tools. Anaemia, in young children defined as haemoglobin concentration <110 g/L, is an indicator of particular interest because, when due to iron deficiency, it is strongly associated with impaired body functions (chapter 1). Less is known about the functional importance of iron depletion without anaemia, or of mild or moderate anaemia due to other causes, including malaria. In areas with a great burden of anaemia, lower cut-off values for haemoglobin concentration (for example, < 90 g/L) may be better to identify population groups in need of intervention and to monitor progress in anaemia control (Stoltzfus 1997).

One drawback of using a cut-off point for haemoglobin concentrations is the tendency to regard individuals without anaemia as healthy, or insufficiently affected to warrant intervention, despite indications that iron depletion without anaemia may already impair mental development of children (chapter 1). One way to bypass this pitfall is to present the entire distribution of haemoglobin concentration against a reference distribution obtained from children free from iron deficiency (Yip et al. 1996).

Haemoglobin concentrations or the presence of anaemia are ideally determined by photometer or haematocrit centrifuges. Particularly when several survey teams are deployed, this may be too expensive or – if electricity is required – not feasible. In those cases, the use of haemoglobin colour scales may give valid estimates of the prevalence of anaemia at virtually no material cost (chapter 6). Their use requires training of survey personnel to standardise measurements. Bias may occur when this device is used to estimate the prevalence of anaemia in (sub)populations with a low burden of anaemia (for example, in adult men), or when low scores are used to determine the prevalence of more severe degrees of anaemia (for example, using cut-off values for haemoglobin concentration <70 g/L or <90 g/L).

The use of dipstick tests to diagnose malaria may save time and money, and is less cumbersome than microscopy (WHO 2000). Thus, one efficient strategy in surveys might be to screen for infection by dipstick test. A negative test result is highly predictive of the absence of infection (chapter 5), and indicates no need for further follow-up or microscopic examination. A positive test result indicates current or recent infection. Patients with such a test result may be presumptively treated; microscopic examination of the blood sample taken may be done at a later stage.

Future research

The degree of clustering, as expressed in the design factor or the intracluster correlation coefficient, may be of research interest because it may indicate genetic predisposition to certain diseases within families or within communities with a high degree of consanguinity, or to common environmental or behavioural factors leading to an increased prevalence of the health outcome within certain households or communities. The geographical clustering observed for malaria corroborates our understanding that the probability of infection in areas of seasonal transmission heavily depends on the availability of breeding sites for malaria mosquitoes.

The use of haemoglobin concentration as an outcome variable allows for multivariate linear regression modelling to identify independent risk factors for anaemia, and to examine

biological interaction (non-additivity of effect estimates on an arithmetic scale) between factors. Logistic regression could be used to model the ln-odds of anaemia, but such an approach has the disadvantage that exponentiation to calculate the odds of anaemia results in a multiplicative model. Thus observation of non-additivity in a ln-odds model of anaemia can be easily misinterpreted as indicating the absence of biological interaction, whereas in fact it implies exactly the opposite, namely super-additivity and biological interaction. Because of these considerations, the assessment of biological interaction is more complicated in logistic regression models. For a more thorough discussion, the reader is referred to a review of this topic by Greenland and Rothman (1998).

Improved diagnosis of anaemia, iron deficiency and malaria

Lessons learnt and conclusions

A newly introduced colour scale to estimate haemoglobin concentrations is inexpensive and does not require chemical reagents or electric power. Its diagnostic performance is satisfactory and for many purposes superior to all other methods for detection and treatment of anaemia at primary care level (chapter 6). Low scores of the scale may give unsatisfactory results when used to detect patients with severe anaemia and to decide on their management.

Accurate detection of iron deficiency in individuals with malaria remains elusive. Absence of stainable iron in macrophages in bone marrow aspirates or biopsy specimens, or serum ferritin concentrations <15 µg/L have a fairly straightforward interpretation, as they are highly predictive for the absence of iron stores (chapter 1). Because serum ferritin concentrations react as acute phase proteins, there is currently no way to diagnose iron deficiency accurately in individuals with malaria and normal serum ferritin concentrations. Erythropoiesis is affected by malaria (chapters 3 and 6), so that serum transferrin receptor concentrations cannot be used to diagnose iron deficiency in individuals with malaria.

Lastly, there were strong indications that a reported history of fever in afebrile children may poorly predict past fever episodes (chapters 7-10). Chemotherapy following such incorrect reports may unnecessarily expose children to adverse drug effects, and lead to selection and spread of drug-resistant parasite strains. In a representative community-based survey, a large proportion (38%) of children tested positive for chloroquine or sulfadoxine, and only a minority of those testing positive for chloroquine had concentrations exceeding the estimated minimum therapeutically effective values (chapter 10).

Implications for public health

The reported findings on the colour scale challenge anaemia control programmes to develop strategies for its use in various target groups. The methodology presented in chapter 6 may facilitate that process.

The findings underscore the need to educate communities about the diagnostic signs of malaria and about proper use of drugs as part of strategies to reduce morbidity and possibly to stretch the 'life span' of antimalarials drugs. A report published subsequently to the report in chapter 10 shows the feasibility of such an approach in Kenya (Marsh et al. 1999). Thermometers should be made available to community health workers to reduce the number of unnecessary treatments for fever.

Future research
The finding that a reported history of fever in afebrile children may poorly predict past fever episodes emphasises the need for better case definitions for malaria, both for use in clinical practice and in epidemiological field studies. In the latter case, such poor prediction may lead to substantial underestimation (Rothman and Greenland 1998) of the effects of interventions on malaria attacks.

Serum concentrations of sTfR might be useful to detect iron deficiency in Africans with inflammatory conditions other than malaria (for example, in those with HIV infection, worm infections or tuberculosis). Several authors, however, reported elevated serum transferrin receptor concentrations indicating increased erythropoiesis in α^{+}-thalassaemia (Rees et al. 1998, Mockenhaupt et al. 1999). If so, sTfR concentrations might not be useful to assess iron deficiency in persons with this disorder, in addition to those with malaria. A study among Africans, however, suggests that this effect might be marginal in homozygotes, and does not occur in heterozygotes (Mockenhaupt et al. 1999). Additional studies are needed to quantify this relationship.

Impaired erythropoiesis in malarial anaemia

Lessons learnt and conclusions
Based on a literature review, new insights were gained in the possible role of inflammation as a cause of malarial anaemia (chapter 1). Thus it was anticipated that combined iron supplementation and SP might be more effective in improving haemoglobin concentrations than the added effects of the interventions alone. Contrary to this expectation, however, the findings pointed to a small antagonistic effect (chapter 5).

Evidence was found that in asymptomatic malaria, the erythropoietic response is adequate for the degree of resulting anaemia. Thus, inflammation and the pathogenic mechanisms involved in the anaemia of chronic disease play no or only a minor role (chapters 3 and 6). Stunting might exacerbate the degree to which malaria produces anaemia (chapter 4). Nutritional inadequacies might explain the latter finding because several deficiencies – notably of zinc, iron and possibly vitamin A – are known to cause stunting and are also known or suspected to impair host immunity.

Implications for public health
The conclusions drawn alleviate initial concerns (chapter 1) that malaria-induced inflammation might reduce the efficacy of iron supplementation programmes in endemic regions in Africa.

Future research
It appears likely that a relative deficit in erythropoietin production or decreased marrow responsiveness to erythropoietin may limit erythropoiesis in acute malaria, and that this mechanism contributes substantially to the resulting anaemia. This should be confirmed in studies that use serum concentrations of erythropoietin and sTfR as indicators of erythropoiesis (chapter 6).

It was postulated that nutritional inadequacies causing stunting also impair host immunity, thus increasing the degree to which malaria is associated with decreased concentrations of haemoglobin (chapter 3). If this effect were due to impaired immunity resulting from iron

deficiency, then this would suggest that malaria and iron deficiency synergistically produce low haemoglobin concentrations. Conversely, iron supplementation might reduce stunting and nutritional anaemia, but also reduce malaria-associated anaemia. This seems in agreement with the findings from the randomised trial (chapter 5).

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Annex 1

Phenobarbital for children with cerebral malaria

Hans Verhoef, Clive E West, Frans J Kok

Lancet 2000; 356: 256-57.

Despite observing a decrease in seizure frequency following phenobarbital prophylaxis in children with cerebral malaria, Joan Crawley and colleagues (2000) advise against such prophylaxis because of the associated increase in mortality. Their analysis suggests that the effect of phenobarbital on mortality is, at least in part, a result of diazepam being administered before or after being given phenobarbital. We are concerned that analysing data with diazepam use as a covariate may have produced severely biased effect estimates of phenobarbital on death and possibly on seizure frequency. In his commentary, Malcolm Molyneux (2000) questioned whether excess mortality would have occurred without using diazepam. Reanalysis may indicate the need for further investigations into the prophylactic use of phenobarbital (20 mg/kg) in combination with other therapeutic drugs.

Crawley and colleagues report that phenobarbital reduces seizure frequency, thereby reducing diazepam use, whereas diazepam, used together with phenobarbital, increases mortality risk. If so, diazepam mediates the effect of phenobarbital on mortality. By reducing seizure frequency, phenobarbital may also reduce mortality directly and not via diazepam. In this situation, stratification for diazepam use will lead to biased effect estimates. Consider a randomised trial with 2000 children, in which 1000 receive phenobarbital and 1000 receive placebo (**table**). Let us presume, as did Crawley and colleagues, that phenobarbital leads to 50% reduction (from 30% to 15%) in seizure frequency. Hence, seizures occur in 150 children receiving phenobarbital, and subsequent use of diazepam leads to high mortality (say 90%). In those not receiving diazepam, 20% die. In the placebo group, seizures occur in 300 children, and subsequent use of diazepam leads to 10% mortality. In those not receiving diazepam, 30% die. Total mortality (including deaths caused by subsequent diazepam use) is higher in the phenobarbital than in the placebo group (305 versus 240 deaths, respectively). Biased effect estimates occur when stratifying for diazepam use. Mortality should be calculated by multiplying stratum-specific estimates by the probability of ending up in each stratum. Comparison between groups 2 and 4 (**table**) shows that phenobarbital directly reduces mortality. The striking reduction in the proportion of children experiencing seizures could decrease the number of children with long-term neurological sequelae. Hence, if seizures occurring in the phenobarbital group could be safely treated by a drug other than diazepam, then phenobarbital prophylaxis would be effective and safe.

TABLE. Estimated mortality in a randomised controlled trial, based on assumptions outlined in the text

Phenobarbital use	Group	Diazepam use	Deaths	Mortality estimate	
				Stratified	Unbiased
Yes = 1000	1	Yes = 150	135	0.90	0.90×150/1000 = 0.14
	2	No = 850	170	0.20	0.20×850/1000 = 0.17
No = 1000	3	Yes = 300	30	0.10	0.10×300/1000 = 0.03
	4	No = 700	210	0.30	0.30×700/1000=0.21

The confusion is compounded by the fact that Crawley and colleagues did not exclude from the analysis 75 children who received diazepam before admission to hospital. Mortality may have been higher in those children given phenobarbital. Hence, the increased mortality reported in the phenobarbital group may be explained by earlier diazepam use. Conventional testing for effect modification by diazepam using multiple regression techniques is inappropriate as discussed above. When using the methods we have outlined above, Crawley and colleagues may find decreased estimates of the total effects of phenobarbital on seizure frequency and mortality, a substantial reduction in the mortality estimate in children receiving both phenobarbital and diazepam, and possibly a reduction of the direct effect estimate of phenobarbital on mortality.

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Annex 2

Effect of HIV-1 infection on malaria parasitaemia

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Lancet 2001; 357: 232-33.

James Whitworth and colleagues (2000) present evidence that HIV-1 infection leads to increased frequency of both symptomatic and asymptomatic malaria. Among cohorts that were followed through repeated surveys, they found that malaria parasite density in HIV-1-positive subjects tended to be higher with advanced immunosuppression, as indicated by nearest and previous CD4-cell counts. We believe that the figure presented is misleading, and we offer possible statistical methods to clarify the nature of the relationship between HIV-1 infection and parasite density.

First, there is a confusing error in the legend to the figure. Contrary to the information provided, it appears from the text that the solid line refers to HIV-1-positive people, and the dashed line refers to HIV-1-negative people.

Second, if the effect of HIV-1 infection on parasite density were mediated by CD4-cell counts alone, then the regression lines in the figure (Whitworth and colleagues 2000) would be parallel and superimposed on one another. The negative slope of these lines would indicate an inverse association between CD4-cell count and parasite density. HIV-1 infection is associated with a decrease in CD4-cell counts, as shown by the position of the closed circles towards the left side of the figure relative to the open circles. Thus, the HIV-1-associated immunosuppression would be associated with increased parasite densities. However, the slopes of these regression lines were reported to be different ($p=0.0076$), with no or perhaps even a positive association between CD4-cell count and parasitaemia in HIV-1-negative subjects. The figure furthermore suggests that this difference in slopes occurred above 300 CD4-cells/μL blood. Thus, at a given CD4-cell count within the range of 300-1200/μL blood, parasitaemia would be higher in HIV-1-positive individuals than in their negative counterparts. On the other hand, at CD4-cell counts >1200/μL blood, parasitaemia would be lower in HIV-1 infected individuals. This findings implies that HIV-1 infection, in addition to leading to decreased CD4-cell counts, also determines the activity of immune cells in limiting malaria parasitaemia.

An alternative and more likely explanation is that the association between CD4-cell count and parasite density is the same for HIV-1-positive and HIV-1-negative subjects, and that the reported differences in slopes are the result of poor fit of the model for HIV-1-positive subjects. Visual inspection of the open circles and closed circles seems to support the notion that at more than 500 cells/μL, there is no substantial association between CD4-cell count and parasite density in either group. The increase in parasite density in HIV-1-positive individuals seems only to occur at values lower than this range. Thus, immunosuppression would affect parasite density only when CD4-cell counts are lower than 500 cells/μL, which is a cut-off point used in the Centers for Disease Control and Prevention classification system of HIV disease (Anonymous 1993). This effect could be assessed statistically by multivariate regression of parasite density as a function of HIV-1 infection and CD4-cell count, whereby the latter is categorised into two or three strata defined by strategically selected cut-off values. Alternatively, the association could be approximated by a second- or probably third-order polynomial.

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Summary

Approximately three quarters of east African children <5 y of age suffer from anaemia, which usually indicates malaria or other underlying infections, deficiencies of iron and other nutrients, or genetic disorders. These factors may cause serious adverse health effects, including reduced physical activity, impaired physical growth and mental development, and death. Successful interventions to control anaemia are therefore assumed to lead to better health.

The potential benefits of iron supplementation depend on the prevalence of children with depleted iron stores and the deficit in functional iron in those children. In children in areas of seasonal malaria, these benefits may not outweigh possible inherent risks of adverse effects caused by malaria. Intermittent administration of sulfadoxine-pyrimethamine (SP) might improve haemoglobin concentrations while allowing children to develop protective immunity against severe disease and subsequent death caused by malaria.

While methods are urgently needed to facilitate diagnosis of anaemia and malaria at primary care level, determination of iron status is complicated in children with infections.

With the aim to contribute to the development of programmes for anaemia control in preschool children in Africa, the immediate objectives were as follows:

1. to measure the efficacy in improving haemoglobin concentrations in children aged 2-36 mo of intermittent iron supplementation and intermittent administration of SP, either alone or when given in combination;
2. to develop and evaluate survey methods for rapid assessment at community level of the burden of anaemia and its risk factors;
3. to contribute to improved methods for diagnosis of anaemia, iron deficiency, and malaria
4. to evaluate the role of impaired erythropoiesis in the pathogenesis of anaemia associated with asymptomatic malaria.

Chapter 1 examines published evidence of iron deficiency and malaria as determinants of anaemia and growth faltering in African children. For this purpose, the following topics were reviewed: the public health importance of growth faltering, anaemia, iron deficiency and malaria; the causal relationships between these factors, especially in relation to the aetiology and pathogenesis of anaemia; and the possibilities for interventions directed at solving the anaemia problem.

Chapter 2 describes the results of a cluster sample survey was carried among asymptomatic children aged 2-36 mo in the study area (n=318). Although such surveys were originally designed to provide valid estimates of descriptive statistics, methods are described to expand their use to assess associations between exposures and health outcomes at a single point in time, also by regression modelling.

Using data from this survey, it was estimated (**chapter 3**) that the prevalences of malaria, anaemia (haemoglobin concentration<110 g/L), iron deficiency (serum ferritin concentration<12 mg/L) and iron deficiency anaemia were 18%, 69%, 53% and 46%, respectively. Malaria was associated with lower mean haemoglobin concentrations (92.7

compared with 104.1 g/L; difference: 11.3 g/L, 95% confidence interval, CI: 6.4-16.3 g/L). Analysis of serum transferrin receptor concentrations and haemoglobin concentrations yielded findings consistent with the notion that malaria-induced haemolysis is accompanied by increased erythropoiesis. Serum transferrin receptor concentration is not useful to detect iron deficiency in individuals with malaria. Individuals with high concentrations of serum C-reactive protein or similar acute phase reactants should be excluded if serum ferritin concentrations<12 mg/L are to be used for measuring the prevalence of iron deficiency in malaria-endemic areas.

Data from the same study were used to examine whether they provided observational support for the hypothesis that stunting modifies the associations between malaria and haematological indicators of iron status and inflammation (**chapter 4**). When adjusted for age and wasting, the malaria-associated decrease in mean haemoglobin concentration was 8.5 g/L and 15.8 g/L in non-stunted and stunted children, respectively (p-value of test for difference: 0.08). It was also found that children with malaria and who were stunted had higher serum concentrations of C-reactive protein and soluble transferrin receptor than would be expected from the combined effect of the two working independently. These results are consistent with the notion that the nutritional inadequacies causing stunting also impair host immunity, thus increasing the degree to which malaria is associated with decreased concentrations of haemoglobin, with increased inflammation, and with increased iron demand in developing erythroblasts. This would suggest that increased intake of micronutrients might not only reduce stunting and nutritional anaemia, but also reduce malaria-associated anaemia.

In addition, the survey data were used to study antimalarial drug use, and to assess the validity of frequency of febrile episodes and drug use reported by mothers or carers (**chapter 10**). Of the children whose blood was studied, 38% (95% CI: 30-47%) tested positive for chloroquine or sulfadoxine. Of chloroquine-positive children, 15% had concentrations exceeding the estimated minimum therapeutically effective values. Among those testing negative for sulfadoxine, chloroquine-positive children were more frequently parasitaemic, and had lower mean haemoglobin concentrations, than chloroquine-negative children. Mothers probably overreported the frequency of malaria or fever episodes as usually defined in medical studies, and underreported antimalarial drug use. It was concluded that antimalarials are frequently given for treatment of malaria or malaria-associated illness, rather than prophylactically or for symptoms unrelated to malaria. Questionnaire surveys cannot replace biochemical markers to obtain information on antimalarial drug use.

The second field study concerned a trial with a 2×2 factorial design in which 328 asymptomatic children (aged 2-36 mo; haemoglobin concentration 60-110 g/L) were randomly assigned tot receive either iron or placebo, and SP or placebo (**chapter 5**). The health surveillance of the children in the study was intense. The effect on change in haemoglobin concentration in the group receiving iron plus SP relative to the placebo group, adjusted for prognostic factors at baseline, was 12.5 g/L (95% CI: 8.5 to 16.4 g/L). In the former group, the estimated prevalence of anaemia reduced from 100% at baseline to 36% at 12 w, and prevalence of iron deficiency reduced correspondingly from 66% to 8%. Administration of SP in addition to iron supplementation gave no haemoglobin response. Survival analysis indicated no evidence of increased risk of malaria following iron supplementation. These findings indicate that iron supplementation over a 12-week period results in a marked improvement of haemoglobin concentrations. Further studies should determine the possible benefits of intermittent administration of SP in reducing the

incidence of symptomatic malaria and associated severe anaemia in areas of seasonal malaria transmission.

Malarial anaemia is associated with a shift in iron distribution from functional to storage compartments. This suggests a relative deficit in erythropoietin production or action similar to that observed in other infections. Longitudinal and baseline data were used from the trial to investigate whether malaria causes increased erythropoiesis in children with asymptomatic malaria, and whether the erythropoietic response appeared appropriate for the degree of resulting anaemia (**chapter 6**). Erythropoiesis was evaluated by serum concentrations of erythropoietin and soluble transferrin receptor. The findings indicated that the erythropoietic response to asymptomatic malaria is adequate for the resulting degree of anaemia. Inflammation probably plays no or a minor role in the pathogenesis of anaemia associated with asymptomatic malaria. Further research is needed to demonstrate the role of deficient erythropoietin production or action in the pathogenesis of the anaemia of acute malaria.

Data collected during the trial also allowed an assessment of the utility of a new and inexpensive colour scale to facilitate anaemia diagnosis in developing countries (**chapter 7**). Scores of the colour scale ≤10 indicated anaemia (haemoglobin concentration <110 g/L) and severe anaemia (haemoglobin concentration <70 g/L) with sensitivities >0.68 and >0.99, respectively. When used in populations with a heavy burden of anaemia, sensitivity estimates were higher, and the scale could provide valid estimates of the prevalence of anaemia. It was concluded that the colour scale is satisfactory and for many purposes superior to all other methods for detection of anaemia at primary care level. Low scores of the scale may give unsatisfactory results when used to detect patients with severe anaemia and to decide on their management. The methodology presented may facilitate development of strategies for its use in various target groups.

Lastly, data collected during both the survey and the trial were used to examine the diagnostic utility of maternal reports of fever in children (**chapters 8-10**). Mothers were questioned whether their child had fever after they had palpated the child's forehead. Their reports were compared with axillary temperatures as recorded by electronic thermometer. The findings suggest that mothers can accurately diagnose the absence of fever without a thermometer. The use of reported fever alone will necessarily result in an unacceptably high proportion of false positives, especially at peripheral levels of health care. A large proportion of unnecessary treatments for malaria can be avoided by confirming the presence of fever by thermometer (**chapter 8**).

In a subsequent study, mothers were questioned whether or not their child had fever, either directly or after they had palpated the child's forehead (**chapter 9**). Either of these two methods of assessment was randomly allocated. Palpation had similar sensitivity (>90%) but better specificity in sick children (50% versus 34%; p=0.05). Fever was more prevalent in children reported sick to a health facility than at community level, where early detection takes place. Mothers' reports of fever are therefore more reliable in sick children. It was shown that, regardless of actual body temperature, mothers are more likely to report fever in sick children. This corroborates earlier findings that mothers probably overreport the frequency of malaria or fever episodes (**chapter 10**; see above). Teaching mothers to palpate their child's forehead cannot improve the sensitivity of detecting fever in sick children, but is likely to increase specificity, thus avoiding unnecessary treatment for febrile diseases.

Finally, the research produced as a spin-off two published letters that are included as annexes to this thesis. The first letter was written in response to an earlier published report that phenobarbital prophylaxis in children with cerebral malaria leads to an increased risk of death. The analysis in this report suggested that the effect of phenobarbital on mortality is, at least in part, a result of diazepam being administered before or after being given phenobarbital. It was shown that stratification for diazepam use, which mediates the effect of phenobarbital on mortality, leads to severely biased effect estimates on death and possibly on seizure frequency (**annex 1**). Subsequent reanalysis by the authors of their data confirmed the occurrence of this bias, but did not result in different conclusions from those originally reported.

The second letter was in response to an earlier published report that HIV-1 infection is associated with an increased frequency of symptomatic malaria and parasitaemia. The findings reported can be interpreted as evidence that the relationship between HIV-1 and immunosuppression, as indicated by nearest and previous CD4-cell counts, is modified by malaria. Thus the data would suggest that HIV-1 infection, in addition to leading to decreased CD4-cell counts, also determines the activity of immune cells in limiting malaria parasitaemia (**annex 2**). As surmised from the authors' reply, the interpretation of these data remains inconclusive.

Samenvatting (summary in Dutch)

Ongeveer driekwart van de kinderen jonger dan 5 jaar in oost Afrika lijdt aan bloedarmoede, hetgeen meestal duidt op malaria of andere onderliggende infecties, gebrek aan ijzer en andere voedingsstoffen, of genetische afwijkingen. Deze factoren kunnen ernstige schade toebrengen aan de gezondheid door verlaagde lichamelijke activiteit, groeiachterstand en geremde geestelijke ontwikkeling, en sterfte. Succesvolle interventies ter bestrijding van bloedarmoede worden daarom geacht de gezondheid te bevorderen.

De werkzaamheid van ijzersuppletie hangt af van de prevalentie van kinderen met ijzergebrek, en het tekort aan functioneel ijzer in die kinderen. In gebieden met seizoensgebonden malaria kan ijzersuppletie van kinderen tot een verhoogd risico van malaria leiden, zodat de baten mogelijk niet opwegen tegen de nadelen. Door regelmatig (elke 4 weken) toedienen van sulfadoxine-pyrimethamine (SP) kan mogelijk de hemoglobine-concentraties worden verhoogd, terwijl kinderen in staat blijven om immuniteit op te bouwen tegen ernstige malaria.

Er is een dringende behoefte aan betere methoden ter diagnose van bloedarmoede en malaria op het niveau van de eerstelijnsgezondheidszorg, en ook is bepaling van ijzerstatus moeilijk in kinderen met infecties.

Het onderzoek dat wordt beschreven in dit proefschrift was erop gericht een bijdrage te leveren aan de ontwikkeling van programma's ter bestrijding van bloedarmoede onder jonge kinderen. De doelstellingen waren als volgt:

1. het meten van de werkzaamheid ijzersupplementen en SP die met tussenpozen werden toegediend – zowel afzonderlijk als in combinatie – ter verbetering van hemoglobine-concentraties in kinderen in de leeftijd tussen 2 en 36 maanden;
2. het ontwikkelen en evalueren van steekproefmethoden waarmee op dorpsniveau het probleem en de determinanten van bloedarmoede snel kunnen worden bepaald;
3. het bijdragen aan verbeterde methoden ter diagnose van bloedarmoede, ijzergebrek, en malaria;
4. het onderzoeken in hoeverre onderdrukte vorming van rode bloedcellen een rol speelt in het ontstaan van bloedarmoede als gevolg van asymptomatische malaria.

Hoofdstuk 1 onderzoekt de gegevens die zijn gepubliceerd over ijzergebrek en malaria als determinanten van bloedarmoede en groeiachterstand in Afrikaanse kinderen. Voor dit doel werden de volgende onderwerpen besproken: het belang voor de volksgezondheid van groeiachterstand, bloedarmoede, ijzergebrek, en malaria; de oorzakelijke verbanden tussen deze factoren, vooral met betrekking tot de oorzaken van bloedarmoede; en de mogelijkheden ter bestrijding van bloedarmoede.

Hoofdstuk 2 beschrijft de resultaten van een clustersteekproefonderzoek onder kinderen in de leeftijd van 2-36 maanden in het studiegebied (n=318). Zulke onderzoeken zijn oorspronkelijk ontworpen om geldige schattingen te verkrijgen van beschrijvende statistische gegevens. In dit hoofdstuk worden methoden beschreven waarmee zulke studies bovendien kunnen worden gebruikt om de verbanden te bepalen tussen

blootstellingen en gevolgen voor de gezondheid op een bepaald tijdspunt. Hierbij kunnen ook regressiemodellen worden gebruikt.

Met behulp van data van dit steekproefonderzoek (**hoofdstuk 3**) werden de prevalenties van malaria, bloedarmoede (hemoglobine-concentratie <110 g/L), ijzergebrek (serum-concentratie van ferritine <12 µg/L) en ijzergebreksbloedarmoede geschat op respectievelijk 18%, 69%, 53%, en 46%. Malaria was geassocieerd met lagere hemoglobine-concentraties (gemiddelde waarden 92.7 vergeleken met 104.1 g/L; verschil 11.3 g/L, 95%-betrouwbaarheidsinterval, BI: 6.4-16.3 g/L). Analyse van de serumconcentratie van transferrine-receptoren en hemoglobine-concentratie gaven resultaten die in overeen-stemming waren met de idee dat door malaria geïnduceerde hemolyse gepaard gaat met een verhoogde erytropoiese. Serumconcentratie van transferrine-receptoren zijn niet bruikbaar om ijzergebrek aan te tonen in mensen met malaria. In gebieden waar malaria endemisch voorkomt, moeten mensen met hoge serumconcentraties van C-reactive protein of vergelijkbare acute fase-eiwitten worden uitgesloten als het criterium van serumconcentratie van ferritine <12 µg/L wordt gehanteerd om de prevalentie van ijzergebrek te meten.

Gegevens van dezelfde studie werden gebruikt om te onderzoeken of ze observationele steun boden voor de hypothese dat achterstand in lengtegroei de associaties tussen malaria en bloedindicatoren van ijzerstatus en ontsteking beïnvloedt (**hoofdstuk 4**). Wanneer leeftijd en vermagering in aanmerking werden genomen, was malaria geassocieerd met een verlaging van de gemiddelde hemoglobine-concentratie van respectievelijk 8.5 g/L en 15.8 g/L voor kinderen zonder en met achterstand in lengtegroei (p-waarde voor test voor verschil: 0.08). Kinderen die malaria hadden maar ook waren achtergebleven in lengtegroei hadden hogere serumconcentraties van C-reactive protein en transferrine-receptoren dan verwacht op grond van het gecombineerde effect van deze factoren afzonderlijk. Deze resultaten zijn in overeenstemming met de idee dat de voedingsgebreken die achterstand in lengtegroei veroorzaken ook de immuniteit van de kinderen ondermijnen. Deze verminderde immuniteit zou ertoe leiden dat malaria in deze kinderen in sterkere mate is geassocieerd met verlaagde hemoglobine-concentraties, met verhoogde ontsteking, en met verhoogde ijzerbehoefte van erytroblasten. Dit zou erop wijzen dat een verhoogde inname van micronutrienten niet alleen kan leiden tot een verminderde achterstand van de lengtegroei en voedingsgerelateerde bloedarmoede, maar ook tot een vermindering van malaria-geassocieerde bloedarmoede.

Het steekproefonderzoek werd bovendien gebruikt om het gebruik van antimalariamiddelen te bestuderen, evenals de validiteit van het vóórkomen van koortsincidenten en medicijnengebruik zoals gerapporteerd door moeders of zorggevers (**hoofdstuk 10**). Van de kinderen wiens bloed werd getest, was 38% (95% BI: 30-47%) positief voor chloroquine of sulfadoxine. Van de kinderen die positief testten voor chloroquine, had 15% bloedconcentraties die de minimum therapeutisch werkzame waarden overschreden. Kinderen met chloroquine in hun bloed waren vaker geïnfecteerd dan kinderen zonder chloroquine in hun bloed, en ook hadden ze lagere gemiddelde hemoglobine-concentraties (bij deze analyse weren diegenen die positief testten voor sulfadoxine uitgesloten). Het is waarschijnlijk dat moeders overschatten hoe vaak malaria of koortsincidenten voorkwamen, althans deze gewoonlijk in biomedische studies worden gedefinieerd, en dat ze het gebruik van geneesmiddelen tegen malaria onder-rapporteerden. De conclusie was dat deze geneesmiddelen veelal worden gegeven tegen malaria of malaria-gerelateerde klachten, in plaats van profylactisch of tegen klachten die niet gerelateerd zijn aan malaria. Steekproefonderzoek gebaseerd op vragenlijsten kunnen

niet gebruikt worden ter vervanging van biochemische metingen om informatie te verkrijgen over het gebruik van geneesmiddelen tegen malaria.

De tweede veldstudie betrof een experiment met een 2x2 factoriële proefopzet waarin 328 kinderen zonder gezondheidsklachten (leeftijd 2-36 maanden; hemoglobine-concentratie 60-110 g/L) random werden toegewezen aan behandeling met ijzer of ijzerplacebo, en SP of SP-placebo (**hoofdstuk 5**). De medische begeleiding van deze kinderen was intens. Het effect op de verandering in hemoglobine-concentratie in de groep die ijzer plus SP ontving ten opzichte van de placebo-groep, gecorrigeerd voor prognostische factoren bij aanvang van de studie, was 12.5 g/L (95% BI: 8.5-16.4 g/L). In de eerstgenoemde groep werd de prevalentie van bloedarmoede teruggebracht van 100% bij aanvang van de studie tot 36% na 12 weken, en de prevalentie van ijzergebrek werd overeenkomstig teruggebracht van 66% tot 8%. Het toevoegen van SP aan deze behandeling bracht geen verandering teweeg in hemoglobine-concentratie. Er was geen aanwijzing dat het risico van malaria was verhoogd als gevolg van de ijzersuppletie. Deze bevindingen duiden erop dat ijzersuppletie over een periode van 12 weken resulteert in een duidelijke verhoging van hemoglobine-concentraties. Verdere studie is nodig naar de mogelijke baten van het regelmatig toedienen van SP in gebieden waar de overdracht van malaria seizoensgebonden is. Met name dient te worden onderzocht of zo'n toediening de incidenties van symptomatische malaria en ernstige bloedarmoede verlaagt.

Malaria-geassocieerde bloedarmoede gaat gepaard met een verschuiving van ijzerverdeling over het lichaam van functionele compartementen naar opslagcompartimenten. Dit wijst op een ontoereikende productie of werking van erytropoietine, vergelijkbaar met wat is waargenomen in andere infecties. Waarnemingen in de tijd of bij aanvang van de experimentele studie werden gebruikt om te onderzoeken of malaria leidt tot een verhoogde aanmaak van rode bloedcellen in kinderen met asymptomatische malaria, en of deze verhoogde aanmaak in verhouding was tot de ernst van de bloedarmoede (**hoofdstuk 6**). De aanmaak van rode bloedcellen werd hierbij beoordeeld met behulp van de serumconcentraties van erytropoietine en transferrine-receptoren. Het bleek dat bij malaria de erytropoiese passend was in verhouding tot de mate van bloedarmoede. Ontsteking speelt waarschijnlijk geen of hooguit een ondergeschikte rol in het ontstaan van bloedarmoede in asymptomatische malaria. Verder onderzoek is nodig om de rol aan te tonen van gebrek in de aanmaak of werking van erytropoietine in de ontwikkeling van bloedarmoede in acute, symptomatische malaria.

De gegevens die tijdens het experiment werden verzameld, maakten het ook mogelijk om de diagnostische waarde vast te stellen van een nieuwe en goedkope kleurenschaal ter bepaling van bloedarmoede in ontwikkelingslanden (**hoofdstuk 7**). Waarden ≤10 van deze schaal hadden sensitiviteiten van >0.68 en >0.99 voor het aantonen van respectievelijk bloedarmoede (hemoglobine-concentratie <110 g/L) en ernstige bloedarmoede (hemoglobine-concentratie <70 g/L). Bij gebruik van de schaal in populaties waarin bloedarmoede veelvuldig voorkomt, waren deze waarden hoger, en werden zuivere schattingen verkregen van de prevalentie van bloedarmoede. De conclusie was dat de kleurenschaal bevredigend werkte, en voor menig doeleinde beter was dan alle andere beschikbare methoden voor het aantonen van bloedarmoede op het niveau van eerstelijnsgezondheidszorg. Lage waarden van de schaal kunnen ontoereikende resultaten geven om ernstige bloedarmoede vast te stellen, en om te beslissen over de verdere behandeling van zulke patiënten. De aangevoerde methodiek kan worden gebruikt ter ontwikkeling van strategieën voor gebruik van de schaal in verschillende doelgroepen.

Zowel tijdens het steekproefonderzoek als het experimentele onderzoek werden gegevens verzameld die gebruikt werden om de diagnostische waarde van koortsmeldingen door moeders te bepalen (**hoofstukken 8-10**). De moeders werd gevraagd of hun kind koorts had nadat ze eerst het voorhoofd van hun kind hadden gevoeld. Hun meldingen werden vergeleken met de okseltemperaturen die werden geregistreerd met een elektronische thermometer. Het bleek dat moeders de afwezigheid van koorts betrouwbaar kunnen vaststellen zonder thermometer. Als koorts slechts wordt vastgesteld op basis van de melding daarvan door de moeder, dan zal dat – mede gezien de relatief lage prevalentie – onvermijdelijk leiden tot een onaanvaardbaar hoog percentage van fout-positieve uitkomsten, vooral op het niveau van de eerstelijnsgezondheidszorg. Een groot aantal onnodige malariabehandelingen kan worden vermeden door het bevestigen van de aanwezigheid van koorts met een thermometer (**hoofdstuk 8**).

In een vervolgstudie werd moeders gevraagd of hun kind koorts had, ofwel direct, ofwel nadat ze eerst het voorhoofd van hun kind hadden gevoeld (**hoofdstuk 9**). Eén van deze twee methoden werd telkens random toegewezen. Voelen had een vergelijkbare sensitiviteit (>90%) maar een hogere specificiteit in zieke kinderen (50% vergeleken met 34%; p=0.05). Koorts kwam vaker voor in kinderen die ziek werden gemeld bij een gezondheidsfaciliteit dan op dorpsniveau, waar de vroegtijdige diagnose veelal plaatsvindt. De melding van koorts door moeders zijn daarom meer betrouwbaar in zieke kinderen. Het bleek dat – onafhankelijk van de feitelijke lichaamstemperatuur – de kans dat moeders koorts meldden groter was als hun kind ziek was. Dit komt overeen met eerdere bevindingen dat moeders waarschijnlijk het vóórkomen van malaria- of koortsincidenten overrapporteren (**hoofdstuk 10**; zie vorige paragraaf). Het leren van moeders zal niet leiden tot een verhoogde sensitiviteit van het vaststellen van koorts in zieke kinderen, maar het verhoogt waarschijnlijk de specificiteit, zodat daarmee onnodig behandelen van koorts wordt voorkomen.

Het onderzoek leverde tenslotte als nevenresultaat twee gepubliceerde brieven op die ingesloten zijn als bijlagen in dit proefschrift. De eerste brief was een reactie op een eerder gepubliceerd verslag dat profylactisch gebruik van fenobarbital in kinderen met cerebrale malaria leidt tot een verhoogd sterfterisico. De analyse die in dit verslag wordt gepresenteerd, duidde erop dat het effect van fenobarbital op sterfte, althans minstens gedeeltelijk, wordt veroorzaakt doordat diazepam werd toegediend voordat of nadat fenobarbital werd gegeven. Het bleek echter dat stratificatie voor het gebruik van diazepam – hetgeen in de causale keten van het effect van fenobarbital op sterfte staat – tot ernstige bias kan leiden in de geschatte effecten op sterfte en mogelijk op het vóórkomen van stuipaanvallen (**bijlage 1**). Een hernieuwde analyse van de gegevens die daarop volgde door de oorspronkelijke auteurs bevestigde het bestaan van deze bias, maar leidde niet tot andere conclusies dan die oorspronkelijk werden gerapporteerd.

De tweede brief was een reactie op een eerder gepubliceerd verslag dat infectie met HIV-1 is geassocieerd met een verhoogde frequentie van symptomatische malaria en van de aanwezigheid van parasieten in het bloed. De gerapporteerde bevindingen kunnen worden geïnterpreteerd als aanduidingen dat de relatie tussen HIV-1 en onderdrukking van de immuniteit, zoals geduid door de meest recente tellingen van CD4-cellen, wordt bepaald door malaria. Deze gegevens zouden zodoende erop wijzen dat infectie met HIV-1, behalve dat het leidt tot verlaagde aantallen van CD4-cellen, ook bepaalt in hoeverre de immuuncellen in staat zijn om de aantallen parasieten in het bloed te verlagen (**bijlage 2**). Zoals kan worden afgeleid van het antwoord van de oorspronkelijke auteurs blijft de interpretatie van deze gegevens onbeslist.

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About the author...

This thesis reflects my scientific interest in the epidemiology of micronutrient deficiencies and malaria. Related areas of interest include the diagnosis and treatment of malaria; the effects of agriculture, land and water use on infectious diseases and nutrition; primary health care in developing countries; and the biology and control of insect vectors of human disease.

Apart from supervising students, I take great pleasure in teaching people from developing countries, particularly at primary care level. A manual that I developed for teaching malaria diagnosis and treatment to community health workers was published in edited form by the World Health Organization. I organized and taught at various international workshops and courses.

I was trained as a clinical epidemiologist at the Netherlands Institute for Health Sciences/Erasmus University Rotterdam, as a medical entomologist at Wageningen University, The Netherlands, and took part in the Graduate Programme in Human Nutrition of the Graduate School on Advanced Studies in Food Technology, Nutrition and Health Sciences, Wageningen University. In addition, I took courses at London School of Hygiene and Tropical Medicine and Utrecht University, The Netherlands.

In my previous work, I was assigned by the World Health Organization to provide technical and training support for the control of malaria in the Solomon Islands, and to the WHO/FAO/UNEP/UNCHS Panel of Experts on Environmental Management for Vector Control in Geneva, Switzerland. In the latter capacity, I had the fortune of undertaking missions to Africa (Benin, Burkina Faso, Côte d'Ivoire, Egypt, Ghana, Kenya, Mali, Niger, The Gambia, Zimbabwe) and Asia (India, Malaysia, Thailand).

As a consultant, I worked for the World Health Organization, the World Bank, the Governments of Canada and the Philippines, the World Conservation Union (IUCN), the West Africa Rice Development Association, and the Research Centre for International Agrarian and Economic Development (Germany). In this capacity, I undertook missions on health issues in the management of the Senegal River and Sahelian floodplains and wetlands (Senegal and Niger), the use of DDT for vector control (Canada), the health effects of irrigation (Madagascar, Mali, Côte d'Ivoire), and resettlement and development after the Onchocerciasis Control Programme for West Africa (France).

At present, I am working as a post-doc fellow on the development of a research programme on agriculture- and food-based approaches to alleviate micronutrient deficiencies in developing countries. In due time, I expect to return to the tropics for field research on malaria and related issues.

Front cover: Children awaiting medical examination in the research project described in this thesis (Kathekani, Kenya, 1998).

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