DR BEHZAD NADJM

TOWARDS AN EVIDENCE BASE IN THE TREATMENT OF SEVERE FEBRILE ILLNESS IN EAST AFRICAN CHILDREN

College of Medicine & Veterinary Medicine, Edinburgh University

MD Thesis 2009

ABSTRACT

Febrile illness is the primary cause of childhood outpatient attendance, admission to hospital and death in Africa. This series of studies were aimed at ascertaining the treatable causes of infection in children admitted to a district hospital typical of those found throughout East Africa, in an area of high transmission of malaria. The studies were also designed to determine the clinical correlates of infection and predictors of mortality, looking in particular at malaria, invasive bacterial disease and HIV infection. These studies also explored to what extent clinical examination by one group of staff was replicable by another.

After informed consent a detailed history and structured examination was performed on all children admitted to the hospital. Blood was drawn for culture, microscopy for malaria, HIV testing, full blood count, bedside haemoglobin, blood glucose and lactate measurement and HRP-2 based rapid diagnostic test for falciparum malaria. Outcomes were recorded at death or discharge.

Sufficient data was available on 3,639 children including 184 deaths (5.1%). Invasive bacterial disease was detected in 341 children (9.4%) and HIV in 142 (3.9%). Children with HIV and those with evidence of recent malaria were significantly more likely to have invasive bacterial disease. The most common organisms isolated were non-typhi *Salmonella* (46.9%), *Strep. pneumoniae* (16.4%) and *Haemophilus influenzae b* (11.4%). The most frequently encountered pathogen was *P. falciparum*, with 2,195 children found to have asexual parasitaemia (60.3%). Falciparum parasitaemia was detected in 100 children with invasive bacterial disease

(29.3%). Falciparum malaria was detected in over half (51.6%) of childhood deaths, invasive bacterial disease was documented in 31.5%.

In children with a positive blood slide for malaria, WHO severe malaria criteria identified 91.6% of the children that died. A multivariate analysis showed that signs of malnutrition, respiratory distress, altered consciousness, hypoxia according to pulse oximetry, hypoglycaemia, raised blood lactate, invasive bacterial disease and female sex were all associated with an increased risk of death. In children with negative blood slides signs of malnutrition, respiratory distress, altered consciousness, hypoglycaemia, raised blood lactate and invasive bacterial disease were all independently associated with mortality by multivariate analysis.

WHO defined criteria of syndromes which would warrant antibiotics predicted 56% of cases of coinfection with invasive bacterial disease and malaria and 69.7% of cases of invasive bacterial disease in slide negative children. Treating all children with severe malaria for bacterial disease would result in 71% of children with coinfection being treated. In children with negative slides including severe anaemia or prostration as syndromes requiring antibiotic therapy would have resulted in 74.7% of children with invasive bacterial disease receiving antibiotic therapy. There was moderate agreement between staff over the presence of clinical signs in children, with hospital nurses performing as well as hospital clinical officers. Agreement was better in children over 18 months of age and in children who were not crying during examination.

Current WHO guidelines on antibiotic use performed poorly in this setting. Gram negative infections were the most common cause of invasive infection and many of

these are likely to be resistant to penicillin and other commonly used antibiotics. Consideration should be given to expanding the indications for antibiotic use and using more broad-spectrum antibiotics in severely ill children.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

STATEMENT OF INVOLVEMENT & ACKNOWLEDGEMENT

Unless stated below all the work submitted has been my own. I was involved in study design and was responsible for writing all standard operating procedures (SOPs). I was also responsible for the recruitment, training, supervision and day to day management of the clinical research staff, data collection and the logistics of the study. I supervised onsite, daily, the collection of samples and all the study data. I was involved in cleaning the data, analysed the data myself and have personally written this manuscript. Where data have been published (Section 4.5) or submitted for publication (Section 4.4) I was first author and responsible for the first & final drafts of the manuscript. Appendix 6 was added at the request of the examiners. It is based on analysis used in a subsequent publication and as such it represents the work of all the authors, with contributions as suggested by reviewers of the paper. I was involved with drawing up SOPs for lab work however the laboratory work itself was overseen by Dr Ben Amos and samples processed by his team as described below.

The study was initially conceived and a first protocol devised with Prof Chris Whitty and Dr Hugh Reyburn at the London School of Hygiene & Tropical Medicine. Clearly a study as large as this required a large number of additional staff I should like to acknowledge. In the assessment unit clinical data was collected by Walii Msuya, Edward Mtili, Christina Kiemi, Emmanwel Swai (Clinical Officers) and Hannah Mwangai, Rosalia Marwa, Simphorosa Silaye and Stella Emmanuel (Nurses). These staff were aided by two assistants Samwel Michael and Moses Nyangweso. A further five lab technicians were involved in the analysis of laboratory samples, supervised by Dr Ben Amos. I was joined later in the study by Dr George Mtove who assisted in the supervision of the more junior staff under my direction. The administration of the study was greatly assisted by Lina Alex and others in the local office of the Joint Malaria Programme, an office that I helped establish. Without the involvement of these staff the study would not have been possible and both the quality of data and the clinical care received by the study patients would have been the poorer. I am eternally grateful for their commitment and hard work.

I am hugely grateful to Dr Hugh Reyburn who, based in Tanzania, provided support and supervision throughout the project, has assisted me in understanding statistical analysis and has helped me writing scientific papers that have arisen from this work. I am also enormously grateful to Prof Chris Whitty who has provided support throughout the project in Tanzania and London, assisted me in statistics and agreed to be my London supervisor, providing me with important suggestions and critique of this manuscript. I would also like to thank Prof. David Taylor for agreeing to be my supervisor in Edinburgh, Sally Clarke for her support through some difficult times during the study period, Clare Chandler for her unending helpful responses to my enquiries and my mother for her help in proof reading this document.

This work has not been submitted for any other degree or professional qualification.

Signed _____ Dr B Nadjm Date 18/5/2011

Hannah Mwangai, Rosalia Marwa, Simphorosa Silaye and Stella Emmanuel (Nurses). These staff were aided by two assistants Samwel Michael and Moses Nyangweso. There were a further five lab technicians involved in the analysis of laboratory samples, supervised by Dr Ben Amos. I was joined later in the study by Dr George Mtove who assisted in the supervision of the more junior staff under my direction. The administration of the study was greatly assisted by Lina Alex and others in the local office of the Joint Malaria Programme, an office that I helped establish. Without the involvement of these staff the study would not have been possible and both the quality of data and the clinical care received by the study patients would have been the poorer. I am eternally grateful for their commitment and hard work.

I am hugely grateful to Dr Hugh Reyburn who, based in Tanzania, provided support and supervision throughout the project, has assisted me in understanding statistical analysis and has helped me writing scientific papers that have arisen from this work. I am also enormously grateful to Prof Chris Whitty who has provided support throughout the project in Tanzania and London, assisted me in statistics and agreed to be my London supervisor, providing me with important suggestions and critique of this manuscript. I would also like to thank Prof. David Taylor for agreeing to be my supervisor in Edinburgh, Sally Clarke for her support through some difficult times during the study period, Clare Chandler for her unending helpful responses to my enquiries and my mother for her help in proof reading this document.

FUNDING

My involvement in these studies was made possible through grants from The Berkley Fellowship, The Sir Halley Stewart Trust and Pfizer Pharmaceuticals. Core funding for the study was obtained from the European Commission (Europaid grant code: SANTE/2004/078-607). I would like to thank all of the funding bodies involved in the study who have contributed not just to this academic work but also to the dramatic and ongoing improvements in clinical care for children at Teule Hospital, Tanzania.

Signed _ Date 12/5/2011

1 INTRODUCTION & OVERVIEW

The majority of childhood deaths globally are due to infectious causes; although data are poor,[2-3] in Africa up to 90% of deaths outside the neonatal period are estimated to be due to infection[3-17] and over a third of deaths in under 5s may be avoided by improving the treatment of childhood infections.[18] Progress towards the 4th Millennium Development Goal of a two-thirds reduction in childhood mortality has been slow and a clear focus on infection is needed if it is to be met.[19-20] Eastern Africa is estimated to contribute 30% of Africa's 5 million deaths in children under 5[21], so gains here will be important and knowledge from this region may be applicable elsewhere.

Febrile illness is the commonest cause of childhood outpatient attendance and admission to hospital in Africa.[22-27] Considerable effort has rightly gone into the community management of febrile disease (focusing largely on malaria), but less attention has been paid to investigating ways of improving hospital care.[28-32] Snow *et al.*, in a study from the Kenyan coast, estimated that childhood mortality had been reduced by 44% as a result of inpatient care in a rural community served by a district hospital.[33] The advantage of such an approach is that it can be tailored to the local health needs. It is likely that as the campaign to eradicate malaria gathers pace there will be areas where there is a reduction in episodes of clinical malaria, through the increased use of insecticide treated bed nets, residual spraying and other interventions.[7 34-37] In these and other areas where malaria is less common other infections can be prioritised.[38] Additionally, a large proportion of children are still taken first to traditional healers or given medicines bought over the counter when

they become sick.[39-45] By sidestepping the medical system these children have no chance of receiving diagnostic tests or effective triage and may be at increased risk of being prescribed inappropriate and substandard (or even counterfeit) drugs.[46-51] Improving the standard of hospital care can only improve the treatment seeking behavior of patients and caregivers; if we wish parents to access care and follow referral advice it is essential that the care provided to children be of a higher calibre.

The Integrated Management of Childhood Infections (IMCI) strategy was set up by WHO and UNICEF to improve children's health and development.[52] The first phase of IMCI addressed the management of children in the community, at health clinics and dispensaries (analogous to primary care). Here it has shown improvements in health worker performance and mortality in most areas,[52-58] though its impact on mortality has not been universal with a recent study from Bangladesh showing no significant mortality benefit.[59] There has been some progress in the design of appropriate interventions for inpatient care. A study from Malawi showed that the introduction of ETAT (emergency triage assessment and treatment)[60] reduced inpatient mortality from 10-18% to 6-8%[61]. In 2000 the IMCI strategy was extended to cover children at the first level of referral (district hospital admissions) with the development of the "referral care manual".[62] In 2005 this was adopted as policy in Tanzania. A study performed over the previous year demonstrated the need for upgrading hospital care, with a high number of missed diagnoses and inappropriate treatments across a range of clinical syndromes; for example malnutrition was diagnosed in only 1 in 5 to 10 of those suffering from it and malaria misdiagnosed in up to 60%.[31]

Improving paediatric hospital care is clearly a complex intervention, with problems in infrastructure, supplies and human resources all needing to be addressed. [28] It is also clear that more research data, and in particular data at a local level, are key to ensuring that guidelines are relevant and up to date.[63] Recently there have been suggestions that local or regional development of guidelines may be necessary if we are going to reach WHO child mortality goals.[64]

Several studies from Africa have shown that the majority of inpatient deaths occur in the 48 hours following admission.[65] [66-70] To impact on this early mortality identifying the children at greatest risk and establishing the correct initial management are clearly of high priority. There are several important questions that need to be addressed to assist in this:

- What is the range of treatable pathogens occurring in children admitted to a hospital in an area of high malaria transmission and how often is a mixture of pathogens found (co-infection)?
- Can those children at risk of death be reliably predicted at the bedside?
- Do current WHO guidelines accurately predict invasive bacterial disease and therefore required treatments?
- Are clinical examination findings, necessary to predict the above, reproducible between different observers?

1.1 THE AETIOLOGY OF FEBRILE DISEASE & THE IMPLICATIONS OF INFECTIONS

1.1.1 THE ROLE OF BLOOD CULTURES AND OTHER DIAGNOSTIC TESTS

The major treatable causes of febrile disease in East Arica are malaria (predominantly due to *Plasmodium falciparum*) and invasive bacterial disease. The exclusion of malaria is in principle relatively straightforward and quick using a simple blood smear or rapid diagnostic test (RDT), the intricacies of these will be explored later. Diagnosing invasive bacterial disease is somewhat more difficult. Whilst bacterial disease can manifest itself as a local collection of infection causing abscesses, pneumonia or meningitis, certainty in diagnosis is only provided by growth of a relevant organism in culture. Normally sterile body fluids, such as blood, cerebrospinal fluid (CSF) or urine are ideal as there can be little debate as to the implications of the finding of pathogenic bacteria (in contrast to the upper respiratory tract where even pathogenic organisms may be found as commensals). Serious infections of any body system will often seed bacteria into the blood, or organisms may pass through the blood before seeding to a specific organ.

Thus, for the clinician a blood culture offers a relatively accessible site from which to determine the aetiology of an infectious process. However the number of organisms present in blood is often small leading to potentially low yields. Studies looking at sequential blood cultures taken from adult patients have shown that not only does the yield depend on the volume of blood cultured, but also the number of cultures drawn – only 65-73% of positive cultures were obtained on the first sample.[71-72] This is in contrast with malaria, where it is considered highly unusual for a patient to have significant (life-threatening) clinical disease with a negative blood slide.[73] Whilst

visitors, or residents in areas where exposure to malaria is less frequent, are likely to have febrile disease related to the finding of parasites on a blood slide,[74-75] in highly endemic areas a positive blood slide is often not associated with a febrile illness at all.[74-85] A study of asymptomatic children with positive slides in Kampala, Uganda demonstrated that the risk of developing symptomatic malaria within 30 days in a child with a positive slide was 50%, and approximately half of these were with the same strain.[86-87] Other studies have shown that children may have asymptomatic infections with multiple strains concurrently.[88-92] These data justify the treatment of all cases where parasites are seen, but clearly care must be taken to treat or look for an alternative cause of fever.

Attempts to improve the specificity of malaria microscopy have mainly focused on the concept of 'attributable fractions'.[82 93-94] This is based on comparing the proportion of children with varying degrees of malaria parasitaemia that have fever.[82 93] A similar process can be done on children admitted to hospital with 'severe malaria' comparing the number of children with only malaria found and the number where an alternative cause for their illness was found at different parasite densities.[94] At best such an approach informs the clinician the probability that in the community (or hospital) a child with a given parasitaemia may have a fever (or severe illness) resulting from it. Such calculations require large numbers in order to have any precision. It is well documented that parasitaemia varies over even short times within a given patient.[88 92] It is also known that the parasite density at which fever is likely to occur varies with the many factors that are associated with the acquisition of immunity.[74 80 95-97] In the light of this, faced with a severely ill child, it would seem

imprudent for a clinician to withhold antimalarial therapy on the basis of a malaria parasite density that was 'too low'.

Rapid diagnostic tests for malaria (RDTs) have high sensitivity but can lack specificity in areas where malaria transmission is high.[98-110] Malaria RDTs rely on the detection of malaria specific antigen in blood. They cannot discriminate between asymptomatic malaria infection and parasites causing clinical disease, currently no available test can. Indeed the absence of a parasite count makes them inferior to well performed microscopy in this respect. The most commonly used RDTs are plasmodium specific Lactate Dehydrogenase (pLDH) and Histidine Rich Protein 2 (HRP-2). HRP-2 tests are possibly more heat stable and are being progressively deployed.[111] However HRP-2 appears to remain detectable in the blood for several weeks after a clinical episode of malaria has resolved.[112-115] This leads to a poor specificity for this group of tests in endemic regions where a large portion of children may have had recent infections.[98] In view of these considerations parasite density cutoffs have not been used in this study, or in any of the published literature that I have reviewed.

The majority of studies exploring the comparative roles of malaria and bacterial disease have focused on blood slides and blood cultures, with some inclusion of CSF culture through lumbar puncture. That this is likely to underestimate the importance of bacterial disease is becoming increasingly recognized,[116] as is the role of both co-infection with invasive bacteria and malaria and recent malarial infection.[117-118]

Although there have been several published studies describing the results of blood cultures performed in African children, there have been few systematic studies

looking at malaria, bacterial disease and HIV in children and their clinical contexts. Such data are essential to draft and assess guidelines on the use of antibiotics by demonstrating the pathogens that need to be covered. Previous studies from the literature are described below and have been grouped according to region and summarized in Table 1.

1.1.2 Eastern Africa

The largest recent study on the epidemiology of invasive bacterial disease in African children admitted to hospital involved 19,339 children admitted to Kilifi District Hospital, Kenya over a 4 year period.[119] All children other than those admitted for elective procedures or for observation following trauma were enrolled. The study found a 12.8% rate of community acquired bacteraemia in children in the first 2 months of life, and a 5.9% rate of bacteraemia in children aged 2 months to 13 years. There was a high contamination rate in this study (14.3% of the total) and contaminants were excluded from the analysis (had these been considered negative the overall bacteraemia rate would have fallen from 6.6% to 5.6%). *S. Pneumoniae* was the most commonly isolated organism with non-typhi *Salmonellae* and *Haemophilus influenza* (Hib) the next most common. Gram negative organisms made up 58.3% of the total isolates. The most common organisms isolated from children are shown in Table 1. Most (98.8%) of the children with bacteraemia were tested for HIV (with 17.8% found to be positive), a randomly selected portion (6.7%) of those without bacteraemia, frequency-matched for age were tested for HIV (with 5.5% found to be positive). Although malaria was not studied directly, the authors did note that over the study period 23.3% of deaths were associated with bacteraemia alone, 18.7% with *Plasmodium falciparum* parasitaemia alone and 4.8% with malaria and

bacterial disease. They noted that 20.5% of the 'malaria' deaths also had positive blood cultures (and I note that 20.8% of the bacterial deaths had a positive blood slide for malaria). Another publication from the same team addressed some of these children in rather more detail.[120] This study found that of 11,847 paediatric admissions recruited over 34 months (children included in the previously cited study) 7.1% had an invasive bacterial infection (positive blood or CSF culture) and 44.7% tested positive for malaria, with 1.3% of all admissions testing positive for malaria and bacterial infection (3.0% of those that had a positive malaria test). Another study from the same hospital looking at outpatient visits found that 22 out of 1,093 (2%) randomly selected children under 5 years age had positive blood cultures when they attended the outpatient clinic, 9 of these children were then admitted. [121]Although the numbers were small, *S. pneumoniae* represented half of the isolates in outpatients.

In Malawi 2,123 children aged 1 day to 14 years, with 'clinically suspected bacteraemia', were enrolled in a study that recruited over one year. [122] 17.2% of children were found to have positive blood cultures. Non-typhi Salmonellae were the most common isolates and again Gram negative organisms made up the bulk (73.2%) of organisms isolated. Although the study claims to be the largest study at the time to report bacteraemia in children with *P. falciparum* parasitaemia, the study design involved performing blood cultures in children with malaria only if they failed to respond to anti-malarial therapy. Thus children that died or resolved their infection spontaneously would not have been picked up and some nosocomial infections may have been wrongly included as community acquired infections. The authors reported that 23% of the bacteraemic children had positive slides for malaria. HIV testing was not performed as part of this study.

Also in Malawi, a study that recruited children for whom the admitting physician had prescribed antimalarial, antimicrobial or antituberculous medication found 35 of 229 children recruited had positive blood cultures.[123] Almost all (225/229) children were tested for HIV and 28% were found to be positive. Malaria was found in 13 (5.7%) children, with positive cultures in 2 of these (15.4%). The study took place in the dry season, which may explain the low rate of malaria parasitaemia. Non-typhi salmonella species were again the most common isolate, found in 77% of the children with positive blood cultures.

A study by Blomberg *et al*, conducted in Tanzania at Muhimbili National Hospital in the economic capital Dar es Salaam looked at 1,828 consecutive admissions with fever or hypothermia or other suspicions of systemic infection.[124] Overall the rate of community acquired bacteraemia was 7.7%, though an additional 0.8% of children were found to have candidaemia. These were not considered contaminants by the authors as they were associated with a significantly increased mortality. The most common pathogens isolated here were again Gram negatives but the lack of any *S. pneumoniae* or Hib is unusual. Hib vaccination was not policy in Tanzania at the time of this study. The setting may explain some of the unusual findings; Muhimbili Hospital serves as a national referral center and it is possible that many of the children admitted had been treated at other centers prior to referral or had underlying malignancy or chronic disease. 51% of children were tested for HIV infection, 16.8% of these children were positive. In contrast to the study by Berkley *et al*., this study found no significant difference in the rate of bloodstream infection between HIV positive and negative children. However no details concerning the method of patient recruitment for HIV testing in the study by Blomberg *et al.* are provided and it is

possible that selection bias may have mitigated against any effect. Malaria slides were performed on 90% of admissions and were positive in 21.6% of these children. Looking at the deaths during the study period, 17.7% of deaths had malaria alone, 22.4% had bloodstream infections (including fungi) alone and 6.5% had both. Once again approximately one fifth of deaths occurring in children with bacteraemia had malaria parasites visible on a blood smear.

An older study by Ghiorghis *et al.* in Addis Ababa, Ethiopia showed that 7.7% of children under 14 years age attending the outpatients department without malaria had bacteraemia.[125] The bacteriology reported was limited, with the authors neither discriminating between NTS and *S. typhi* nor between *S. pneumoniae* and other *Streptococci*. Nonetheless Enterobacteriacae made up the majority of isolates. Malaria was excluded in these children and there was no systematic testing for HIV.

1.1.3 WEST AFRICA

A study in rural Gambia, found much lower rates of non-typhi *Salmonella* amongst 1,162 children being investigated for pneumonia, meningitis or septicaemia.[126] This study used lung aspirates and urinary antigen detection (for pneumococcus and Hib) in addition to blood culture. These techniques are likely to have emphasized the importance of these two pathogens, as well as picking up children who would be missed by simple blood culture. The authors found that there were seasonal changes in the aetiology of invasive bacterial disease – with a rise in the isolation of non-typhi Salmonellae and coliforms during the rainy season, also the season with high malaria transmission. Similar seasonality trends in invasive NTS disease have been noted in studies from Eastern Africa. [118 127] The researchers found that 31.3% of the

children had positive tests for malaria, and noted that 19% of the children presenting during the rainy season with Salmonella infections and 29% of those presenting with Hib infections had positive blood slides for malaria, this difference was not statistically significant. However they also noted that leukocytes with malaria pigment were identified in significantly more children with Salmonella bacteraemia (relative risk 4.05, $p<0.005$), possibly indicating a link between recent malaria infection and invasive salmonellosis.

A 15 month study from an urban setting in The Gambia, looking at admissions of all ages in whom the attending clinician felt a blood culture was indicated found that 11.4% of the 686 children aged 2 months to 15 years had positive cultures.[128] As in the previous Gambian study[126] the most common pathogen isolated was *S. Pneumoniae*, with NTS playing a relatively minor role. It is telling to note that whilst in the study by O'Dempsey *et al.*, [126] prior to the introduction of the Hib vaccine to The Gambia, this organism was the second most prevalent isolate, by the time of this study in The Gambia (after the roll out of Hib vaccine) there was only 1 case of invasive Hib reported. HIV testing was not performed in a systematic way, but rather where the clinicians felt this was clinically indicated, 36 of 119 (30.3%) patients tested positive for HIV, though figures for how many of these were children are not given. Results of malaria films were not provided, nor were there enough NTS isolates to explore seasonality.

An early study in Benin City, Nigeria by Akpede *et al.* sought to address the problem of fever without localizing signs in children presenting to hospital[129]. Their study, running prospectively over a year from 1988 to 1989 looked at all children with a fever of less than 7 days duration and no localizing signs. Of 642 children enrolled

the overall prevalence of bacteraemia was 10.4%; 62.8% had a positive malaria slide, 3.7% had bacteraemia and 6.7% had a combination of malaria and bacteraemia. The investigators found Staphylococcus aureus to be the most common pathogen, occurring in 43% of children. The next most common organisms were coliforms (undifferentiated by the authors). There were no cases of Hib and S pneumoniae was only isolated in 3 % of bacteraemic children.

Meremikwu *et al.* looked at blood culture results from children admitted to hospital in Calabar, Nigeria over a six year period.[130] Children had blood cultures performed when the attending clinician felt it was indicated. 1,201 children were included in the study with a large cohort of neonates (44.4% of the total). This may explain why the most frequent organism identified was *Staphylococcus aureus* (in 48.7% of positive cultures) and the high overall rate of positive cultures (46%). However the rate of coagulase negative Staphylococus (9 isolates from 1201 patients, 0.7%) seems surprisingly low in a group that often have a high rate of contamination and must raise some question over the quality of reagents used. The study did not look at HIV infection, nor did it comment on malaria infection.

1.1.4 Central Africa

Bahwere *et al* studied 932 consecutive admissions over a one year period to a rural hospital in the Democratic Republic of Congo, obtaining blood cultures in 779.[131] Of these children 15.9% were found to have a pathogenic bacterial isolate at admission. Similar to the East African studies, Enterobacteriacae were the most common finding and *S. pneumonia* was isolated in only 4% of positive cases. The authors found that 28.8% of 632 children tested had a positive blood slide for malaria. A study from Kigali, Rwanda by LePage *et al*, now over 20 years old, is interesting in that the most

frequent isolate was *Salmonella typhi*, rather than NTS.[132] Although the majority of bacteraemic children were later admitted, the study took place in an outpatient setting, making the high proportion of positive cultures more worrying. In Rwanda, the investigators found that 27% of children with bacteraemia also had malaria parasitaemia, in the DRC study this figure was 42.1%, with bacterial disease significantly more common in the children with positive malaria films than those with negative slides, a finding not replicated in any of the other studies where malaria parasitaemia was documented. (Table 2)

1.1.5 Southern Africa

The only study from a malaria endemic region in Southern Africa found that 8% of 19,896 children admitted over a 5 year period had invasive bacterial disease.[133] All children under 15 years were included in the study and 84% had blood collected for culture. Non-typhi salmonella species were the most commonly isolated organisms in children aged less than 5 years, but outside the neonatal period. 44% of the children with positive cultures had malaria parasites on blood film. 64% of children had malaria parasites seen on microscopy with bacterial co-infection found in 8.9% of these. HIV status was not documented, though the level in pregnant women attending the antenatal clinic was high at 23.6%.

A second study from Southern Africa by Cotton *et al* from Cape Town, South Africa was performed in a setting with a Mediterranean climate and no malaria.[134] The study considered all 8,524 paediatric admissions as eligible, but fails to describe how many actually had blood cultures taken – leading to some doubt as to whether the figure of 1.5 cases of community bacteraemia per 100 admissions is an accurate one.

Nonetheless the distribution of disease is interesting in that this is the only site reporting a significant prevalence of *N meningitides* (11.4% of positive isolates).

1.1.6 STUDIES IN SEVERE MALARIA

The studies outlined above and in Table 1 were looking at all children (with exceptions as described). Other studies have chosen to look only at severely ill children, often with severe malaria (either confirmed by blood test or clinically suspected), and determine what the aetiology of disease in these children is. These studies are reviewed below and in Table 3.

Berkley *et al.*, working in Kilifi, Kenya reviewed the clinical and laboratory features of 783 Kenyan children admitted with a diagnosis of severe malaria between April 1993 and May 1996.[117] Data were collected prospectively and criteria for inclusion were prostration (the inability to breast feed in a child aged under 1 year or inability to sit unaided in a child aged 1 year and older), impaired conscious level (as defined by the Blantyre coma score[135]) or respiratory distress. Importantly children who were severely ill, with a positive malaria film, but had another primary diagnosis (*e.g.* meningitis diagnosed by lumbar puncture) were not included. 643 of the children had blood cultures drawn, the rate of contamination was again high (103 cultures, 16%) and these children were excluded. Of the remaining 540 children with severe malaria a pathogenic organism was grown in 42 cases (7.8%). The pattern of organisms grown was similar to that seen in the larger study of all children from this site,[119] with *S. pneumoniae* the most frequently isolated bacterial pathogen. The investigators found that the mortality in children with a clinical picture of severe malaria and both bacteraemia and a positive malaria blood slide was significantly

higher than that in children with malaria alone (33.3% vs. 10.4%, $p<0.001$). They found that younger children were more at risk, with 18.5% of children with severe malaria under 6 months of age having positive blood cultures. Based on their findings the authors proposed that all young children with severe malaria be treated with antibiotics in addition to antimalarials. It should be noted that the definition of severe malaria adopted was narrower than the WHO definition, [1] most notably by the exclusion of children with severe malarial anaemia only. Further the group found that in children with severe anaemia (Hb <5g/dl) and prostration, but without impaired consciousness or deep breathing, there were no cases of bacteraemia (though there were only 25 children in this group).

A study of 251 children presenting with signs and symptoms of severe malaria in Kumasi, Ghana showed quite different results.[136] The definition for severe malaria used by the group was more in line with those proposed by the WHO; coma or prostration, severe anaemia or respiratory distress. Only 182 of the children had parasites detected on a blood film. Although 23 (12.6%) of the children with severe disease and a positive blood slide for malaria had a positive blood culture, this group did not have a worse outcome when compared with the children with severe malaria alone (mortality 8.7% vs. 8.8%). Of the children who tested negative for malaria 40.6% had positive cultures with very high mortality (39.2%). Few details of how the children were managed are given, though one can speculate that the children with negative slides would have been treated for bacterial disease, the situation for those with a positive blood slide is less clear. One would expect that this would, if anything, worsen the outcomes for those with co-infection yet this was not the case. NTS was the most common isolate in both slide positive and slide negative children.

However the authors themselves remarked that the laboratory had difficulty in growing *S. pneumonia* and the results may have been skewed as a result.

A third study in Blantyre, Malawi between 1996 and 2005 analysed blood culture results from all children admitted with severe malaria during the rainy season.[137] The study recruited 1, 388 children with severe malaria. The criteria for eligibility for the study are vague – the authors stating that not all children with severe malaria were admitted – but 88% of the children described had cerebral malaria (Blantyre coma score ≤ 2) with or without severe malarial anaemia. All children were at least 6 months of age. Bacteraemia was detected in 4.6% of children, with NTS the most commonly isolated organism. Of the total 1,119 children (80.6%) were tested for HIV, with 15.9% of the children testing positive. HIV positive children with severe malaria were at significantly increased risk of NTS bacteraemia (4.5% of HIV positive children vs. 2% of HIV negative children, p=0.48) but not at increased risk of bacteraemia overall. The study found that although there was no significant association between either HIV infection or bacteraemia and death in this patient group there was a non-significant increase in mortality in children co-infected with malaria and invasive bacterial disease (21.8%) compared with those with malaria alone (16.0%, p=0.22).

1.1.7 SUMMARY

There is clearly variation between sites in the spectrum of bacteria causing invasive disease. This may relate to local vaccination rates, malaria endemicity or other factors.[138-140] In most of Africa there is little if any community surveillance to

establish the relative roles of infectious agents in population morbidity and mortality. In such a situation the best results may be obtained through the use of high quality inpatient data to guide policy makers on targets for vaccination and other preventive strategies. However most hospitals in the region have no microbiology services, with reliance instead being placed on sentinel sites for bacterial surveillance supported by groups such as the Hib-Paediatric Bacterial Meningitis Surveillance Network (Hib-PBM), the Pneumococcal Vaccines Accelerated Development Programme (Pneumo-ADIP) and in East Africa, the Network for Surveillance of Pneumococcal Disease in the East Africa Region (netSPEAR).[141] These sites tend to be inpatient facilities where committed clinical and laboratory staff have been identified who can ensure that data and microbiological samples are collected. Nonetheless resources for such surveillance are scarce, with finances often available for education and database support, but little for increased staff and supplies that are necessary for expanded surveillance through blood culture. Of 14 sites involved with netSPEAR only 5 sites collect blood culture data, at least 2 of which are supported largely by research funds (including this study).[141]Additionally, local data is invaluable in the preparation of antibiotic guidelines appropriate to local sensitivity patterns. We set out to produce such data for national and regional use.

An important unresolved issue remains the impact of bacteraemia on mortality in children with severe malaria, with different results emerging from different sites, as outlined above. Much of the evidence discussed has become available only recently, thus current guidelines are a little vague on this important point. The WHO guidelines on the treatment of malaria suggest a low threshold for antibiotic treatment in children with severe malaria.[142] Perhaps because of the lack of firm

guidance on which clinical signs should trigger antibiotic therapy, or what that therapy should be, the national malaria treatment guidelines of Kenya, Tanzania, Uganda and Malawi make little mention of antibiotic treatment. The more integrated "WHO Management of the child with a serious infection or severe malnutrition: guidelines for care at the first level of referral in developing countries"[62] suggests that antibiotic treatment be given to children with malaria and signs of meningitis in whom meningitis cannot be excluded and those with signs of circulatory shock. There is a clear need for further data on this issue and it was the intention of these studies to provide these data.

BLANK PAGE

	Site	N	Selection method	Bacteraemia Prevalence	S. pneumo.	Staph. Aureus	Grp A Strep	Other Strep.	NTS	H. Influenzae	E coli	Other Gram Neg.
Berkley *et al* 2005[119]	E Kenya	16,570	All medical	1094 (6.6%)	24.9%	6.9%	4.2%	5.7%	14.7%	12.0%	10.7%	20.9%
Walsh *et al* 2000[122]	Malawi	2,123	Suspected bacteraemia	365 (17.2%)	16.2%	1.9%	1.6%	6.3%	38.4%	5.8%	-*	29%*
Archibald et al 2000[123]	Malawi	229	All suspected infection	35 (15.3%)	0	0	0	0	77%	0	11.4%	5.7%
Blomberg *et al* 2007[124]	Tanzania	1,828	All suspected infection	155 (8.5%)	0	8.4%	0	1.9%	16.8%	0	15.5%	47.7%
Ghiorghis *et al* 1992[125]	Ethiopia	634	Febrile, not malaria	49 7.7%	16%	14%	†	†	57%[b]	2%	6%	57%[b]
O'Dempsey *et al* 1994[126]	Gambia	1,162	Suspected bacterial infection	187‡ (16.1%)	55.1%	2.78%	-	2.1%	10.2%	17.1%	3.7%	9.1%
Hill *et al* 2007[128]	Gambia	686	Clinicians judgment	78 (11.4%)	46.2%	16.7%	-§	9%§	6.4%	-	11.5%	10.3%
Akpede *et al* 1992[129]	Nigeria	642	Fever without focus	67 (10.4%)	3%	43%	0	0	1.5%	0%	23.4%*	13.9%**
Meremikwu *et al* 2005[130]	Nigeria	1,201	Clinicians judgement	552 (46%)	††	48.7%	2.2%	2.5%††	1.6%	††	††	43.3%††
Bahwere *et al* 2001[131]	DRC	779	All medical	124 (15.9%)	4%	4.8%	0	0	42.1%	0.8%	15.1%	32.6%
Lepage *et al* 1987[132]	Rwanda	900	Consecutive febrile	112 (12.4%)	12.5%	8%	0.9%	0	32.1%	2.7%	0.9%	42.9%
Siguaque *et al* 2009[133]	Moz'bique	19,896	All medical	1550 (7.8%)	25%	12%	3%	6%	26%	7%	10%	10%
Cotton *et al* 1992[134]	S. Africa	8,524	All children	132 (1.5%)	33%	13.6%	‡‡	‡‡	‡‡	‡‡	‡‡	‡‡

Table 1. Studies in African children showing rates of community acquired bacterial disease & distribution of pathogens. Footnotes on facing page

	P. falciparum prevalence	Co-infection prevalence		
		P.falciparum and IBD in total	IBD in *P.falciparum*	*P.falciparum* in IBD
Berkley *et al* 2005[120]	44.7%	1.3%	3.0%	18.8%
Walsh *et al* 2000[122]	N/R[1]	N/R[1]	N/R[1]	23%
Blomberg *et al* 2007[124]	21.6%	N/R[1]	N/R[1]	N/R[1]
O'Dempsey *et al* 1994[126]	31.3%	N/R[1]	N/R[1]	N/R[1]
Akpede *et al* 1992	62.5%	6.7%	9.7%	64.2%
Bahwere *et al* 2001[131]	28.8%	7.1%	19.8%	42.1%
Lepage *et al* 1987[132]	N/R[1]	N/R[1]	N/R[1]	26.9%
Siguaque *et al* 2009[133]	65.1%	3.1%	5.8%	44.2%

Table 2. Prevalence of malaria and proportion of children with invasive bacterial disease with malaria in different studies

[1] Not recorded in study

	Definition of severe malaria	Age	N	Bacteraemia prevalence	CFR Malaria (%)	CFR Co-infection (%)
Berkley *et al* 1999[117]	Prostration, impaired consciousness, resp. distress	All	540	7.8%	10.4*	33.3*
Evans *et al* 2004[136]	Coma, Severe anaemia, Resp. Distress, Prostration	All	182	12.6%	8.8†	8.7 b†
Bronzan *et al* 2007[137]	Coma, Severe anaemia	>6months	1, 388	4.6%	16 †	21.8 †

Table 3. Prevalence and case fatality of bacteraemia in severe malaria studies

*p<0.001

† Not significant

1.2 CAN THOSE CHILDREN AT RISK OF DEATH BE RELIABLY PREDICTED AT THE BEDSIDE?

In resource limited environment it is clearly important to be able to target therapies to those children who are most in need. District hospitals in East Africa are commonly understaffed with overcrowded wards, drug shortages and poor facilities.[29-31] Clear guidelines for clinicians on which patients have the poorest prognosis are essential or health care workers will become overwhelmed. There have been several studies that have addressed risk factors for death in children with malaria, pneumonia and other illnesses but few studies that have looked at all children systematically. Children commonly present with multiple problems and the clinician involved may not be able to describe the nature of each at the outset as many of the features of these illnesses overlap. Difficulties will always arise as the high sensitivity desirable to ensure that all children at risk are identified is usually at the expense of specificity. There is no simple answer to this – the optimal balance will vary from location to location depending on staff levels and expertise and other local factors.

1.2.1 STUDIES IN CHILDREN WITH MALARIA

Early studies identified hypoglycaemia, deep coma, hyperparasitaemia and raised CSF lactate with poor outcome in falciparum malaria.[135 143-146] An early classification of severe malaria defined it as the presence of asexual Plasmodium falciparum parasites with any one of 10 features (Table 4).[147] However it was not until a landmark publication in 1995, describing the clinical features associated with death in children with malaria in Kenya, [65] that the sensitivity of such clinical and bedside criteria were explored in a large study of African children. This study of 1,844

children with a primary diagnosis of malaria, demonstrated that impaired consciousness, respiratory distress, hypoglycaemia and jaundice were independently associated with an increased risk of death. The presence of either impaired consciousness or severe respiratory distress predicted 84.4% of the fatalities. With the addition of severe anaemia over 90% of deaths were predicted. Children with none of these features had a low mortality (0.6%). Risk of death seemed to be additive, with the highest mortality occurring in children with all three features. This Kenyan study gathered together threads from previous studies, prior to and following the 1990 WHO guidelines, which had shown an increased mortality in children with cerebral malaria, severe anaemia, hypoglycaemia and acidosis. [69 135 145 148-149] Subsequently, other studies have emphasized the importance of acidosis (best demonstrated through the clinical sign of deep breathing)[150] in addition to circulatory shock. [151] Based on these and other studies the WHO Communicable Diseases Cluster published revised definitions for the classification of severe malaria (Table 5).[1]

Across a range of settings in Africa[65 67-68 70 152-157] and elsewhere[158] (Table 6), further studies have also shown high mortality in children with impaired consciousness and respiratory distress, with lower mortality in severe anaemia. Four African studies describe mortality in the group that was not labeled as having severe malaria.[65 67 70 157] In a Tanzanian study the authors added a further criteria of 'dehydration', though it is unclear how many deaths occurred in children with dehydration alone. Mortality in children without respiratory distress, severe anaemia or cerebral malaria was 1%.[70] A study from Mozambique found that only 45/62 (73%) deaths in children with malaria were predicted by the WHO severe malaria criteria.[67] Whilst in Yemen no

children died outside of those identified by the WHO definition as having severe malaria and all deaths occurred in children with severe anaemia, respiratory distress or neurological features.[158] The study by Taylor *et al.* involved the collection of data from 6 well established research sites across Africa. Overall the mortality in children with malaria that did not have severe anaemia, cerebral malaria, hyperlactataemia or deep breathing was 1.2%, though this varied from site to site (range 0-2.2%). It is interesting to note that even in these well established research centers there was considerable variation in the sensitivity of WHO criteria of severe malaria for predicting death, ranging from 68.6% in Kenya, at the site of the previous study by Marsh *et al.,* to 95% in Malawi.[157] Sensitivities and specificities of prognostic categories for identifying at admission children that went on to die, using the different strategies in studies where all children with malaria were enrolled, are shown in Table 7.

1.2.2 Studies in children with pneumonia

Acute lower respiratory tract infection is the most common cause of death in young children worldwide.[159-160] As such, considerable attention has been paid to identifying which children should have focused care because of an increased risk of death. The WHO has produced guidelines on diagnostic categories for pneumonia, aiming to allow clinicians to prioritise care. [62 161] These guidelines base the clinical diagnosis of pneumonia on a history of cough or difficulty in breathing supplemented by clinic findings, such as tachypnoea or chest wall indrawing, that allow the child to be ranked in term of severity (Table 8). Studies have sought to evaluate these guidelines in prospective observational studies, though relatively few in an inpatient African setting where the clinical signs of malaria and pneumonia can overlap.[162-164]

A study from the Central African Republic, using earlier WHO guidelines,[165] found 49 (12.4%) inpatient deaths amongst 395 children, aged 2months to 5 years, with a clinical diagnosis of pneumonia or severe pneumonia.[166] They found that 47/48 (97.9%) children with a history of cough or difficulty in breathing who died showed chest wall indrawing (defined as either lower chest wall or intercostals indrawing) when first assessed (in one child who went on to die there was no record). This gives an impressive sensitivity for such a simple observational sign. Of children who survived, 204 showed chest indrawing giving a positive predictive value of 18.1%. Chest indrawing is a component of both the 1990 guidelines,[165] and the updated 2000/2005 guidelines,[62 161] indicating a clinical diagnosis of severe pneumonia. This should have resulted in treatments aimed at reducing the mortality, potentially underestimating the importance of this sign. Other clinical signs found to be significant and predictive of inpatient mortality included hepatomegaly (Adjusted Odds Ratio 6.7), aged between 2 and 11 months (AOR 6.4), grunting (AOR4.5), acute malnutrition (AOR 2.7) and a moderate or severe alteration in 'general status' (AOR 3.2). In the same multivariate model chest indrawing had an AOR of 8.4. The authors went on in a separate publication to specifically address the WHO pneumonia and severe pneumonia categories for their sensitivity and specificity in identifying inpatient deaths.[167] They found that 43/47 (91.5%) of deaths were in children assigned to the severe pneumonia category. The source of discrepancy with the initial publication, where 47/48 inpatient deaths amongst children with a history of cough or difficulty in breathing had chest indrawing, is unclear – though may be due to different interpretations of the term 'chest indrawing'. The authors also proposed that a 'second tier' of severity be added to allow the more precise targeting

of scarce resources, such as oxygen, to those at the highest risk of dying. This group of 'very severe pneumonia' would include all children that had chest indrawing (lower chest wall or intercostals) and in addition showed either nasal flaring or respiratory grunting. They calculated that this group would comprise 72% of the inpatient deaths, whilst treating intensively only 3.4 children for each child that died (as opposed to 5.5 if every child with indrawing received intensive treatment).

As part of a study to determine their utility in targeting appropriate treatment, a large study of 11,847 children admitted to hospital in Kenya gathered data on the performance of the more recent WHO guidelines [62] in predicting outcome.[120] These data demonstrated that, in children with clinical pneumonia, WHO 'severe' or 'very severe pneumonia' predicted 108/123 inpatient deaths (sensitivity of 87.8%). However 1,766 children were assigned to these two severity categories – requiring that 16.4 children receive a high level of care for each death targeted. The more selective category of 'very severe pneumonia' included only 56/123 (45.6%) of deaths in children with pneumonia, but had a very high mortality (56/296, 18.9%).

In South Africa, McNally *et al* found that WHO 'very severe pneumonia' was significantly associated with both treatment failure and in-hospital mortality compared with children admitted with 'severe pneumonia' (OR 3.5 and 6.1 respectively), as was HIV positive status (OR 2.6 for treatment failure and 8.1 for inpatient death).[168] As all children with pneumonia were not assessed it is not possible to determine how the categories performed in predicting mortality generally.

A smaller study of 132 children with 'severe' or 'very severe pneumonia' admitted to hospital in Zambia found 21 deaths (mortality of 16%) and though HIV infection was

associated with an increased risk of a fatal outcome (OR 2.6) this did not reach clinical significance (p=0.079).[169]

Three studies from outside Africa have addressed the issue of predicting death in children with pneumonia in the developing world. Spooner *et al.* assessed the utility of clinical signs in predicting death in 897 children aged under 5 years presenting to hospital in Papua New Guinea.[170] Cyanosis, poor feeding grunting, nasal flaring and younger age were all associated with a significant increased risk of mortality, similar to many of the studies from Africa. Chest indrawing was an eligibility criteria for the study and therefore not amenable to analysis. In contrast to previously mentioned studies bronchial breathing, a sign associated with consolidation of the underlying lung, was also significantly associated with inpatient death (OR 3.35, p<0.001). Cyanosis was found to be the strongest predictor of death with 89% of deaths in children noted to be cyanosed. This study recorded signs present at any time during a child's admission, potentially biasing the study towards the demonstration of signs associated with the process of death, rather than true predictors.

Shann *et al.*, working at the same hospital in Papua New Guinea in the 3 years prior to the cohort enrolled in the study by Spooner *et al.*, prospectively studied 748 children admitted with severe pneumonia (defined as cough and chest indrawing plus one of: severe indrawing; a pulse rate over 160/minute with hepatomegaly; failure to feed; bronchial breathing; grunting; cyanosis; severe chest radiograph changes; or a total white blood cell count over 30,000 cells/μl).[171] The study identified 9 criteria that predicted a high risk of death in children with severe pneumonia: prolonged illness (more than 5 days); failure to feed; cyanosis; absence of fever; hepatomegaly; malnutrition; pulse rate of less than 160; leukocytosis; severe chest radiograph

changes. Two features that appear incongruous warrant further explanation; absence of fever was associated with severe malnutrition, a group that had a high mortality; pulse <160 was likewise associated with an absence of temperature and consequently severe malnutrition. The most sensitive signs were a history of over 5 days illness and severe chest radiograph changes (72% and 67% respectively), though the signs felt by the authors to be most amenable to assessment by paramedical staff were inability to feed and cyanosis (together picking up 77% of children who went on to die).

Djelantik *et al.*, working in three hospitals in Lombok, Indonesia, prospectively studied 4,351 children aged under 2 years hospitalised with pneumonia to identify screening criteria for mortality.[172] There were 505 (11.6%) inpatient deaths amongst children with pneumonia, in a multivariate model hypoxia, young age (<4months), low respiratory rate, raised white blood cell count and severe anaemia were all associated with death. The most common factors associated with death were hypoxia and young age, occurring in 83% of children who died. Several important variables associated with death in other studies, such as cyanosis, bronchial breathing, grunting and malnutrition, were not addressed in this large study.

It is always difficult to generalize from one location to another; this is made more difficulty when malaria endemic and non-malarial regions are compared. The clinical similarity between pneumonia and malaria may result in many children diagnosed with clinical pneumonia having malaria[162-163], with different risk factors for death. Possibly as a result of this clinical similarity hypoxia is less prevalent in malaria endemic regions[173] and many clinical signs consequent on it may have less prognostic significance in children fitting the case definitions of pneumonia but

suffering from malaria (clearly the same is true of children with malaria parasites who are actually suffering pulmonary infection).

Several studies have addressed the prediction of hypoxemia as a surrogate of predicting mortality, as it has been strongly associated with outcome.[167 172 174-175] This has the advantage that the factor being predicted (hypoxemia) will not be affected by subsequent interventions in the same way that subsequent treatment based on clinical severity will affect outcome. This topic was reviewed by Usen & Weber[176] in 2001, since then a further 3 studies were identified in the course of writing this thesis. Features of these studies are shown in Table 9. Published combination models for predicting hypoxaemia are shown in Table 10.

Reviewing these tables it is clear that there is significant heterogeneity between the studies, though there are several consistent features. Amongst single signs, head nodding and cyanosis are consistently specific findings, whilst chest indrawing and crepitations appear to be sensitive in the majority of studies. However, given that the children enrolled in most studies were children with a diagnosis of pneumonia, for which chest indrawing is a marker of eligibility; it is not surprising that many children had this sign. The high sensitivity of crepitations may similarly also represent selection bias, and another study found no connection between crepitations audible on auscultation in children and lobar consolidation on chest X-ray.[177]

Only one study looked at indicators of hypoxia in children that were not preselected by a diagnosis of pneumonia or lower respiratory tract infection. This study, by Duke *et al.* in Papua New Guinea, showed that cyanosis was the most specific sign (98.1%), but lacked sensitivity (37.9%), whilst a combination model of cyanosis or

poor feeding or a respiratory rate >60bpm had a sensitivity of 81.9% and specificity of 49% when applied to children with acute lower respiratory tract infection but 82.8% and 58.2% respectively when applied to children with other diagnoses.[178] The high specificity of cyanosis is worthy of further consideration, yet the physical sign is estimated to require between 4 and 6g/dl of deoxygenated haemoglobin in the capillary beds viewed.[179] In malaria endemic parts of Africa up to 40% of children in the community may have a blood haemoglobin under 8g/dl, [180-181]and this proportion will be higher amongst admissions, requiring a level of hypoxia before cyanosis can be detected that may not be compatible with life.

Combinations of signs again show a wide variation, with sensitivities ranging from 59% to 82% and specificities from 49% to 94% depending on the site and signs used. In terms of the provision of scarce oxygen supplies the specificity of the Weber model (Table 10) is appealing, although there needs to be an awareness that many children at increased risk of death will not be covered by such a definition.

One might expect specificity of clinical signs to detect hypoxia to be lower in studies conducted in malaria endemic regions as many of the clinical signs can be caused by acidosis relating to malaria, rather than hypoxia. This did not appear to be the case; with the exception of nasal flaring in the study by Usen *et al.* in the Gambia there appeared to be no real distinction between studies from areas endemic for falciparum malaria and those not.[182]

1.2.3 STUDIES ADDRESSING ALL CHILDREN ADMITTED TO HOSPITAL

Only two studies have systematically assessed all paediatric admissions with a view to assessing the factors at presentation associated with death. Both studies are from the same site and involved largely the same children.[66 120]

In their earlier study, Berkley *et al.* prospectively assessed factors associated with immediate (within 4 hours of admission), early (within 4-48 hours of admission) and late deaths (after 48 hrs) in all 8,477 children aged over 90 days admitted to a district hospital in Kenya between July 1st 1998 and June 30th 2000.[66] The clinical features identified were then used to construct a prognostic score which was validated on 4,802 children admitted over the following year. The features associated with death and their assigned individual prognostic scores are shown in Table 11. The mortalities associated with summed prognostic scores, when applied to the children admitted in the third year of the study, are shown in Table 12. Thus a cut off of an 'early death' score of 3 or above had a sensitivity of 24/26 (92%) in identifying children that died with a specificity of 79%. The same 'late death' score would give a sensitivity and specificity of 81% and 72% respectively for late deaths. Early deaths were more evenly spread amongst the summed prognostic score, a score of 3 or above would only give a sensitivity of 43% but a specificity of 95%. The sensitivity improved to 87.5% if the cut off was reduced to a score of 1 or above, but specificity dropped to 59%.

In their later study Berkley *et al.* assigned WHO clinical diagnoses[62] to all 14, 987 children aged 0 to 13 years admitted between February 1999 and December 2001.[120] The definitions of clinical diagnoses used are shown in Table 13. This study will be described further in the next section, but the authors found that the presence of any

one of the WHO clinical diagnoses had a 93% sensitivity and specificity of 50% for inpatient death. When children outside the neonatal age are excluded the sensitivity fell to 89% and specificity rose to 55%.

The chief detraction from these impressive studies, and the data that they have generated, is simply that they have been performed in a single center, and one with a well established, highly skilled and expert research team. The need to establish how such findings would translate to other settings was one of the reasons for the studies described in this thesis.

Defining criteria of severe malaria
Cerebral Malaria (unrousable coma)
Severe anaemia (Haematocrit <15%/Hb<5g/dl in the presence of >10 000parasites/µl
Renal failure (urine output <12ml/kg/24hrs)
Pulmonary oedema
Hypoglycaemia (whole blood glucose <2.2mmol/l)
Circulatory collapse/shock
Spontaneous bleeding/disseminated intravascular coagulation
Repeated convulsions
Acidaemia/acidosis
Malaria haemoglobinuria
Other manifestations
Impaired consciousness, but rousable
Prostration, extreme weakness
Jaundice
Hyperpyrexia (rectal temp >40°C)

Table 4. 1990 WHO criteria of severe malaria

Classification of severe malaria in children		
Group 1		
	Prostration	
		With full consciousness
		With impaired consciousness
		With coma
	Respiratory distress	
		Mild
		Severe
Group 2		
	Severe anaemia	
	Multiple convulsions	
Group 3		
	Persistent vomiting	

Table 5. 2000 WHO classification of severe malaria in children

	Country	Inclusion	Age	Participants (CFR %)	Impaired Consciousness Prevalence % (CFR%)	Resp. Distress Prev % (CFR%)	Sev. Anaemia Prev % (CFR%)
Marsh *et al.*1995 [65]	Kenya	All malaria	0-13[†]	1, 844 (3.5)	10 (16.8)	13.7 (13.9)	17.6 (4.7)
Schellenberg *et al.1999* [70]	Tanzania	All malaria	1mo-5y	2, 080 (3.4)	8.2 (14.1)	14.7 (8.2)	8.5 (5.6)
Bassat et al. 2008 [67]	Mozambique	All malaria	0-15y	3, 859 (1.6)	0.6 (18.2)	11.1 (6.1)	4.6 (5.7)
Taylor *et al.* 2006 [157]	Multiple[‡]	All malaria	N/A	7, 134 (4.9)	9.0 (19.3)	10.4 (18.7)[§]	21.2 (8.4)
Al-Taiar *et al. 2006* [158]	Yemen	All malaria	6mo–10y	1332 (2.0)	13.1 (12.6)	24.2 (4.7)	21.8 (3.4)
Idro *et al. 2006* [154]	Uganda	Sev malaria	0-5y	617 (4.4)	14.1 (16.1)	25.6 (10.1)	28.5 (6.3)
Giha *et al.*2005 [153]	Sudan	Sev malaria	All ages[**]	110 (6.5)	16.4 (39.0)	N/A	45.5 (0)
Dzeing-Ella *et al.*2005 [68]	Gabon	Sev malaria	0-10y	583 (8.9)	24.4 (22.5)	31.0 (17.1)	67.8 (6.1)
Mockenhaupt *et al.* 2004 [155]	Ghana	Sev malaria	6mo-9y	290 (11.2)	19.3 (37.0)	22.8 (20.0)	55.2 (10.1)
Biemba *et al.* 2000 [152]	Zambia	SMA/CM	0-5y	876 (12.1)	13.3 (18.9)	N/A	27.4 (8.8)
Ranque *et al. 2008* [156]	Mali	SMA/CM	0-15y	455 (15.8)	66.4 (23.3)	17.4 (44.3)	41.1 (5,0)

Table 6. Mortality associated with malaria and severe malaria syndromes.

[†] 86% under 4 years age
[‡] Multiple sites – Kenya, Malawi, Ghana, Gabon & Gambia
[§] Defined as 'Deep Breathing'
[**] 75% under 12 years

	Criteria	Country	Severe malaria prevalence	Sensitivity	Specificity	PPV	NPV
Marsh *et al.*[65]	Impaired consciousness *or* severe resp. distress *or* severe anaemia	Kenya	746/1773 (42.1%)	90.3%	59.7%	7.5%	99.4%
Schellenberg *et al.*[70]	Impaired consciousness *or* severe resp. distress *or* severe anaemia *or* dehydration	Tanzania	828/2080 (39.8%)	84.3%	61.7%	7.1%	99.1%
Bassat *et al.*[67]	Coma *or* prostration *or* multiple convulsions *or* resp. distress *or* hypoglycaemia *or* severe anaemia	Mozambique	1016/3859 (26.3%)	72.6%	74.4%	4.4%	99.4%
Taylor *et al.*[157]	Coma *or* severe anaemia *or* deep breathing	Kenya	519/2060 (25.2%)	68.6%	76.3%	9.2%	98.6%
	Coma *or* severe anaemia *or* deep breathing *or* hyperlactataemia	Malawi	453/1208 (37.5%)	95%	64.5%	8.4%	99.7%
		Ghana	789/1445 (54.6%)	87.5%	46.7%	6.2%	98.9%
		Gabon a	125/338 (37.0%)	50%	63.3%	2.4%	98.6%
		Gabon b	179/355 (50.4%)	100%	51.3%	5.6%	100%
		Gambia	925/1483 (62.4%)	91.9%	40.9%	14.8%	97.8%
Al-Taiar *et al.*[158]	Impaired consciousness *or* severe resp. distress *or* severe anaemia	Yemen	605/1337 (45.4%)	100%	56.7%	2.6%	100%

Table 7. Sensitivity, specificity and test characteristics of different criteria of severe malaria at admission as predictors of death in hospital applied in different settings

1990 WHO clinical categories[165]		2000/2005 WHO clinical categories[62 161]	
Very severe pneumonia	Cough/difficulty breathing plus *one of:* Central cyanosis Unable to feed/drink Chest indrawing usually present	**Very severe pneumonia**	Cough/difficulty breathing *plus one of:* Central cyanosis Vomiting everything Unable to feed Convulsions Lethargy Unconsciousness Severe respiratory distress
Severe Pneumonia	Cough/difficulty breathing *plus* Chest indrawing *With* no signs of very severe pneumonia	**Severe Pneumonia**	Cough/difficulty breathing *plus one of:* Lower chest wall indrawing Nasal flaring Grunting With no signs of very severe pneumonia
Pneumonia	Cough/difficulty breathing *plus* a raised respiratory rate for age *With* no signs of very severe or severe pneumonia	**Pneumonia**	Cough/difficulty breathing *plus* a raised respiratory rate for age *With* no signs of very severe or severe pneumonia

Table 8. Outline of WHO clinical diagnostic categories for pneumonia.

Location	Peru[183]	Kenya[175]	PNG[184]	Columbia[185]	Gambia[186]	Gambia[182]	Zambia[187]	India[188]	PNG[178]	PNG[189]
Hypoxia def	82-85%	90%	85%	88%	90%	90%	92%	90%	86-88%	85-90%
Prev Hypoxia	132/423 (31%)	151/256 (59%)	47/91 (52%)	125/200 (62.5%)	69/180 (38%)	63/1072 (6%)	55/185 (30%)	28/109 (26%)	257/491 (52%)	90% - 20/77 (26%) 85% - 10/77 (13%)
Age	2-60 mo	7d-36 mo	3-36 mo	7d – 36 mo	2-60 mo	2-33 mo	1-60 mo	<60 mo	<60 mo	1-60 mo
Resp. Rate ≥ 70/min		48, 70 (<2mo) 51, 83 (3-11mo) 32, 90 (≥12mo)		16, 100 (0-11mo) 4, 100 (12-36mo)		54, 80	57, 91			
Rep rate ≥ 60/min		76, 35 (<2mo) 86, 56 (3-11mo) 65, 76 (≥12mo)		40, 86 (0-11mo) 12, 100 (12-36mo)		81, 47	72, 67		33, 71 (<1mo) 97, 71 (1-60mo)	75, 68 (<90%) 80, 78 (<85%)
Cyanosis	13, 99	20, 100 (<2mo) 9, 96 (3-11mo) N/A (≥12mo)	42, 84		39, 100	25, 95		14, 96	72, 89 (<1mo) 38, 98 (1-60mo)	70, 75 (<90%) 80, 71 (<85%)
Chest indrawing	35, 94	96, 20 (<2mo) 97, 29 (3-11mo) 88, 30 (≥12mo)	98, 7	76, 43	74, 47			36, 86		
Grunting		48, 65 (<2mo) 64, 73 (3-11mo) 56, 76 (≥12mo)	42, 89	45, 72	15, 94	46, 86		14, 92	23, 92 (<1mo) 22, 87 (1-60mo)	90, 61 (<90%) 100, 56 (<85%)
Nasal flaring			56, 84	63, 65	71, 56	98, 17				85, 56 (<90%) 100, 53 (<85%)
Head nodding					29, 95	57, 84			23, 92 (<1mo) 11, 96 (1-60mo)	30, 96 (<90%) 60, 97 (<85%)
Crepitations	50, 92	91, 50 (<2mo) 77, 40 (3-11mo) 91, 36 (≥12mo)	90, 16	79, 53	93, 23	86, 30	79, 53	68, 68		
Unconscious		68, 50 (<2mo) 63, 67 (3-11mo) 56, 78 (≥12mo)								
Unable to feed		66, 47 (<2mo) 50, 75 (3-11mo) 40, 75 (≥12mo)		35, 60	16, 98	33, 91			67, 49 (<1mo) 42, 76 (1-60mo)	

Table 9. Sensitivity & specificity of different clinical signs to predict hypoxia in studies from a variety of sites.

Combination	Study	Prevalence Hypoxia	Sensitivity	Specificity	PPV	NPV
Cyanosis/head nodding	Usen et al.[182]	63/1072 (6%)	68.3	80.4	17.8	97.6
Cyanosis/grunting/reduced consciousness/resp. rate >90	Dyke et al.[184]	47/91 (52%)	70.2	68.2	70.2	68.2
Cyanosis/head nodding/inability to cry	Weber et al.[186]	69/180 (38%)	59.4	93.7	85.4	78.8
Cyanosis/not feeding/resp. rate >60	Duke et al.[178]	162/223 (73%)	81.9	49.0	82.4	48.1

Table 10. Test characteristics of combinations of signs in detecting hypoxia.

	Immediate Deaths (<4 Hrs)		Early Deaths (4-48 Hrs)		Late deaths (>48 Hrs)	
	Indicator Present	Indicator Absent	Indicator Present	Indicator Absent	Indicator Present	Indicator Absent
History >7days					+1	+0
Severe anaemia	+1	+0				
Jaundice	+1	+0	+1	+0		
Subcostal indrawing	+1	-1	+1	+0		
Prostrated with seizures	+1	+0	+2	+0	-1	+0
Prostrated without seizures	+3	+0	+2	+0	+0	+0
Impaired consciousness with seizures	+2	+0	+2	+0	+1	+0
Impaired consciousness without seizures	+3	+0	+3	+0	+1	+0
Temp <36	+1	+0			+1	+0
Temp >39	-1	+0			-1	+0
Wasting			+1	+0	+1	+0
Kwashiorkor			+1	+0	+1	+0
Constant	+2		+0		+2	

Table 11. Prognostic scores for death in children admitted to hospital in Kenya from Berkley *et al.*

Scores are summed in columns for each of immediate, early and late death.

	Immediate		Early		Late	
Score	**No**	**Deaths (%)**	**No**	**Deaths (%)**	**No**	**Deaths (%)**
0	698	0	2779	11 (0.4)	15	0
1	2196	1 (0.1)	1101	16 (1.5)	1081	2 (0.2)
2	657	1 (0.2)	668	23 (3.4)	2310	26 (0.8)
3	612	5 (0.8)	183	23 (13)	869	26 (3.0)
4	217	4 (1.8)	55	10 (18)	347	38 (11)
5	72	3 (4.2)	15	5 (33)	170	22 (13)
6	34	3 (8.8)	1	0	10	2 (20)
7	31	4 (13)	0	0	0	0
8	12	3 (25)				
9	2	1 (50)				
10	1	1 (100)				

Table 12. Numbers of admissions, deaths and CFR (%) in children admitted to hospital in Kenya, data from Berkley *et al.* Score based on sum of prognostic scores in Table 11.

1.3 Do current WHO guidelines accurately predict invasive bacterial disease and therefore required treatments?

Whereas rapid diagnostic tests for malaria (RDTs) can provide bedside information on the likelihood that *P. falciparum* has a role in a child's febrile illness, there are no such tests for bacterial disease. Even when available, blood culture is insensitive and takes a minimum of 24 hours before results become available. This means clinicians are reliant on clinical examination, supplemented by whatever bedside tests are available, to make decisions on what, if any, antibiotic they should prescribe. In developed countries hospitals often have in-house protocols based on local data on the prevalence of different bacteria and antibiotic sensitivity patterns. As I have discussed such local data are lacking in much of East Africa. To fill this void WHO have developed protocols on the presumptive use of antibiotics in various clinical scenarios.[62 161]

Only one study, in an area of moderate malaria transmission, has systematically assessed the ability of these guidelines to accurately target invasive bacterial disease.[120] This study, by Berkley *et al.* in Kenya, has been briefly described in the preceding section. All 11,847 acute paediatric admissions over a 34 month period were assessed clinically and had blood drawn for culture and malaria slide microscopy. Children were categorized into WHO syndromes associated with presumptive antibiotic therapy. (Table 13) The authors excluded children with an obvious diagnosis (such as trauma, tetanus, sickle cell crises etc.), children with missing data and 1,752 children with contaminated blood cultures. Of the remaining 11,847 children 843 (7.1%) had evidence of invasive bacterial disease (positive culture of a pathogen from CSF or blood). Of all admissions 52.8% had clinical

features associated with one of the clinical syndromes; this group also included 80% of all invasive bacterial disease and 93% of all inpatient deaths. This is initially impressive, but a closer analysis by the authors shows that 24% of cases of laboratory proven meningitis would not have received parenteral antibiotics under syndromic treatment rules. Moreover sensitivity of the syndromes for identifying invasive bacterial disease in children co-infected with malaria was only 66% compared with 83% in aparasitaemic children.

It is not clear how intense transmission of *P.falciparum* might change the clinical presentation of invasive bacterial disease; infection with non-typhi salmonella is relatively common in areas where malaria transmission is intense and these infections have been associated with non-specific presentations,[190] severe anaemia and HIV infection,[118-119] any of which may be missed by the current guidelines on the use of antibiotics. In view of these considerations this study aimed to assess whether current WHO guidelines could accurately predict invasive bacterial disease in those children at risk of malaria in a site of high intensity malaria transmission.

Syndrome	Definition	Recommended Antibiotic
Sick young infants	Any child <60d old admitted with febrile disease	Penicillin/ampicillin with gentamicin
Meningitis/encephalopathy	Neck stiffness, bulging fontanelle or impaired consciousness*	Penicillin with chloramphenicol
Severe malnutrition	Severe wasting†/ kwashiorkor	Penicillin/ampicillin with gentamicin
Very severe pneumonia	Respiratory distress‡ *plus one of* prostration§, ** cyanosis *or* hypoxia**	Chloramphenicol
Severe pneumonia	Respiratory distress	Penicillin
Mild pneumonia	Tachypnoea for age†† *plus* a history of cough *or* difficulty in breathing	Oral amoxicillin
Skin/soft tissue infection	Cellulitis, abscess, pyomyositis	Cloxacillin

Table 13. Definitions of clinical syndromes used by Berkley et al.

* Blantyre coma score <3
† Weight for age Z score <-4
‡ Lower chest indrawing or abnormally deep breathing
§ Inability to sit in child ≥ 1yr or inability to breastfeed if aged <1yr
** SaO_2 < 90% on air by pulse oximeter
†† ≥50 breaths per minute if aged 60d – 1yr, ≥ 40 breaths per minute if ≥ 1yr old

1.4 ARE CLINICAL EXAMINATION FINDINGS REPRODUCIBLE BETWEEN DIFFERENT OBSERVERS?

A relatively small number of standard clinical signs are in common use to assess children admitted to district hospitals in resource-poor settings. Many of these are specified in the integrated management of childhood illness (IMCI) and some have originated from clinical research (e.g. prostration or the Blantyre coma scale).[62 135] I have discussed the use of many of these clinical signs in the early assessment of children with fever to determine the severity of their illness, its potential aetiology and possible treatments. Application of case management guidelines, such as those formulated by WHO, is dependent on the accurate assessment of these signs. It is therefore critical that such signs are perceived in the same way by different observers (low inter-observer variability), or their sensitivity and specificity may be lost.

Studies published on this have tended to use the κ (Kappa) statistic, which is a measurement of the agreement between observers beyond that expected by chance. K values vary from -1 to +1, with +1 demonstrating perfect agreement and -1 perfect disagreement, 0 would be the result if the level of agreement was purely that achieved by chance. A scale for the interpretation of κ has been proposed; values of 0- 0.20 indicate slight agreement; 0.21 – 0.40 fair agreement; 0.41 – 0.60 moderate agreement; 0.61 – 0.80 substantial agreement and 0.81 – 1.00 almost perfect agreement.[191]

Two studies have addressed this by using video recordings, thereby standardizing the clinical signs. A study looking at respiratory signs in children admitted to a hospital in Kenya found that agreement between 5 observers on 51 clinical cases for

recession, nasal flaring and severe recession was moderate (κ values in the range 0.41-0.60)[191] whilst that for deep breathing and a summary impression of respiratory distress was substantial (κ values in the range 0.61 – 0.80).

A second study, using 104 video recordings of 11 clinical features, analysed agreement between 32 leading paediatric clinical researchers (expert panel).[192] The agreement between the expert panel was slight to fair (κ <0.4) for 29/104 videos. No particular signs were highlighted by the authors as showing consistently poor agreement, indeed for almost all of the signs there were high consensus videos, where κ values were above 0.61 and low consensus videos, where κ values were below 0.2. The only exceptions to this were for the signs 'delayed skin pinch' and responsive only to pain, where there were no low consensus videos. A selection of videos was then also shown to a range of clinicians from interns to consultants, performance tended to be poor in the same videos where consensus among experts was lacking. Amongst the high consensus videos the sensitivity and specificity of the heath workers was reassuringly good in picking up the abnormal signs (sensitivities between 90% and 98%, specificities between 81% & 89%).

Together these studies appear to show there is room for optimism that, potentially, clinicians can pick up the same signs present in ill, febrile children. Studies from the bedside have shown that signs that are detected by inspection tend to have a higher level of agreement than signs that require either palpation or auscultation, but that across a range of signs clinicians appear able to show acceptable levels of agreement.[193-196]

Despite their importance these signs are often not documented, or looked for in children being assessed for admission to hospital.[29 31 197] Possible reasons include the lack of time to examine children in busy outpatient departments with extreme constraints on staff numbers. In such a situation nurses may be able to assist in assessing children on the wards. As part of the study I sought to determine the ability of nurses, with a small amount of extra training, to assess signs in febrile children admitted to hospital.

2 STUDY SITE

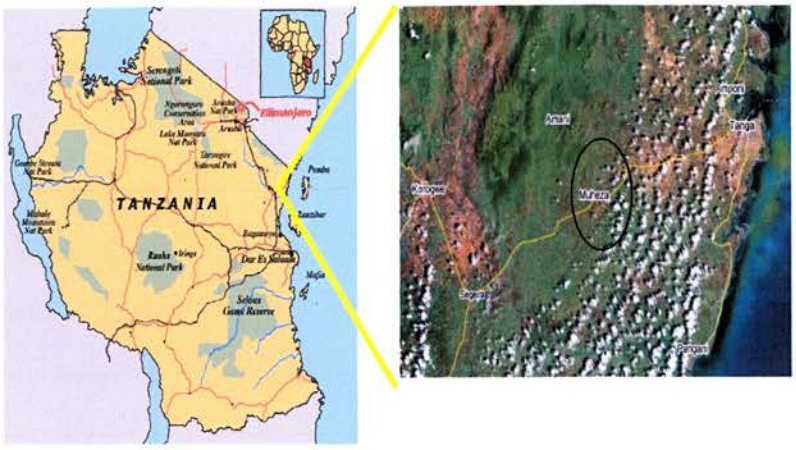


Figure 1. Map of Tanzania showing the study site on the coastal plain near the Kenyan border.

The studies described were conducted at Teule Hospital, Muheza, NE Tanzania. Teule hospital functions as the referral hospital for the population of Muheza district (approx 277,000). The population is largely rural. 17% of the population are under 5 years of age and child mortality is 165/1,000 (Tanzanian census 2002). Malaria transmission is intense (previously measured as between 50 and 700 infected bites/person/year) and perennial, with two peaks coinciding with the short and long rains (October and April).[198] HIV prevalence was 10.2% in antenatal clinic attendees (hospital data, 2004-5). The hospital has two paediatric wards each of 35 beds, though the wards often run at over capacity. Children are admitted based on a decision by hospital staff (clinical officers) working in the outpatient clinic.

3 METHODS

The study was a prospective observational study. To facilitate the study an Acute Assessment Unit (AAU) was established (Figs 2 - 4), staffed by four Tanzanian clinical officers (a non-physician clinician grade of staff who receive 3 years basic training in Tanzania), four nurses, two support workers and myself. It was felt that, although costly and time consuming, setting up such a unit would provide a destination for all children in whom an admission was requested. This would ensure full recruitment during the study period. This set up also allowed reproducibility of examinations to be enhanced as the conditions that the children were being examined in were similar. By using staff specifically recruited for the task the number of examiners could be minimized and their skills could be more easily monitored.

After approximately six months of recruitment (just over 1 year after my arrival) a Tanzanian doctor joined the team (GM). The research staff received training in the recognition of clinical signs, using classroom, video and bedside methods. One member of staff (clinical officer) had previously received IMCI training at an approved government facility, but received the additional training to ensure that study definitions, where different to IMCI definitions, were followed. IMCI had been part of Clinical Officer training in Tanzania since 2000 but was not in routine use at the hospital prior to the study.

Classroom training lasted one week, with bedside training continuing throughout the piloting period. Two members of staff (1 nurse and 1 clinical officer) were recruited and received the classroom training prior to my arrival. All other staff involved were recruited, selected and subsequently trained by me.

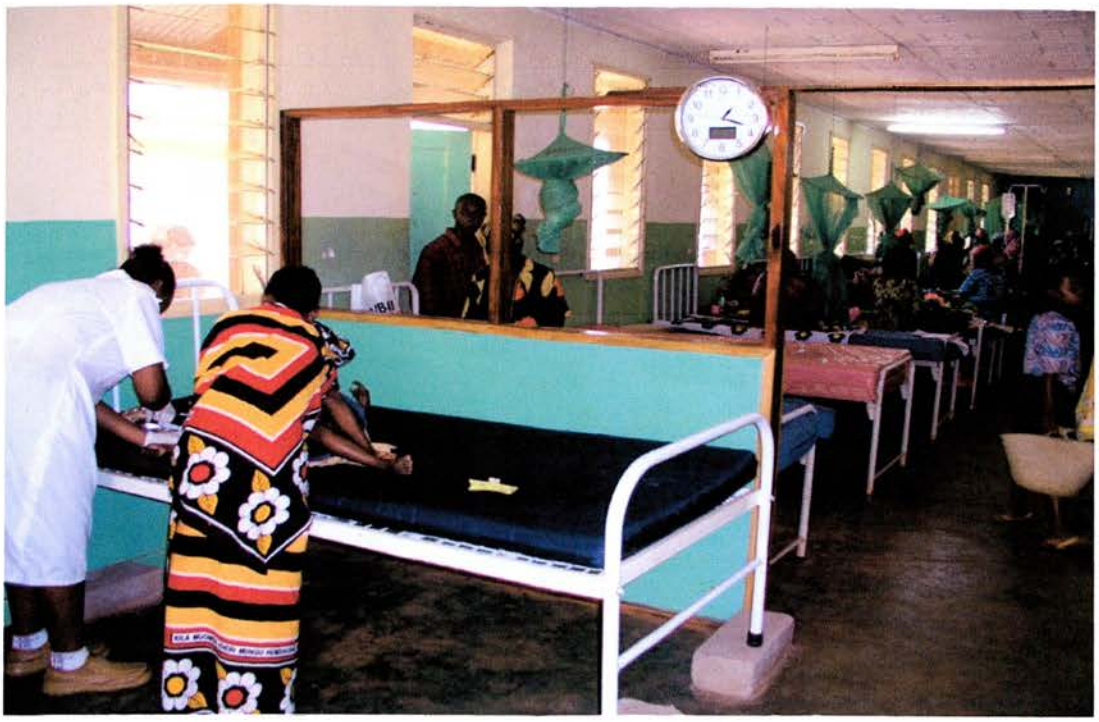


Figure 2. Child having blood drawn in the AAU

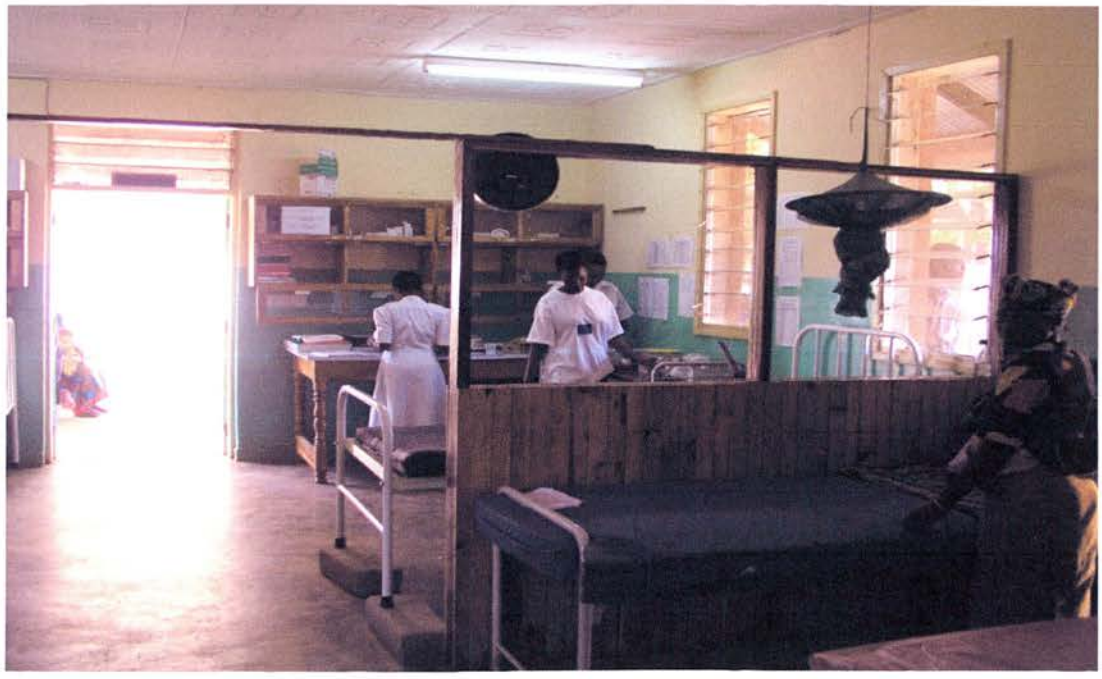


Figure 3. The AAU from the ward

Figure 4. Waiting area for the AAU

Research staff were also trained in how to fill out the research proforma, the principles of research data collection and the principles and importance of obtaining informed consent. The clinical support workers were trained by me in standardized anthropometry and in filling in relevant parts of the case record forms.

I supervised the research clinical staff onsite, later aided by my Tanzanian colleague (GM). The study was piloted for three months prior to data collection. All initial resuscitation, assessments and early treatment was performed in the AAU by research staff.

3.1 Enrolment & the study population

The study ran for one year from June 2006 – June 2007. Children were judged as needing admission by hospital staff at the Mother and Child Health (MCH) clinic. These hospital staff were also trained by me in Emergency Triage Assessment and Treatment (ETAT) principles [161] and were provided with colour coded triage cards to enable children who were judged to be in need of more urgent care to be identified on arrival at the AAU. During this period consecutive admissions from the hospital outpatients department with fever or history of fever were triaged for their need for emergency resuscitation on arrival at the AAU according to ETAT guidelines. Children were then screened for eligibility. (Box 1) Children admitted for trauma, surgery or those with known severe congenital abnormalities were excluded. Children under the age of two months were also not eligible. Children involved in a birth cohort study (MOMS) that was ongoing early in the study were initially not enrolled at the request of the MOMS study PI. We enrolled patients over 5 consecutive days each week. During the first seven months of the study these were

Monday – Friday, for the last 5 months the study enrolled Wednesday - Sunday. Tanzanian weekend holidays are Saturday and Sunday. Children were enrolled between 8am and 6-8pm on the days of enrolment (the exact times depending on staffing constraints).

Eligibility Criteria
Fever $\geq$ 37.5°C or history of fever within 48hrs
Age 2months to 13 years
No known malignancy or severe congenital abnormality
Not admitted for surgery or trauma
Not part of 'MOMS birth cohort study'

Box 1. Study eligibility criteria.

3.2 Clinical Data Collection

All patients were first assessed using ETAT criteria and where required were given resuscitation prior to enrolment in the study. All parents or guardians received HIV pre-test counseling as part of the consenting procedure, prior to the participation and HIV testing of their children. Parents or guardians who were unable to write were asked to provide a thumbprint.

Height and weight were recorded for all children using scales calibrated daily; a check on inter-observer and intra-observer variability was within recommended WHO limits. In addition to demographic details, medical history and examination findings were recorded on a proforma (Appendix 1). Findings recorded were based on the IMCI referral care handbook, adapted for local conditions using other guidelines or studies. [165]

3.3 Methods for the comparison of reproducibility of clinical signs between different grades of staff

This part of the study was conducted in the third and fourth month of the 1-year study. Staff consisted of clinical officers and hospital nurses (with 3-4 years of training in nursing). Clinical officers were either those specifically employed to gather research data (RCOs) for the 1-year study or were employed by the hospital to provide routine care (HCOs). Hospital nurses (HNs) were all regular hospital staff. Both HNs and HCOs regularly worked on the paediatric wards.

RCOs had received didactic teaching supported by video clips that demonstrated the signs in the study; these were assembled from recordings that had been used for

IMCI training or had been recorded by experienced paediatricians to demonstrate specific signs. In addition RCOs received bedside supervision on clinical examination for 3 months before the start of the study (the piloting period) and their assessments of children in the main study were regularly checked by me and the second study physician (GM) when he joined.

HCOs and HNs had a 1-hour training session on clinical examination using the same training materials as had been used for RCOs followed by an interactive teaching session. In addition, each staff member received a 30-minute individual training session where they examined children on the ward under supervision. Definitions of clinical signs were consistent throughout and are shown in Box 2.

3.3.1 Repeat examination procedure

On arrival at the ward all children were triaged and resuscitated as needed before the first examination. Following consenting procedures, children were examined initially by a research clinical officer (RCO) followed within 1 hour by a second examiner (RCO, HCO or HN according to a duty roster). Examinations were performed with no other staff present in a well-lit, quiet part of the ward, with results recorded on a standardised form listing the 17 clinical signs defined in Box 2. All examiners were blind to any clinical information (previous clinical examination, investigations or treatment) at the time of examination.

3.4 Bedside tests and laboratory methods

Blood was taken for blood culture (BactAlert, Biomerieux), full blood count (Coulter Act/Dif, Beckman-Coulter) and acute serology/serum save and research malaria slide. All children were tested for HIV antibodies using two rapid tests (Capillus HIV-1, HIV-2 Test, Trinity Biotech PLC, County Wicklow, Ireland & Determine HIV-1/2 Test, Abbott Laboratories, Abbott Park, IL, USA). Discordant samples in children aged over 18 months were resolved by ELISA (Vironistika UniForm II Plus O Test, bioMerieux, Durham, NC.). Plasma from children aged less than 18 months, positive by either Capillus or Determine tests were also analysed for HIV-1 RNA using the Abbott Real-Time m2000 System (Abbott Molecular Inc., Des Plaines, IL, USA). Patients also had bedside tests for haemoglobin and blood glucose (Hemocue™, Anglholm, Sweden), lactate (Lactate-Pro™, Arkray Inc, Kyoto, Japan) and a rapid test for P falciparum (Paracheck™, Orchid Biomedical, Mumbai, India).

The research protocol did not include lumbar puncture or radiography, both of which were available at the site. Lumbar puncture was performed as indicated based on

regional guidelines (NETSPEAR – The Network for Surveillance of Pneumococcal Disease in the East African Region). Where performed, lumbar punctures were processed by the research lab and results were recorded for research purposes. Chest radiography (CXR) was performed where indicated in children who were stable. CXR results were not available for the purposes of this thesis.

3.4.1 MICROBIOLOGY

Venous blood was taken from all patients after cleaning the skin first with a methylated spirit/alcohol mix then with iodine. A sample was first inoculated into BactAlert Paediatric blood culture vials before being used for other tests. Bottle tops were cleaned with iodine prior to inoculation and the original needle was discarded into a sharps bin and replaced with a sterile one prior to inoculation. Staff were encouraged to obtain 4-5ml of blood for the purpose of culture. Staff indicated their name on samples that they had drawn and inoculated, a procedure that allowed staff who began to run a high contamination rate to be identified and the problem discussed with them. Blood culture bottles were weighed before and after sampling to determine inoculum volume. Samples were cultured for 5 days in an automated system. Samples that flagged positive were sub-cultured onto commercially sourced chocolate, horse blood and CLED agar (Oxoid Ltd, UK). Samples that flagged positive but showed no subsequent growth were noted and reincubated. Positive specimens were identified by standard laboratory methods including API test kits where appropriate (bioMerieux, France). Antimicrobial resistance patterns were determined by NCCLS procedures. Initial typing and antibiotic sensitivity was performed on site. Laboratory work was carried out by a Tanzanian team of research lab technicians, trained and supervised by an accredited microbiologist (BA).

CSF was examined microscopically for determination of cell count, gram stain and Indian ink stain. Biochemical tests for determination of glucose and protein were also performed. In addition latex agglutination for *Haemophilus influenza* type b and *Streptococcus pneumoniae* was performed if the results of microscopy, glucose or protein were abnormal. Acid fast staining for *Mycobacteria* was performed when clinically indicated/suspected. Samples were then plated onto chocolate and horse blood agar and positive results identified as above.

Blood or cerebrospinal fluid (CSF) samples positive for coagulase negative *Staphylococcus*, Micrococcus, Corynebacterium, Bacillus sp or viridans group Streptococci were considered contaminants. If a known pathogen was amongst a mixed growth of 2 organisms it was considered positive. Cultures with three or more organisms were considered contaminated.

3.4.2 Malaria Slides

Blood slides were stained with Geimsa and double read. Parasites were counted by experienced Tanzanian lab technicians. Parasites were counted against 200 white blood cells on thick film microscopy. Slides were declared negative only after 100 high power fields had been viewed. Staining and first reads were supervised by me. Slides that were positive/negative discordant between the two slides went for a third readings, as did slides where there was a greater than twofold discrepancy in slide density above 400 parasites/μL. Slide readings were geographically and temporally apart to ensure independence. Blood slides were read after the study was over and were therefore not available to assist in patient care.

3.5 Quality control

The rapid test for malaria used in the study (Paracheck) and the Determine HIV rapid tests both use internal control lines. Samples where the control line was not visible were discarded and the test repeated. The Capillus rapid test utilizes positive and negative control samples that were run daily and the results recorded. The Hemocue Hb and glucose machines use an internal control that runs when the machine is activated.

Control samples were run daily for the for the Coulter Act/Dif full blood count machine and the machine recalibrated accordingly.

Bacterial isolates of S pneumoniae and Haemophilus were sent to a regional reference laboratory (NETSPEAR, Kilifi, Kenya) where they were checked, serotyped and sensitivities were repeated. Isolates of Salmonella (typhi or non-typhi) were sent to the University of Queensland Centre for Clinical Research and Pathology, Australia.

3.6 Outcome

At discharge a form detailing treatment provided and outcome (time of death or discharge) was filled for all patients (Appendix 1). Where this form was missing the information was obtained from hospital records.

3.7 Data Management

Data were scanned using Teleforms (Verity software Inc.) into an Access database (Microsoft). Data for the sub-study looking at the reproducibility of clinical signs

between different grades of staff were separately double entered into an Access database.

3.8 Statistical Analyses

Data analysis was performed using Stata-10 (Stata Corp, College Rd, Tx). Nutritional Z-scores were calculated from NCHS/WHO reference data in Epi-6. (CDC, Atlanta) We used the χ^2 test for the comparison of proportions. We used the t-test for comparison of normally distributed data (means) and the Wilcoxon rank sum test for non-parametric data. Forward logistic regression was used to determine associations between potential risk factors and outcomes; variables were added sequentially to a model in order of those with the highest unadjusted OR first, variables that became non-significantly associated with the outcome were dropped. Correlation of findings between examiners for the reproducibility of clinical signs analysis was assessed using Cohen's Kappa score and classified by categories proposed by Landis and Koch.[191]

3.9 Ethics

The study was approved by the Ethics Committees of the National Institute for Medical Research, Tanzania and the London School of Hygiene and Tropical Medicine, UK.

Variable (sign)	Definition
Axillary temperature	Measured by digital thermometer in the axilla for 2 bleep cycles.
Respiratory Rate	Counted over 1 minute in a non feeding child who is not crying.
Lethargy	A conscious child who takes no interest in its surroundings.
Ability to sit	Ability to sit unsupported if aged 8 months or more.
Ability to breast feed/drink	Observed ability to breast feed or drink from a cup.
Sunken eyes	A subjective assessment of sunken eyes.
Very slow skin pinch	A full thickness pinch of skin taken in a longitudinal plane midway between umbilicus and the side of the body. Longer than two (2) seconds to return to normal is considered a positive response.
Central cyanosis	Bluish tinge to the buccal mucosa.
Blantyre score	
Eyes	Directed eye movements, score 1 Undirected or closed eyes, score 0
Motor	Movements to localise pain, score 2 Other movements without localisation, score 1 No movement or abnormal flexion/extension, score 0
Verbal	Normal cry / speech, score, 2 Weak/high pitched cry. score 1 No vocal response' score 0
Jaundice	A subjective assessment based on inspection of the sclera or palms.
Severe Pallor	Noted by examination of the conjunctiva and palms.
Bulging fontanelle	The anterior fontanelle is assessed by palpation.
Neck stiffness	Assessed by observing resistance to voluntary neck flexion and passive flexion of the neck by the researcher
Lower chest wall indrawing	Inward movement of the lower chest wall on inspiration in a non-crying child.
Slow Capillary refill	Assessed by depressing the fingernail or fingertip pulp for 3 seconds and counting the time it takes to re-perfuse. A count of greater than 3 seconds is considered positive.
Temperature gradient	Detected by running the back of the hand down the limb.
Oedema of both feet	Detected by pressing a thumb on the dorsum of the foot.

Box 2. Definitions of clinical signs used in the study of reproducibility of clinical signs between different grades of staff.

4 RESULTS

4.1 OVERVIEW

During the one year of the study (June 2006 and May 2007) 6,470 children were admitted, 4,334 (67%) during study hours, and 695 (16%) were ineligible or excluded due to missing data or refusal to participate.

Breaking the non-enrolled patients down further; 529 children (12.2%) were not eligible because they had no history of fever, were a trauma or surgical admission, had severe congenital abnormality or malignancy, there were 30 deaths in this group (CFR 5.7%); in 58 (1.5%) of the eligible children consent was withheld by parents or guardians, 4 of these children died (CFR 6.9%). Review of the hospital death register at the end of the study revealed a further 39 children who appeared to have been admitted and died during study hours, but were not included in the study database; these children have been considered as having missing screening data. There were 187 deaths amongst the 2,136 children admitted out of hours (CFR 8.8%).

In 69 (1.9%) enrolled children there was missing data on outcome (25 children), HIV result (7 children), malaria blood slide result (33 children) or blood culture result (2 children); in two children the hard copy of their case record form had gone missing - these children were excluded from the final data analysis; there were 2 deaths in this group (CFR 2.9%). Amongst children enrolled into the study, with full data for analysis, there were 184/3,639 deaths (CFR 5.1%). Of the deaths in study children where the time of death was recorded 98/177 (55.4%) occurred in the first 24hrs of

admission, with 84.2% of deaths occurring within 72 hours of admission. The breakdown of admissions and exclusions over the year is shown in Fig 5.

There were 1,970 male children (54.1%). Male children had a lower mortality, though this did not reach statistical significance (CFR 4.4% in male children compared with 5.8%, p=0.056). The median age was 19 months. A graph of the distribution of children by age, with associated case fatality rates, is shown in Fig 6.

4.1.1 Anaemia

The median (mean) haemoglobin in study children was 8.2g/dl (7.9g/dl). The prevalence of severe anaemia (haemoglobin < 5g/dl) was 15.8%. Severe anaemia was significantly more prevalent in children less than 5 years of age (543/3327, 16.3% compared with 33/312, 10.6% in those over 5 years, $p<0.05$). Mortality was significantly higher in children with severe anaemia (53/576, 9.2%) than those without (131/3,063, 4.3%, $p < 0.001$).

4.1.2 Malnutrition

Malnutrition was defined as a weight for height Z score of < -3 or visible severe wasting or bilateral pitting oedema of the ankles. Case fatality amongst children with malnutrition was 18/110 (16.4%) compared with 4.7% in children without malnutrition ($p<0.001$).

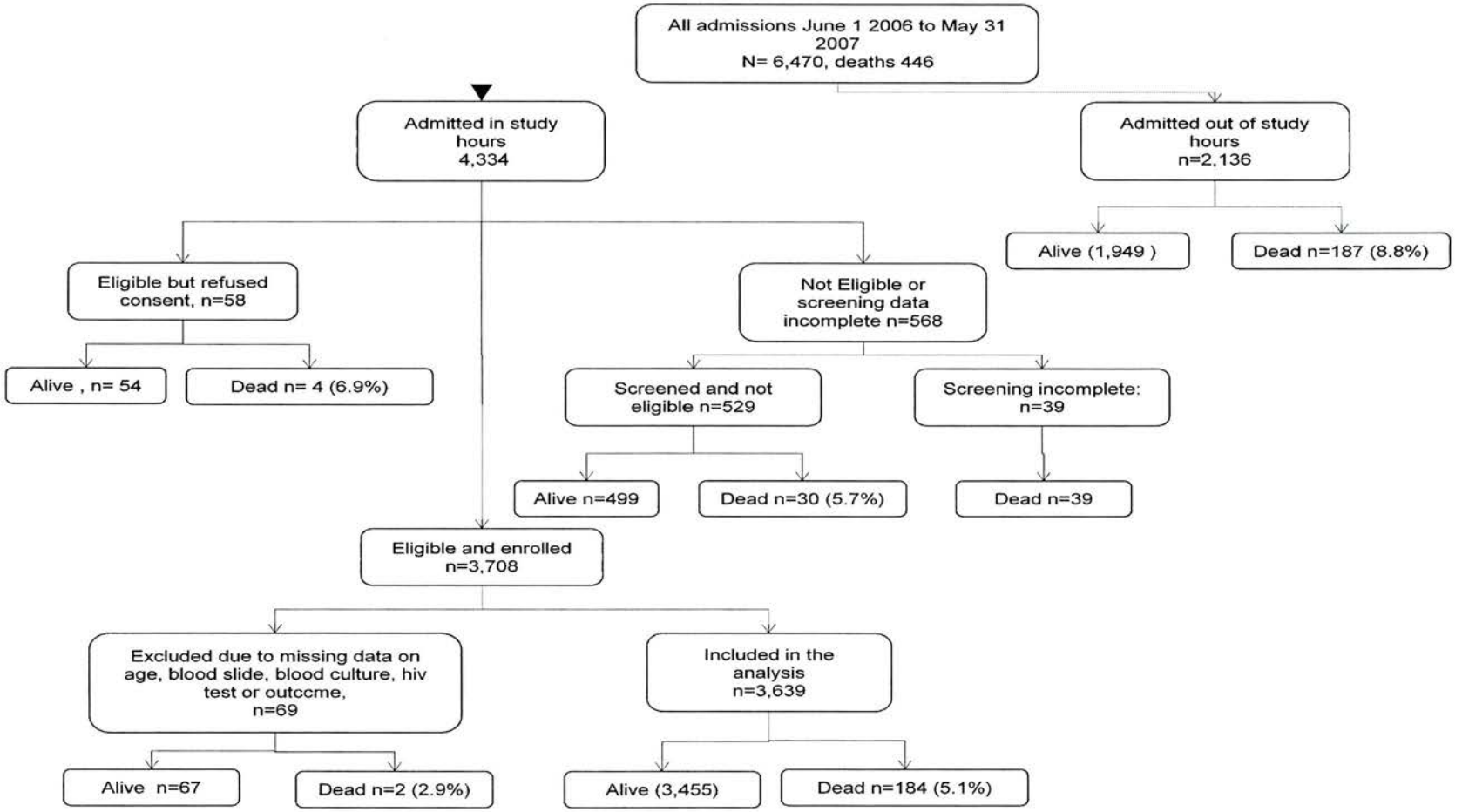


Figure 5. Breakdown of admissions over the study period.

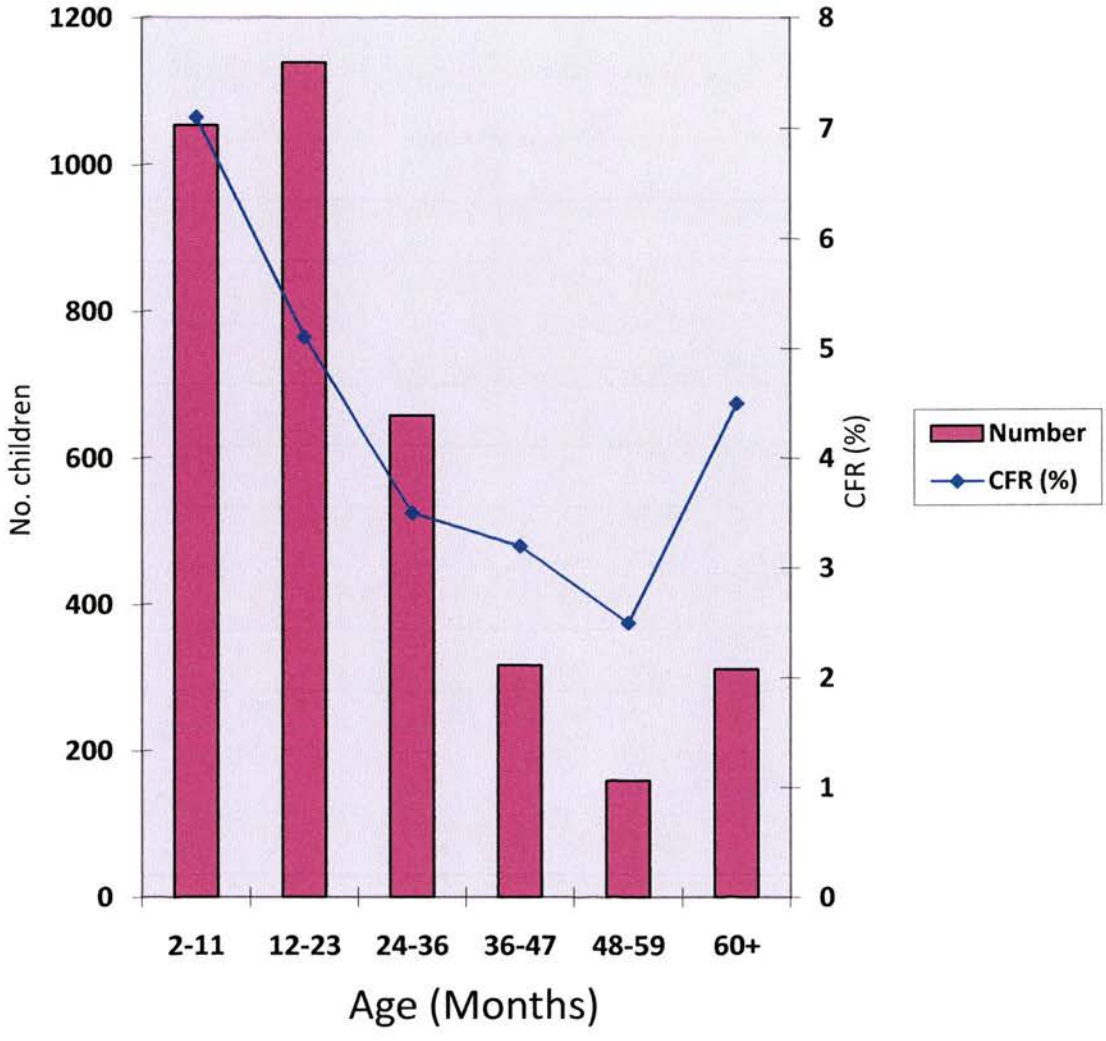


Figure 6. Distribution of children by age with case fatality rate

Characteristic	Frequency n/N (%)
Referred from an inpatient facility	151/3,635 (4.2%)
Fever for over 7 days	180/3,635 (5.0%)
Diarrhoea	534/3,638 (14.7%)
Vomiting	892/3,637 (24.5%)
Cough or difficulty breathing	2,364/3,638 (65.0%)
Convulsions	444/3,631 (12.2%)
Taken oral antimalarial within 48hrs	1, 102/3,624 (30.4%)
Taken parenteral antimalarial within 48hrs	236/3,616 (6.5%)
Taken antibiotics within 48hrs	450/3,600 (12.5%)
Unconscious*	124/3,639 (3.4%)
Lethargic	507/3,637 (13.9%)
Meningitic†	54/3,639 (1.5%)
Respiratory distress‡	444/3,639 (12.2%)
Signs of circulatory shock§	825/3,446 (23.9%)
Signs of dehydration**	138/3,638 (3.8%)
Signs of malnutrition††	110/3,639 (3.0%)
Hypoxia (PaO2 < 90%)	78/3,574 (2.2%)
Severe anaemia (Hb <5g/dl)	576/3,639 (15.8%)
Hypoglycaemia (glucose<2.5 mmol/l)	117/3,639 (3.2%)
Hyperlactataemia (lactate>5mmol/l)	429/3,248 (13.2%)
WBC >15,000/µl	891/2,957 (30.1%)

Table 14. Characteristics of study children on admission.

* Blantyre coma score <3
† Neck stiffness or bulging fontanelle
‡ Chest indrawing or deep breathing
§ Systolic blood pressure <70-mmHg, capillary refill <3 sec or cool peripheries
** Sunken eyes or skin pinch > 2 seconds
†† Weight for height Z score <-3, visible severe wasting or bilateral pedal oedema

4.2 THE AETIOLOGY OF FEBRILE DISEASE & THE IMPLICATIONS OF INFECTIONS - RESULTS

The blood cultures from 252/3639 (6.9%) children yielded organisms that were considered contaminants or were polymicrobial (≥3 organisms). The case fatality rate in children with contaminated blood cultures was 6.7%, and did not differ significantly from that in those with non-contaminated blood cultures (CFR 4.9%, p=0.2). Children with contaminated blood cultures were considered to have a negative culture result for further analysis. One child with a contaminated blood culture had a significant growth from lumbar puncture (*Haemophilus influenzae* group b), this child has been considered to have invasive bacterial disease (see below). One child grew non-typhi salmonella from blood and *Strep. pneumoniae* from CSF, this child has been considered as having invasive pneumococcal disease for the purposes of analysis.

An infectious agent was identified in 2,507/3,639 (68.9%) of the study children. The most commonly found pathogen was *P.falciparum;* asexual parasitaemia being found in 2,195 (60.3%) children. Invasive bacterial disease (IBD), defined as a bacterial pathogen cultured from blood (336, 9.3% of children) or CSF (25, 0.7% of children), was found in 341 (9.4%) children and HIV in 142 (3.9%). Infections, coinfections and associated mortality are summarised in Fig 7.

Children with invasive bacterial disease were significantly more likely to die than those without bacterial disease (age adjusted OR 4.9; 95% confidence interval 3.5 – 6.9; p<0.001). This was true for children with positive blood slides for falciparum malaria (AOR 3.5, 95% confidence interval 1.9 – 6.5, p<0.001) and also children

with negative malaria slides (AOR 6.2, 95% confidence interval 4.0 – 9.7). In contrast the presence of falciparum malaria on a blood slide had no significant effect on the risk of death in children with invasive bacterial disease, though there was a trend to a reduced risk in children with positive blood slides (AOR 0.8, 95% confidence interval 0.6 – 1.0, p=0.089). This trend disappeared if invasive bacterial disease was adjusted for (AOR 1.05, p=0.770).

The importance of invasive bacterial disease as a cause of mortality appeared to be greatest in children under 1 year of age, where it was associated with more deaths than malaria. Table 15 describes the total number of deaths and case fatality rate by infectious agent and age for falciparum malaria and invasive bacterial disease. Although the numbers were small, children with falciparum malaria appeared to die sooner after admission than children with IBD alone (Table 16). Although children with malaria and IBD coinfection had a mortality rate dictated by the IBD, the time to death was more similar to that of the children with malaria alone.

4.2.1 THE SPECTRUM OF INVASIVE BACTERIA

Overall, the most common bacterial isolates were non-typhi Salmonellae (46.9%), followed by *Strep. pneumoniae* (16.4%) and *Haemophilus influenzae* (11.4%). The only age group in which non-typhi salmonella (NTS) was not the most common isolate was in children over 5 years age, where both *Strep. pneumoniae* and *S. typhi* were more common. Non-typhi salmonella were also associated with more deaths than any other organism. (Table 17) *Salmonella enterica* serovar Typhimurium made up the bulk of the NTS isolates (137/161, 85.1%), with *Salmonella enterica* serovar Enteritidis the next largest group (11/161, 6.8%).

4.2.2 CHILDREN WITH NEGATIVE BLOOD SLIDES FOR MALARIA

Mortality in children without malaria was significantly higher than in children with malaria (89/1,444, 6.2% compared with 95/2,195, 4.3%, p=0.013). Children with negative blood slides had a higher incidence of invasive bacterial disease (241/1,444, 16.7% compared with 100/2195, 4.6%, p<0.001), however the mortality of children with invasive bacterial disease was no higher in children with malaria parasitaemia (13/100, 13%) compared with children without malaria (45/241, 18.7%, p=0.204). The most common bacterial pathogen isolated in slide negative children remained non-typhi Salmonella (108/241, 44.8%) with *Strep. pneumoniae* and *Haemophilus influenzae* accounting for 18.7% and 14.5% of positive isolates in children with negative blood slides.

Children with a positive *P. falciparum* HRP-2 based rapid diagnostic test and a negative blood slide can be considered to have had recent malaria.[118] Children with recent malaria had a higher risk of invasive bacterial disease than other slide negative children (AOR 1.4, p=0.028). In these children almost 1 in 5 children had invasive bacterial disease (98/501, 19.6%); NTS was the most common bacterial pathogen, accounting for 68.4% of isolates. In contrast only 28.7% of bacterial isolates in children with negative slides and rapid diagnostic tests were NTS. (Table 18)

4.2.3 COINFECTION WITH P. FALCIPARUM.

Falciparum malaria was present in 100/341 (29.3%) children with invasive bacterial disease. Co-infected children thus represented 100/2,195 (4.6%) of the total with malaria. Invasive bacterial disease was more common in children at low densities of

P. falciparum infection than at high density; in children with a *P. falciparum* parasite density of between 1 and 5000 parasites/µl a bacterial pathogen was cultured in 33/405 (8.1%) as compared with 67/1,790 (3.7%) of children with over 5,000 parasites/ µl ($p<0.001$). However the rate of invasive bacterial disease observed in children with parasite densities above 50,000/µl (31/917, 3.4%) did not differ significantly from that in children with between 5000 and 50,000 parasites/µl (36/873, 4.1%, $p=0.408$). There was a significant increase in mortality associated with invasive bacterial disease in children with low (1-5000 parasites/µl) density malaria (AOR of 5.0, 95% confidence interval 1.6 – 15.6, $p=0.006$) and in children with a high (> 5000 parasites/µl) parasite density (AOR 3.2, 95% confidence interval 1.4 – 6.9, $p=0.004$). (Fig 8)

Gram negative organisms made up the bulk of the organisms isolated regardless of the results of slide or rapid test results. However in children with neither current nor recent malaria NTS made up 28.7% of the positive isolates as compared with 68.4% in children with evidence of recent malaria and 52% of those with a current parasitaemia. In children with no evidence of current or recent malaria *Haemophilus influenzae* and *Strep. pneumoniae* caused a correspondingly larger share of invasive bacterial disease. (Table 18) Analysis of the organisms isolated by parasite density shows that NTS causes a smaller proportion of bacterial infections at higher densities of falciparum malaria although Gram negative isolates still dominated with a wider array of organisms in this group. (Fig 9)

Severe malaria

Children were retrospectively categorized as severe malaria cases according to a number of definitions used in the published literature[117 136-137] and the current WHO

definition.[1] It was then possible to assess the effect of changing definitions of severe malaria on the prevalence of bacterial co-infection and its effect on mortality. Other than for this comparison we have used the WHO definitions of severe malaria.[1] One 25 month old child with lethargy, a stiff neck and hypoglycaemia grew *Haemophilus influenzae* from blood and CSF and had a concurrent malarial parasitaemia of 20,000 parasites/μl. There were no other cases of meningitis amongst the severe malaria patients.

Prevalence of invasive bacterial disease remained fairly constant across the definitions, though some of the definitions for severe disease selected children with a higher mortality. In all definitions, other than the 'Bronzan criteria' (defining severe malaria as children in coma or with severe anaemia and asexual parasites on a blood film), children with severe malaria and invasive bacterial disease had a case fatality rate significantly higher than those with severe malaria alone (Table 19).

Gram negative organisms caused a similar proportion of the invasive bacterial disease in children with severe malaria (35/47, 74.5%) as non-severe malaria (43/53, 81.1%, p=0.422). NTS made up the bulk of these (22/35, 63%). (Table 20)

The prevalence of invasive bacterial disease in children aged 2 months to 1 year was 6.5%, in children aged 1 to 2 years 6.8% and in children aged 2 to 5 years 5.1%. There were no cases of invasive bacterial disease in children with severe malaria aged 5 years and above.

Invasive bacterial disease in severe malaria was associated with severe anaemia, with or without other clinical features, in 31/47 (66%) cases. (Fig 10) NTS comprised

18/31 (58%) of the isolates in children with severe malaria and severe anaemia but only 4/16 (25%) of those with severe malaria without severe anaemia.

4.2.4 HIV ASSOCIATED INFECTION

There were 142/3,369 (3.9%) cases of confirmed HIV infection. In children over 5 years age HIV infection was confirmed in 30/312 (9.6%) as compared to 112/3,327 (3.4%) children under 5 years age ($p<0.001$). Invasive bacterial disease was more common in children with HIV, occurring in 27/142 (19.0%) children with HIV infection but only 314/3,497 (9.0%) of children without HIV (AOR 2.5, 95% confidence interval 1.6 – 3.9, $p<0.001$). In contrast only 48/142 (33.8%) of children admitted with HIV had a *P.falciparum* parasitaemia as compared to 2,147/3,497 (61.4%) of children without HIV (AOR 0.3, 95% confidence interval 0.2 – 0.5, $p<0.001$). The caregivers of 15/142 children admitted with HIV told research staff the child had been given cotrimoxazole prior to admission.

In children admitted with HIV infection the most common isolate was *Strep pneumoniae*, with a prevalence of 9.2% in this population. NTS remained common in this group with a prevalence of 5.6%, though this was not statistically different from its prevalence in admissions without HIV (4.3%, $p=0.463$). (Table 21)

Children with HIV had a higher case fatality rate (18/142, 12.7%) than children without HIV (166/3,497, 4.7%, $p<0.001$). Out of the 18 deaths in children with HIV infection 6 children had a falciparum parasitaemia (3 with >50,000 parasites/µl, 2 with 5,000 – 50,000 parasites/µl and 1 with ≤5,000 parasites/µl); 6 children had invasive bacterial disease (2 cases of NTS, 1 each of *Strep. pneumoniae*, *Haemophilus influenzae*, *Staph aureus* and *E. coli*).

4.2.5 INFECTIONS ASSOCIATED WITH MALNUTRITION

Severe acute malnutrition (SAM), defined as a weight for height Z score less than -3, severe wasting or bilateral pedal oedema, was found in 110 children. Children with SAM were significantly more likely to have invasive bacterial disease than children without SAM (AOR 3.1, 95% confidence interval 1.9 – 4.9, p<0.001). Although non-typhi salmonellae (NTS) were the most common bacterial isolate in children with SAM, the proportion of positive isolates in children with SAM that were NTS (8/25, 32%) was lower than in children without SAM (152/316, 48.1%), in contrast *Strep. pneumoniae* was more common in children with SAM (7/25, 28%) than children without SAM (49/316, 15.5%) although neither of these differences reached statistical significance. (Table 22)

Children admitted with SAM had a higher prevalence of HIV infection (25/110, 22.7%) compared to those without SAM (117/3,529, 3.3%, p<0.001). There were only 4 HIV positive children with SAM and proven invasive bacterial disease, in 3 of these children NTS was isolated from blood, in one child *Strep. pneumoniae* was isolated from blood.

Mortality in children with SAM was raised (18/110, 16.4%) compared with children without SAM (166/3,529, 4.7%, p<0.001). Of the 18 children who died with SAM, 8 were associated with invasive bacterial disease (1 of whom had concurrent falciparum parasitaemia), 4 had falciparum malaria alone, 2 had HIV infection with no other pathogens found and in 4 children no pathogen was isolated.

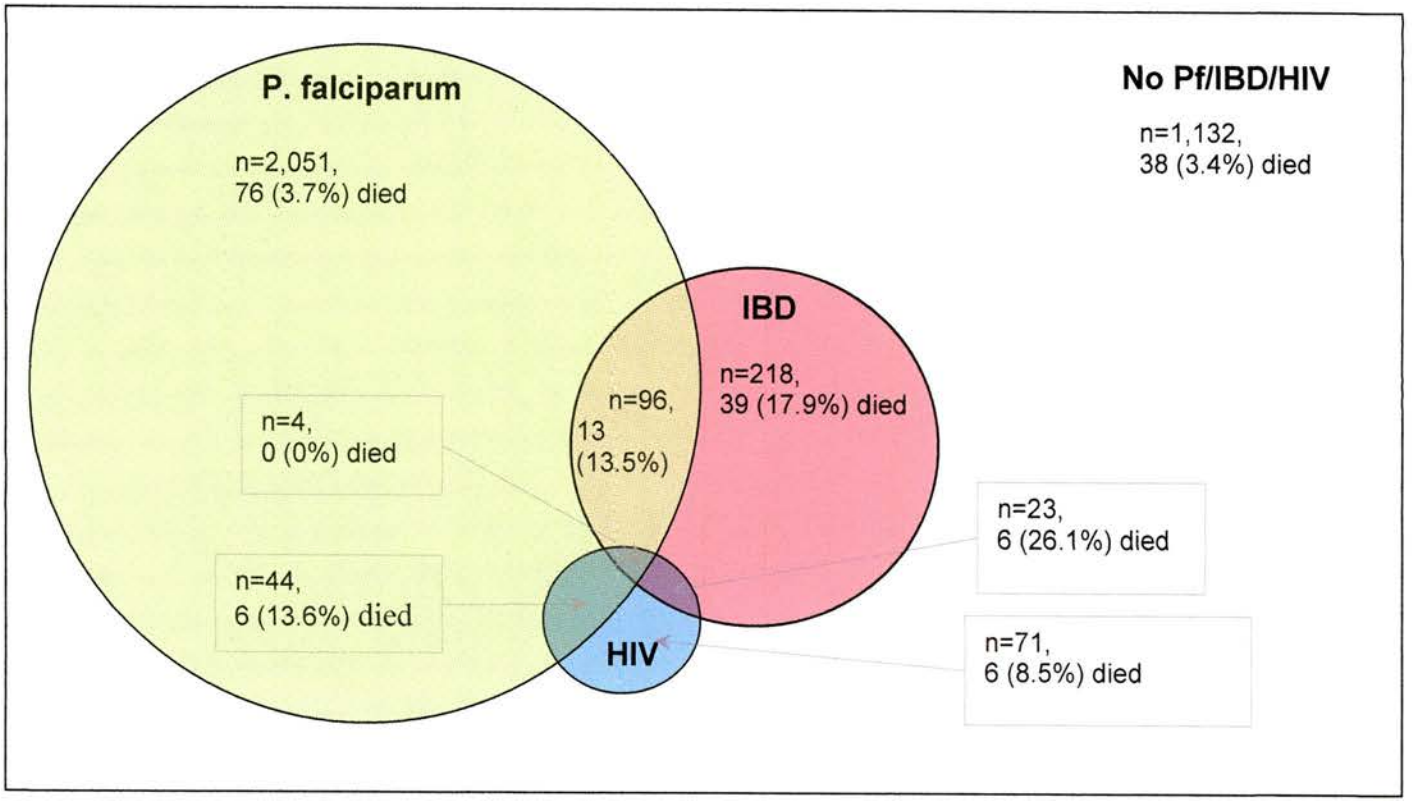


Figure 7. Numbers and deaths (CFR) of children infected with *P.falciparum*, IBD or HIV infection.

	Deaths/Total cases (CFR %)				
Age	**P.f only**	**IBD only**	**Co-infection**	**Nil found**	**Overall**
2mo-1yr	20/461 (4.3%)	22/95 (23.2%)	6/26 (23.1%)	27/472 (5.7%)	75/1054 (7.1%)
1-2yr	32/695 (4.6%)	12/68 (17.6%)	4/45 (8.9%)	10/331 (3%)	58/1139 (5.1%)
2-5yr	25/782 (3.2%)	5/53 (9.4%)	3/29 (10.3%)	4/270 (1.5%)	37/1134 (3.3%)
5yr+	5/157 (3.2%)	6/25 (24%)	0	3/130 (2.3%)	14/312 (4.5%)
Total	82/2095 (3.9%)	45/241 (18.7%)	13/100 (13%)	44/1203 (3.7%)	184/3639 (5.1%)

Table 15. Distribution of deaths and cases by infection with malaria and invasive bacterial disease (IBD). Case fatality rates in parenthesis.

	No. deaths	**Median time to death**
P.falciparum only	73	11 hr 54min
P. falciparum & IBD	11	12 hr 20min
IBD only	39	21 hr 25min
IBD & HIV	5	31hr 58min
HIV alone	6	51hrs 25min
P.falciparum & HIV	6	59hr 9min
P.falciparum & HIV & IBD	0	N/A

Table 16. Median time to death for different infections where time of death was recorded

BLANK PAGE

Bacterial Isolates	Age Group				Total N (%)	Deaths/Total (CFR %)
	2-11mo	1 – 2 yrs	2-5 yrs	5 yrs +		
Gram positive						
Strep. pneumoniae	18 (1.7)	16 (1.4)	13 (1.1)	9 (2.9)	56 (1.5)	11/56 (19.6)
Staph. aureus	4 (0.4)	6 (0.5)	4 (0.4)	3 (1.0)	17 (0.5)	5/17 (29.4)
Other Gram pos*	3 (0.3)	3 (0.3)	2 (0.2)	0	8 (0.2)	1/8 (12.5)
Gram negative						
NTS	55 (5.2)	60 (5.3)	41 (3.6)	4 (1.3)	160‡ (4.4)	18/160 (11.25)
H. influenzae b	17 (1.6)	12 (1.1)	7 (0.6)	3 (1.0)	39 (1.1)	13/39 (33.3)
E. coli	13 (1,2)	7 (0.6)	3 (0.3)	0	23 (0.6)	3/23 (13.0)
S. typhi	0	2 (0.2)	3 (0.3)	6 (1.9)	11 (0.3)	2/11 (18.2)
Other Gram neg†	11 (1.0)	7 (0.6)	9 (0.8)	0	27 (0.7)	5/27 (18.5)
Total (any IBD)	121 (11.5)	113 (9.9)	82 (7.2)	25 (8.0)	341 (9.4)	58/341 (17.0)
No IBD	933 (88.5)	1,026 (90.1)	1,052 (92.8)	287 (92.0)	3,298 (90.6)	126/3,298 (3.8)
Total	1,054 (100)	1,139 (100)	1,134(100)	312 (100)	3,639 (100)	184/3,639 (5.1)

Table 17. Bacterial isolates by age with prevalence and case fatality. Footnotes facing page.

Bacterial isolate	Slide negative RDT negative	Slide negative RDT positive*	Slide Positive
Gram positive			
Strep. pneumoniae	34 (23.8%)	11 (11.2%)	11 (11%)
Staph. aureus	6 (4.2%)	3 (3.1%)	8 (8%)
Other Gram pos	4 (2.8%)	1 (1%)	3 (3%)
Gram negative			
NTS	41 (28.7%)	67 (68.4%)	52 (52%)
H. influenzae b	29 (20.3%)	6 (6.1%)	4 (4%)
E. coli	11 (7.7%)	9 (9.2%)	3 (3%)
S. typhi	9 (6.3%)	0	2 (2%)
Other Gram neg	9 (6.3%)	1 (1.0%)	17 (17%)
Total (any IBD)	143 (100%)	98 (100%)	100 (100%)

Table 18. Bacterial isolates by blood slide and rapid diagnostic test results.

* Considered as having 'recent malaria'

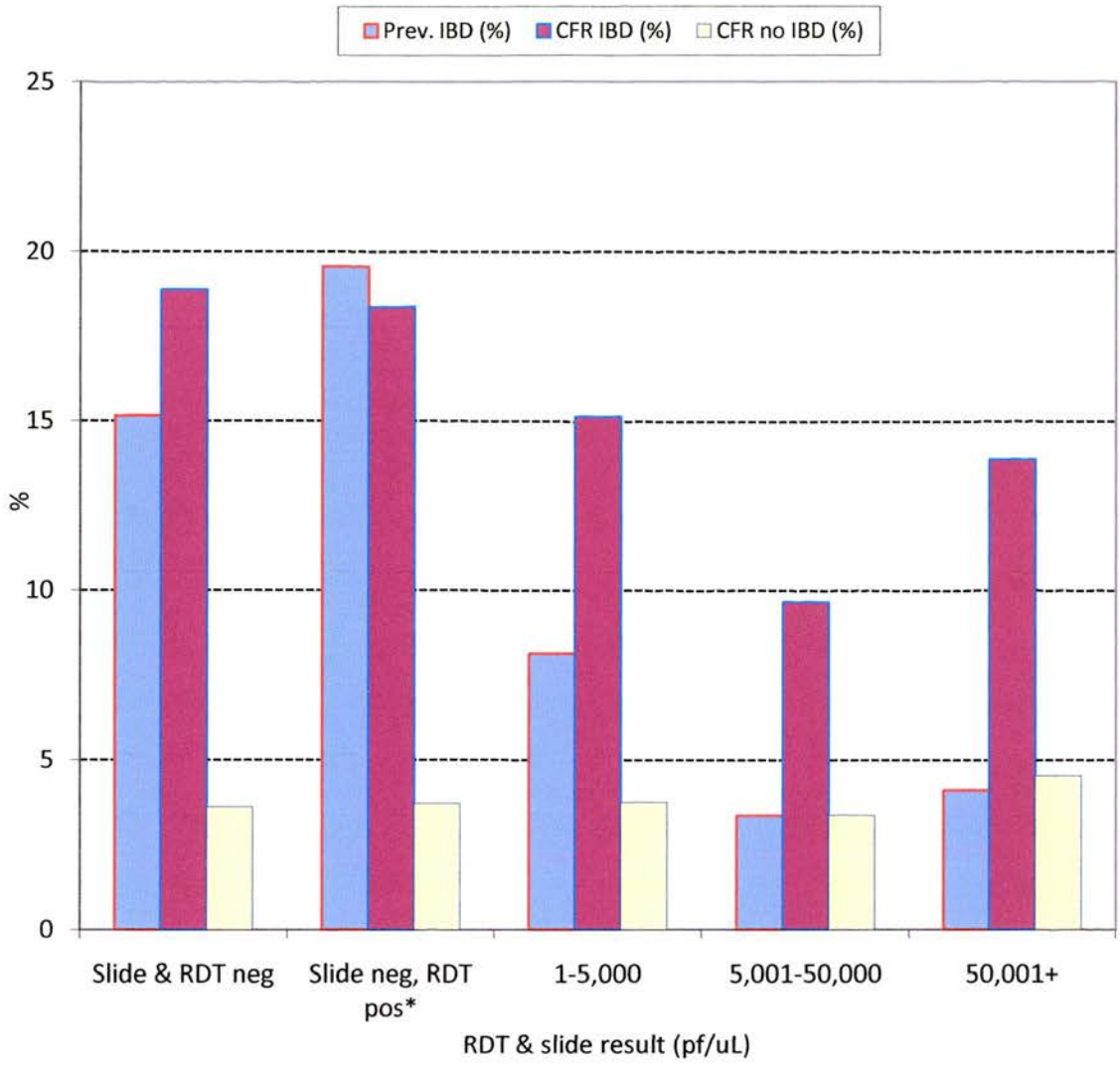


Figure 8. Prevalence of invasive bacterial disease and case fatality rate in children with and without invasive bacterial disease by slide and malaria rapid diagnostic test result.

* Probable recent malaria

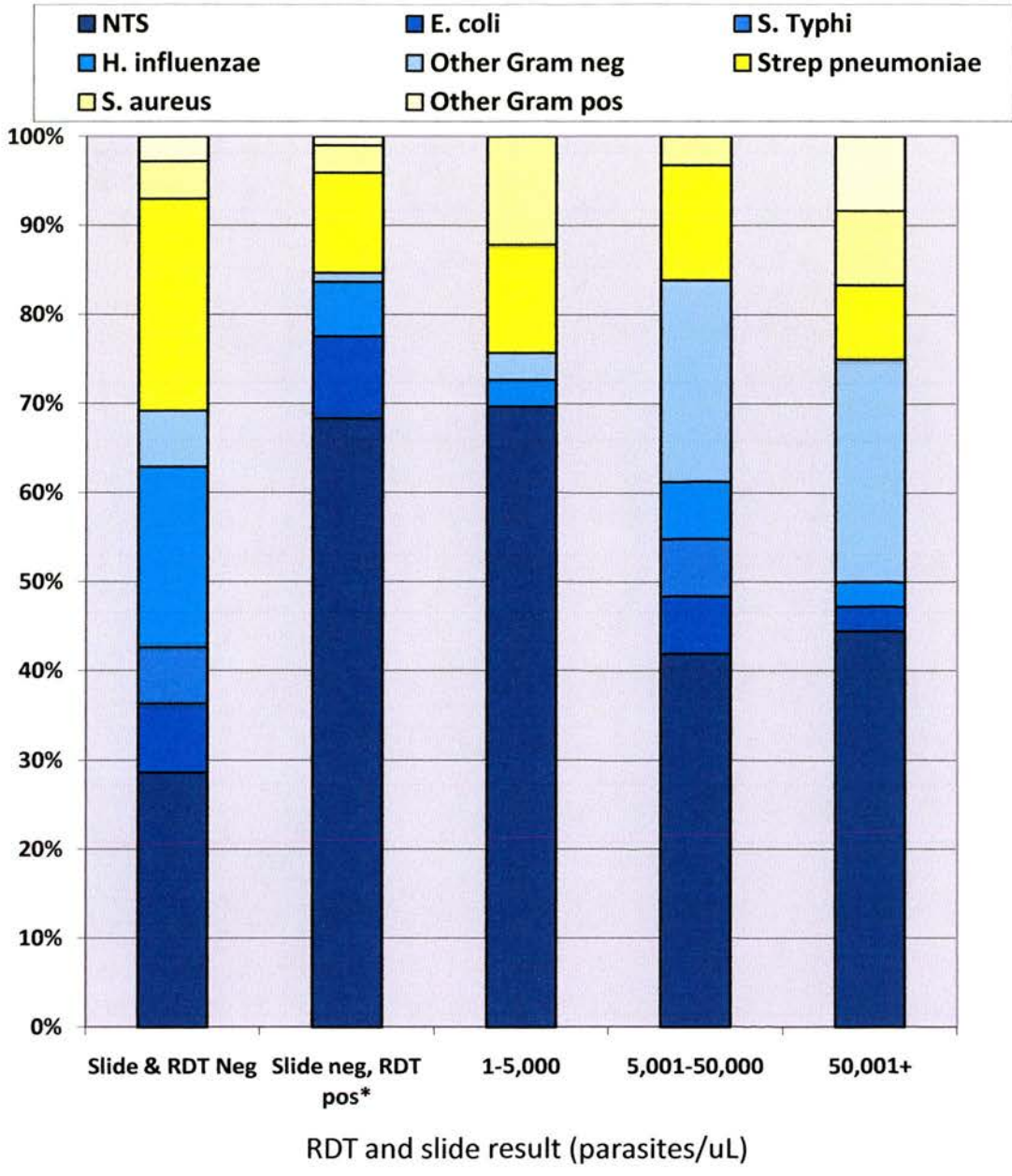


Figure 9. Proportion of isolated bacterial organisms by P falciparum density and rapid diagnostic test results.

* Probable recent malaria

	This study			Historic studies			
	'Berkley criteria'[a]	'Evans Criteria'[b]	'Bronzan Criteria'[c]	WHO criteria[d]	Berkley et. a.l[117]	Evans et. al.[136]	Bronzan et. al.[137]
Prevalence of IBD	30/443 (6.8%)	45/671 (6.7%)	30/446 (6.7%)	47/823 (5.7%)	42/540 (7.8%)	23/182 (12.6%)	64/1388 (4.6%)
Mortality Deaths/Total (CFR%)							
No IBD	66/413 (16.0%)*	68/626 (10.9%)*	45/416 (10.8%)†	75/776 (9.7%)*	10.4%*	8.8%	16.0%‡
IBD	10/30 (33.3%)*	12/45 (26.7%)*	5/30 (16.7%)†	12/47 (25.5%)*	33.3%*	8.7%	21.8%‡
OR	2.5 (1.1-5.7)	3.0 (1.4–6.0)	1.6 (0.6–4.3)	3.2 (1.6–6.5)			

Table 19. Prevalence of invasive bacterial disease (IBD) and mortality in children with severe malaria by several definitions, with comparison to historic studies.

[a] Prostration, impaired consciousness or respiratory distress
[b] Coma, prostration, respiratory distress or severe anaemia
[c] Coma or severe anaemia
[d] Coma, prostration, multiple convulsions, respiratory distress, severe anaemia, jaundice, hypoglycaemia, hyperlactataemia.
* $p<0.05$
† $p=0.327$
‡ $p=0.22$

	Severe malaria		Non-severe malaria	
Bacterial Isolate	**No. isolates**	**Deaths**	**No. isolates**	**Deaths**
Gram positive				
Strep. pneumoniae	6	2	5	1
Staphylococcus aureus	3	1	5	0
Group A Streptococcus	2	1	0	0
Group B Streptococcus	1	0	0	0
Gram negative				
Non-typhi Salmonella	22	4	30	0
H. influenzae b	4	1	0	0
Escherichia coli	2	0	1	0
Salmonella typhi	1	0	1	0
Acinetobacter sp	1	0	3	0
Pantoea sp.	1	0	2	0
Citrobacter	1	0	1	0
Kingella kingii	1	1	0	0
Klebsiella pneumoniae	1	1	0	0
Pseu. aeruginosa	1	1	1	0
Campylobacter	0	0	1	0
Neisseria sp.	0	0	1	0
Other Gram neg orgs	0	0	2	0

Table 20. Bacterial isolates in children with severe & non-severe malaria (WHO criteria)

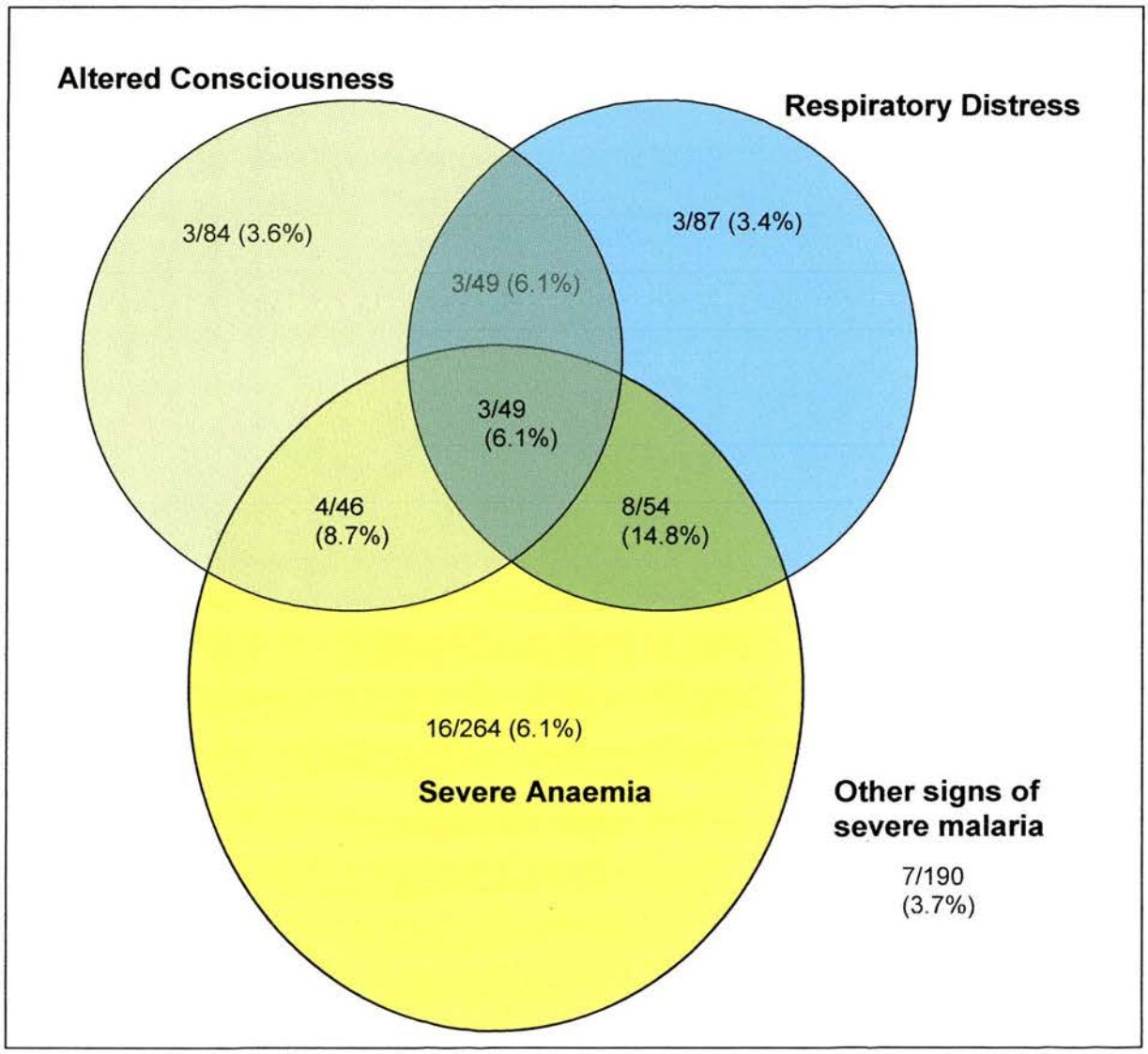


Figure 10. Prevalence of invasive bacterial disease amongst childrenwith severe malaria, by syndrome.

	HIV Positive		HIV negative	
Bacterial Isolate	**No. isolates**	**Deaths**	**No. isolates**	**Deaths**
Gram positive				
Strep. Pneumoniae	13	1	43	10
Staph. aureus	1	1	16	4
Other Gram pos. orgs	-	-	8	1
Gram negative				
Non-typhi Salmonella	8	2	152	16
H. influenzae b	1	1	38	12
Escherichia coli	2	1	21	2
Salmonella typhi	-	-	11	2
Other Gram neg. orgs	2	0	25	5
Total	27	6	314	52

Table 21. Bacterial isolates in children with and without HIV infection.

	Severe Acute Malnutrition		No Severe Acute Malnutrition	
Bacterial Isolate	**No. isolates**	**Deaths**	**No. isolates**	**Deaths**
Gram positive				
Strep. Pneumoniae	7	4	49	7
Staphylococcus aureus	2	1	15	4
Other Gram positive orgs	1	0	26	4
Gram negative				
Non-typhi Salmonella	8	0	152	18
Haemophilus influenzae b	2	1	37	12
Escherichia coli	3	1	20	2
Salmonella typhi	1	0	10	2
Other Gram neg. orgs	1	1	7	1
Total	25	8	316	50

Table 22. Bacterial isolates in children with and without severe acute malnutrition.

4.3 CAN THOSE CHILDREN AT RISK OF DEATH BE RELIABLY PREDICTED AT THE BEDSIDE?

4.3.1 MISSING DATA

Supply problems resulted in a proportion of children not having glucose or lactate measurement performed. For both blood lactate and glucose these children represented a cohort defined by time rather than selected by the research team. In 391/3,639 (10.7%) children supply problems resulted in no blood lactate being available, in these children acidosis was defined clinically. Mortality in children who had no blood lactate measured (20/391, 5.1%) was not significantly different to mortality in children in whom lactate was measured (164/3,248, 5.0%, p=0.955). In 412/3,639 (11.3%) children there was no record of blood glucose available. In children in whom a blood glucose was not recorded mortality (17/412, 4.1%) did not differ significantly from those in whom it was recorded (167/3,227, 5.2%, p=0.36).

4.3.2 CHILDREN WITH POSITIVE MALARIA SLIDES

Of 184 deaths in study children 95 (51.6%) were in children with falciparum parasitaemia. Girls had a higher mortality from malaria (54/1,009, 5.4%) than boys (41/1,186, 3.5%, age adjusted OR 1.59, p=0.03). There were several clinical features associated with death in age-adjusted analysis (Table 23). These features were added sequentially to a logistic regression model in order of those with the highest OR first, features that became non-significantly associated with death were dropped. The resulting multivariate model showed that severe malnutrition and prostration or coma were the clinical signs with the highest association with death (AOR 8.82 and 5.24

respectively). Bedside laboratory tests showing hypoglycaemia (blood glucose <2.5mmol/l), hyperlactataemia (blood lactate >5mmol/l) and hypoxia by oximetry (oxygen saturations <90%) were all associated with increased mortality, whilst children with severe anaemia had a significantly reduced risk of death in the multivariate model (AOR 0.50, 95% confidence interval 0.26 – 0.98, p=0.044). Female sex remained a significant risk factor for death from malaria in children (AOR 1.89, p=0.026). (Table 24)

We retrospectively assigned children into severe and non-severe malaria categories by WHO criteria[1] as defined in Box 3; 823/2,195 (37.5%) parasitaemic children were thus considered as having severe malaria. Equal proportions of male (435/1,186, 36.7%) and female (388/1,009, 38.5%, p=0.392) parasitaemic children were considered to have severe malaria. Mortality in children with severe malaria was 87/823 (10.6%) compared with 8/1,372 (0.6%) in children with non-severe malaria (OR 20.15, 95% confidence interval 9.72 – 41.81, p<0.001). Whilst there was no difference between case fatality rates for non-severe malaria between boys (4/751, 0.5%) and girls (4/621, 0.6%, p=0.787), girls with severe malaria had a higher case fatality rate (50/388, 12.9%) than boys (37/435, 8.5%, p=0.041). Severe malaria identified 87/95 (91.6%) of deaths in children with a positive blood slide for malaria. Of the 8 deaths not identified 2 had concomitant HIV infection and 1 had invasive bacterial disease (*Strep. pneumoniae*), 6 died 3 days or more after admission. Of the children that died with severe malaria, 75/87 (86.2%) had evidence of acidosis, 60/87 (69%) had altered consciousness and 36/87 (41.4%) had severe anaemia. Mortality in children with severe anaemia alone was low (2/195, 1%) and one of these children was admitted hypoxic with kwashiorkor and pneumococcal pneumonia in addition to

severe anaemia and malaria. A larger proportion of boys with severe malaria had severe anaemia alone (122/435, 28%) than girls (73/388, 18.8%, p=0.002). There were no significant differences between the sexes in the proportions of children with severe malaria that had either acidosis or altered consciousness. The mortality of children with severe malaria by syndrome is depicted in Fig 11.

When the severe malaria definitions were altered to a set that involved clinical findings only with maximal sensitivity (any of severe pallor, deep breathing, lower chest wall or intercostal recession, nasal flaring, prostration, lethargy or coma and jaundice) 859 children were identified including 84/95 (88.4%) deaths in children with malaria parasitaemia and giving a group with a case fatality rate of 9.8%.

4.3.2.1 Children with malaria and altered consciousness

Of children with altered consciousness and malaria 92/235 (39.1%) had true 'cerebral' malaria (Blantyre coma score ≤ 2). Children with cerebral malaria had a significantly higher case fatality rate (34/92, 37.0%) compared with children with malaria and less severe altered consciousness (26/143, 18.2%, p=0.001). Factors associated with mortality in children with malaria and altered consciousness included acidosis (OR 6.38, 95% confidence interval 2.75– 14.81, p<0.001), hypoglycaemia (OR 5.61, 95% confidence interval 1.83 – 11.12, p<0.001) and invasive bacterial disease (OR 5.23, 95% confidence interval 1.64 – 16.68, p=0.005). Severe anaemia was not associated with mortality in children with altered consciousness and malaria (OR 1.07, 95% confidence interval 0.59 – 1.94, p=0.820).

4.3.2.2 Children with malaria and acidosis

Acidosis was defined as respiratory distress (deep breathing and/or chest wall indrawing) or a raised blood lactate (>5mmol/l). Of parasitaemic children with clinical or laboratory signs of acidosis 400/434 (92.2%) had a blood lactate measured and 338/434 (77.9%) of these children had a blood lactate over 5mmol/l. Both raised lactate (>5mmol/l) and respiratory distress in parasitaemic children were associated with mortality by univariate and multivariate analysis (Table 23 & 24). Higher values for blood lactate were associated with increasing mortality; mortality in parasitaemic children with a blood lactate between 5 and 10mmol/l was 26/230 (11.3%), rising to 22/91 (24.2%) of children with blood lactate between 10mmol/l and 15mmol/l and 9/17 (52.9%) in children with a blood lactate >15mmol/l ($p<0.001$).

4.3.2.3 Children with severe malaria anaemia

Of parasitaemic children with severe anaemia 36/413 (8.7%) died compared with 59/1,782 (3.3%) of parasitaemic children with haemoglobin results over 5g/dl at admission ($p<0.001$). Mortality in parasitaemic children with lower haemoglobin had a higher mortality (15/113, 13.3% for those with admission haemoglobin under 3.5g/dl died compared with 21/300, 7% of those with admission haemoglobin between 3.5 and 5g/dl, $p=0.044$).

4.3.3 Children with negative malaria slides

Although there were fewer deaths in slide negative children, the mortality in slide negative children was higher than in slide positive children (89/1,444, 6.2% compared with 95/2,195, 4.3% in slide positive children, $p=0.013$). In contrast to

children with positive slides, there was no difference in mortality between male (46/784, 5.9%) and female children (43/660, 6.5%, p=0.61) with negative slides for malaria. Most factors associated with death in slide positive children by age-adjusted analysis were also associated with death in slide negative children. In addition, signs of meningism (a bulging fontanelle or stiff neck) were associated with an increased risk of death by age-adjusted analysis in slide negative children, but not in slide positive children. (Table 25) A similar methodology to that performed in slide positive children for creating a multivariate model for mortality was performed in slide negative children. Hypoglycaemia and prostration or coma were the features most strongly associated with death with odds ratios of 8.47 (95% confidence interval 2.49 – 28.79, p=0.001) and 6.31 (95% confidence interval 3.18 – 12.52, p<0.001) respectively. (Table 26)

Children with any one of the factors associated with mortality in the multivariate model available at the bedside (hypoglycaemia, prostration/coma, hyperlactataemia, severe malnutrition and respiratory distress) had a significantly higher risk of death (OR 10.76, 95% confidence interval 6.53 – 17.71, p<0.001) but only accounted for 67/89 (75.3%) of deaths in aparasitaemic children.

When the clinical features of WHO-defined severe malaria were applied to slide negative children the resulting group of children also included 67/89 (75.3%) of deaths in aparasitaemic children. When the two definitions were combined, because of significant overlap, 70/89 (78.7%) of deaths in aparasitaemic children were identified.

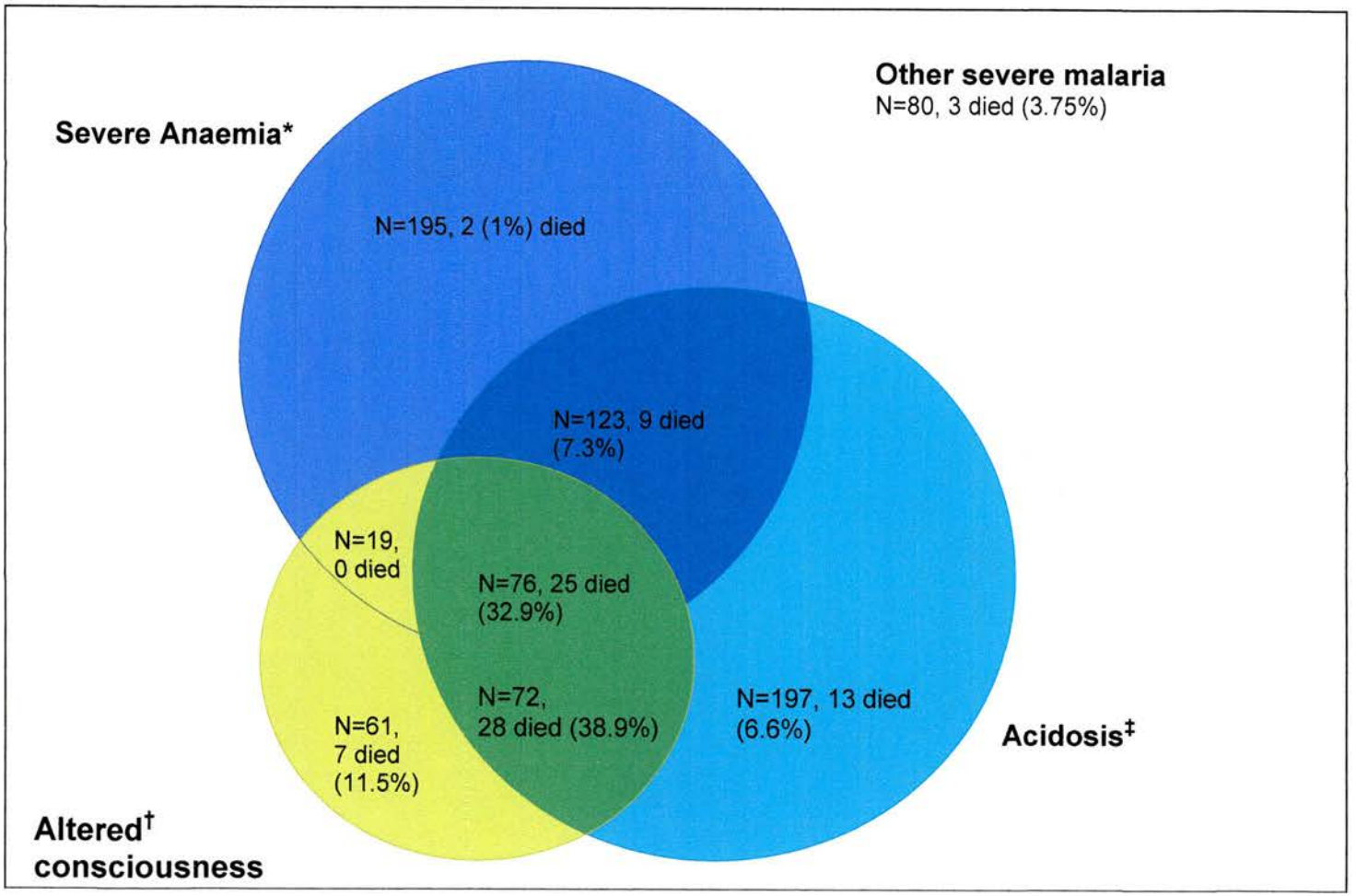


Figure 11. Distribution of children with severe malaria by syndrome. Case fatality rate in parenthesis.
*Hb <5g/dl
†Blantyre coma score <5
‡ Respiratory distress or lactate >5mmol/L, in 274 parasitaemic children no blood lactate was available and acidosis was based on clinical signs alone.
8 deaths occurred in the 1, 372 children with non-severe malaria (CFR 0.6%)

Characteristic	CFR n/N (%)	AOR	95% CI	P
Female sex	54/1,009 (5.4%)	1.59	1.05 – 2.41	0.030
Convulsions	36/329 (10.9%)	3.84	2.48 – 5.92	<0.001
Vomiting everything	3/40 (7.5%)	1.86	0.56 – 6.18	0.309
Unable to drink	53/180 (29.4%)	21.96	13.92 – 34.66	<0.001
Lethargy	64/363 (17.6%)	13.32	8.48 – 20.93	<0.001
Respiratory distress*	53/239 (22.2%)	13.01	8.37 – 20.22	<0.001
Signs of circulatory shock†	35/548 (6.4%)	1.83	1.16 – 2.89	0.009
Prostration or coma‡	65/308 (21.1%)	19.13	11.97 – 30.57	<0.001
Meningism§	1/19 (5.3%)	1.14	0.15 – 8.75	0.898
Signs of malnutrition**	5/30 (16.7%)	4.64	1.73 – 12.49	0.002
Hypoxia (PaO2 < 90%)	13/39 (33.3%)	14.15	6.93 – 28.89	<0.001
Severe anaemia (Hb <5g/dl)	36/413 (8.7%)	2.71	1.76 – 4.18	<0.001
Hypoglycaemia (glucose<2.5mmol/l)	30/85 (35.3%)	18.35	10.89 – 30.91	<0.001
Hyperlactataemia (Lactate >5mmol/l)	57/338 (16.9%)	12.53	7.67 – 20.46	<0.001
Invasive bacterial disease	13/100 (13%)	3.49	1.86 – 6.55	<0.001
HIV positive	6/48 (12.5%)	3.85	1.57 – 9.47	0.003

Table 23. Clinical features with associated case fatality rates and age-adjusted odds ratios for death in children with positive blood slides for malaria.

*Lower chest wall indrawing or deep breathing
† Systolic blood pressure <70-mmHg, capillary refill <3 sec or cool peripheries
‡ Blantyre coma score <3 or unable to sit/drink
§Stiff neck or bulging fontanelle
** Weight for height Z score <-3, visible severe wasting or bilateral pedal oedema

Characteristic	AOR	95% CI	P
Signs of malnutrition*	9.32	2.62 – 33.22	0.001
Prostration or coma†	5.06	2.66 – 9.63	<0.001
Hypoglycaemia (glucose<2.5mmol/l)	4.59	2.22 – 9.49	<0.001
Hyperlactataemia (Lactate >5mmol/l)	3.98	2.10 – 7.55	<0.001
Hypoxia (PaO2 < 90%)	3.96	1.55 – 10.10	0.004
Invasive bacterial disease	3.83	1.61 – 9.11	0.002
Respiratory distress‡	3.55	1.91 – 6.58	<0.001
Female sex	1.89	1.08 – 3.32	0.026
Severe anaemia (Hb <5g/dl)	0.50	0.26 – 0.98	0.044

Table 24. Multivariate model showing risk factors associated with death in slide positive children.

* Weight for height Z score <-3, visible severe wasting or bilateral pedal oedema
† Blantyre coma score <3 or unable to sit/drink
‡Deep breathing/lower chest wall indrawing

Characteristic	CFR n/N (%)	AOR	95% CI	P
Female sex	453/660 (6.5%)	1.11	0.72 – 1.72	0.623
Convulsions	18/115 (15.7%)	3.50	1.98 – 6.17	<0.001
Vomiting everything	2/48 (4.2%)	0.61	0.15 – 2.57	0.5
Unable to drink	31/77 (40.3%)	17.51	10.09 – 30.38	<0.001
Lethargy	41/144 (28.5%)	10.82	6.75 – 17.34	<0.001
Respiratory distress*	45/205 (22.0%)	6.95	4.39 – 10.99	<0.001
Signs of circulatory shock†	32/277 (11.6%)	2.73	1.68 – 4.45	<0.001
Prostration or coma‡	37/102 (36.3%)	15.97	9.59 – 26.58	<0.001
Meningism§	7/35 (20%)	3.62	1.50 – 8.72	0.004
Signs of malnutrition**	13/80 (16.25%)	3.76	1.95 – 7.24	<0.001
Hypoxia (PaO2 < 90%)	12/39 (30.8%)	2.91	3.74 – 16.21	<0.001
Severe anaemia (Hb <5g/dl)	17/163 (10.4%)	2.18	1.24 – 3.82	0.007
Hypoglycaemia (glucose<2.5mmol/l)	16/32 (50%)	26.45	11.95 – 58.55	<0.001
Hyperlactataemia (Lactate >5mmol/l)	35/91 (38.5%)	4.57	10.02 – 28.80	<0.001
Invasive bacterial disease	45/241 (18.7%)	6.19	3.96 – 9.67	<0.001
HIV positive	12/94 (6.2%)	2.60	1.34 – 5.01	0.005

Table 25. Clinical features with associated case fatality rates and age-adjusted odds ratios for death in children with negative blood slides for malaria.

*Lower chest wall indrawing or deep breathing
† Systolic blood pressure <70-mmHg, capillary refill <3 sec or cool peripheries
‡ Blantyre coma score <3 or unable to sit/drink
§Stiff neck or bulging fontanelle
** Weight for height Z score <-3, visible severe wasting or bilateral pedal oedema

Characteristic	AOR	95% CI	P
Hypoglycaemia (glucose<2.5mmol/l)	8.47	2.49 – 28.79	0.001
Prostration or coma	6.31	3.18 – 12.52	<0.001
Hyperlactataemia (Lactate >5mmol/l)	5.45	2.77 – 10.70	<0.001
Invasive bacterial disease	4.84	2.73 – 8.59	<0.001
Signs of malnutrition	3.38	1.49 – 7.65	0.004
Respiratory distress	3.18	1.72 – 5.88	<0.001

Table 26. Multivariate model showing risk factors associated with death in slide negative children.

4.4 Do current WHO guidelines accurately predict invasive bacterial disease and therefore required treatments?

4.4.1 Diagnostic syndromes

Retrospectively applied, 1,767/3,639 (48.6%) children had clinical features that would have led them to meet WHO criteria for antibiotic treatment. (Table 27) This group included 224/341 (65.7%) cases of proven invasive bacterial disease and 47/58 (81.0%) of the deaths associated with invasive bacterial disease. Children with any of the WHO criteria had a 2.2-fold increase in odds of actual invasive bacterial disease (95% confidence interval 1.7 – 2.8, $p<0.001$) and a 4.3-fold increased risk of death (95% confidence interval 3.0 – 6.3, $p<0.001$).

The organism most commonly isolated in children with a meningitis/encephalopathy syndrome was *Haemophilus influenzae*, which accounted for 46% of the positive isolates in children with this syndrome. In 69/255 children (27.1%) with a clinical diagnosis of meningitis/encepahalopathy no lumbar puncture was performed. In 20/186 (10.8%) children with a clinical diagnosis of meningitis/encephalitis who had a lumbar puncture meningitis was proven by the growth of a pathogenic bacteria from CSF. There were 5 further cases of culture proven bacterial meningitis in children without a clinical diagnosis of meningitis/encephalopathy (4 children with very severe pneumonia and 1 child that was not assigned to a WHO clinical diagnostic category).

Non-typhi salmonellae were the most common isolates in children with WHO clinical pneumonia, accounting for 83/178 (46.6%) of isolates. In children with a clinical diagnosis of severe or non-severe pneumonia 79/110 (71.8%) isolates were

Gram negative and consequently likely to be resistant to recommended antibiotic therapy. (Table 28)

Although there were 25 cases of invasive bacterial disease in the 110 children with severe malnutrition (22.7%), 18 of these children had clinical features of other WHO diagnoses (1 meningitis/encephalopathy, 11 very severe pneumonia, 6 pneumonia), leaving 7/42 (16.7%) children with no other clinical signs suggesting a WHO diagnosis that were culture positive for invasive bacterial disease. (Table 27)

4.4.2 Do current guidelines adequately identify children with invasive bacterial disease and malaria parasitaemia?

The sensitivity of WHO-defined syndromes for identifying IBD in children with *P.falciparum* parasitaemia was 56.0% (95% CI 53.9 – 58.1%) compared to 69.7% (95% CI 67.3 – 72.1%) in aparasitaemic children. In identifying fatal cases, these sensitivities rose to 69.2% (95%CI 67.3 – 71.2%) and 84.4% (95%CI 82.6 – 86.3%) respectively, though numbers were small. The sensitivity of WHO guidelines to detect IBD dropped as the parasitaemia rose; at over 50,000 parasites/μl sensitivity was 47%, whilst the prevalence of IBD in this group was similar to that in lower parasitaemias. (Table 29)

Amongst children with malaria, both children who were correctly identified as having invasive bacterial disease by the WHO guidelines, and those in whom bacterial disease was 'missed' by the guidelines, had an increased risk of death associated with their bacterial disease (OR 2.7 and 6.0 respectively, $p<0.05$ for both).

4.4.3 WHAT ARE THE RISK FACTORS FOR IBD IN CHILDREN WITH AND WITHOUT FALCIPARUM PARASITAEMIA?

Factors associated with invasive bacterial disease in an age-adjusted analysis of slide negative children included a history of cough or difficulty breathing, respiratory distress, prostration/coma, lethargy, meningism, hepato- or splenomegaly, severe malnutrition, severe anaemia, hypoglycaemia, hyperlactataemia, leukocytosis, positive HRP-2 falciparum malaria rapid diagnostic test and HIV infection. (Table 30)

In slide positive children there were fewer factors associated with invasive bacterial disease; surprisingly neither meningism nor malnutrition were associated with invasive disease, nor were a history of cough or difficulty breathing, respiratory distress or hypoxia. Circulatory shock was associated with invasive bacterial disease in slide positive (but not slide negative children). Whilst splenomegaly was associated with invasive bacterial disease only in slide negative children, hepatomegaly was associated with bacterial disease in both slide negative and slide positive children. Factors relating to bedside laboratory tests (severe anaemia, hypoglycaemia and hyperlactataemia) were associated with invasive bacterial disease in both groups of children. HIV infection, associated with invasive bacterial disease in slide negative children did not reach significance in slide positive children (AOR 2.72, p=0.066). (Table 31)

Factors significant in age-adjusted analysis were sequentially added to a multivariate model, starting with those at the highest AOR. Factors were dropped when they no longer showed significance. In slide negative children meningism, lethargy, hepato-

or splenomegaly, severe malnutrition and chest crepitations were all independent markers for invasive bacterial disease. In slide positive children jaundice, the clinical feature associated with the highest OR in the age-adjusted analysis, when analysed with any other positive features became non-significant. In view of this, factors were sequentially added to hypoglycaemia. Only hypoglycaemia, leukocytosis and signs of circulatory shock remained positively associated with invasive bacterial disease. The final models with significant features are shown in Table 32.

4.4.4 How can we improve the guidelines in the detection of IBD in children with P. falciparum parasitaemia?

The addition of clinical features found to be independently associated with invasive bacterial disease in multivariate analysis (raised white blood cell count, hypoglycaemia and signs of circulatory shock) to the WHO guidelines improved sensitivity in the detection of invasive bacterial disease in slide positive children from 56% to 78% (95% confidence interval 76.3 – 79.7%). However specificity was poor (37.8%) which would have hypothetically resulted in treating 63% of slide positive children with antibiotics. Additionally a white blood cell count is not available in most district hospitals. In view of these constraints, the effect of adding variables from another widely used set of clinical and basic laboratory indices (the WHO criteria for severe malaria) was assessed.

In order to make the results more relevant the WHO severe malaria criteria were adjusted by removing hyperlactataemia and hyperparasitaemia as both are unlikely to

be accurately recorded in most African district hospitals (Box 3). The addition of WHO criteria for severe malaria to the WHO criteria for presumptive use of antibiotics improved sensitivity from 56.0% (95%CI 53.9 – 58.1%) to 70.0% (95% CI 68.1 – 71.9%) in identifying children with IBD-*P. falciparum* coinfections, and 92.3% (95% CI 91.2 – 93.4%) when identifying fatal cases. However addition of WHO criteria for severe malaria as an indication for antibiotic use would result in a modest (0.7%) increase in the number of children that would be treated with antibiotics per child treated with proven bacterial coinfection.

The contribution of the addition of individual components of the WHO definition of severe malaria (prostration, anaemia, respiratory distress and hypoglycaemia) to increasing the sensitivity of current WHO guidelines for antibiotic use was then assessed both individually and in combination. The addition of severe anaemia resulted in the largest increase in sensitivity. There was a further increase in sensitivity with the addition of severe anaemia or prostration, however there were no further improvements in sensitivity with any further additions. (Table 33) The improvement in sensitivity was gained with a small reduction in the number of children that would have been treated with antibiotics to treat one with IBD (NNT), as the prevalence of IBD in the children with malaria and severe anaemia or prostration not otherwise identified by the WHO categories was 14/218 (6.4%) as compared with 56/1000 (5.6%) in parasitaemic children meeting WHO criteria for presumptive antibiotic treatment.

4.4.5 Can we improve the sensitivity of the guidelines in aparasitaemic children?

The sensitivity of the WHO guidelines for detecting IBD in slide-negative children was 69.7% (95% CI 67.3 – 72.1) and 4.6 children would have been treated for each case of IBD (NNT). The addition of severe anaemia or prostration to the WHO criteria increased the sensitivity to 74.7% (95% CI 72.5 – 76.9%) with a slight increase in the NNT to 4.7. (Table 33) Addition of axillary temperature over 38°C to the standard WHO criteria increased sensitivity to 82.2% (95% CI 80.2 – 84.1) but would have involved treating 68.1% of children with antibiotics.

The rise in sensitivity from the addition of severe anaemia and prostration was largely due to the ability of these findings to pick up infection in children who may have recently had malaria (positive HRP-2 based rapid test and negative blood slide); 9/12 (75%) of the additional cases identified for presumptive antibiotic use by the addition of severe anaemia or prostration were in children in this group.

4.4.6 Are the differences in observed sensitivity of the WHO guidelines in children with positive and negative blood slides related to different Pathogens or differing clinical presentations?

For most bacterial pathogens the WHO guidelines missed a larger proportion of cases of invasive disease in children with malaria parasitaemia than in aparasitaemic children. This was most marked for NTS, where 52% of slide positive infections

were missed compared with 38% of slide negative, but was also true for other Gram negative organisms and *Strep. pneumoniae*. (Table 34)

Looking at differences between pathogens, the guidelines had low sensitivity in identifying *S typhi,* NTS and *Staph aureus* compared with other organisms. Applied to all children, 68/160 (42.5%) of invasive NTS were missed compared to 15/95 (15.8%) of infections due to *Strep. pneumoniae* or *Haemophilus influenzae* and 6/23 (26.1%) for *E coli*. This also contributed to the difference in the sensitivity of the WHO guidelines between slide positive and slide negative children as the spectrum of infecting organisms differed between the two. In slide negative children, *Strep. pneumoniae*, *Haemophilus influenza* and *E. coli*, organisms detected well by the guidelines, together accounted for 100/241(41.5%) of isolates, while NTS and other Gram negative organisms accounted for 108/241 (44.8%) and 19/241 (7.9%) respectively; in slide positive children *Haemophilus influenza, Strep. pneumoniae* and *E. coli* were less common, accounting for only 18/100 (18%) of infections whilst NTS were more common, accounting for 52/100 (52%) of invasive bacterial disease as were other Gram negative organisms (19/100, 19%), both groups of organisms that the guidelines identified poorly. Table 34 summarises the pathogens that were missed by WHO guidelines and amended guidelines in slide positive and negative children.

Syndrome	Definition	Prevalence of IBD n/N (%)
Meningitis/encephalopathy	Neck stiffness, bulging fontanelle, coma or multiple convulsions	39/255 (15.3)
Very severe pneumonia	Cough/difficulty breathing *plus* multiple convulsions, coma, lethargy, vomiting everything, unable to drink, cyanosis *or* severe respiratory distress*	68/406 (16.7)
Severe pneumonia	Cough/difficulty breathing *plus* lower chest indrawing, nasal flaring *or* grunting	30/204 (14.7)
Uncomplicated severe malnutrition	Bilateral oedema, severe wasting *or* weight-height z-score <-3	7/42 (16.7)
Mild pneumonia	Cough/difficulty breathing *plus* raised respiratory rate for age	80/860 (9.3)

Table 27. Definitions of WHO diagnostic categories used in this study with prevalence of invasive bacterial disease.

* Severe respiratory distress defined using WHO criteria for oxygen administration (SaO2 <90% or respiratory rate ≥70bpm).

	Non-typhi salmonella	*S Pneumo*	*H influenzae*	Other Gram neg	Other Gram pos	No IBD	Total
Meningitis/encephalopathy	7 (2.7)	5 (2.0)	18 (7.1)	5(2.0)	4 (1.6)	216 (84.7)	255 (100%)
Very Severe Pneumonia	31 (7.6)	15 (3.7)	6 (1.5)	11 (2.7)	5 (1.2)	338 (83.3)	406 (100%)
Severe Pneumonia	13 (6.4)	9 (4.4)	5 (2.5)	2 (1.0)	1 (0.5)	174 (85.3)	204 (100%)
Pneumonia	39 (4.5)	15 (1.7)	4 (0.5)	16 (1.9)	6 (0.7)	780 (90.7)	860 (100%)
Uncomplicated SAM	2 (4.8)	2 (4.8)	1 (2.4)	2 (4.8)	0	35 (83.3)	42 (100%)
No syndrome	68 (3.6)	10 (0.5)	5 (0.3)	25 (1.3)	9 (0.5)	1, 755 (93.8)	1,872 (100%)
Total	160 (4.4)	56 (1.5)	39 (1.1)	61 (1.7)	25 (0.7)	3, 298 (90.6)	3, 639

Table 28. Prevalence of bacterial isolates in study children by WHO clinical diagnosis.

	N	IBD n (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV	NNT*	% all children treated†	Proportion of fatal IBD cases treated (%)†
Slide negative	1,444	241 (16.7)	69.7% (67.3–72.1)	50.1 (47.6-52.7)	22%	89%	4.6	53.2%	38/45 (84%)
RDT and slide neg‡	943	143 (15.2)	72.0% (69.2-74.9)	49.1% (45.9-52.3)	20%	91%	5.0	54.1%	24/27 (89%)
RDT Pos, slide neg§ (recent malaria)	501	98 (19.6)	66.3% (62.2-70.5)	52.1% (47.7-56.5)	25%	86%	4.0	51.5%	14/18 (78%)
Slide positive	2,195	100 (4.6%)	56.0% (53.9-58.1)	55.0% (52.9-57.1)	6%	96%	17.8	45.5%	9/13 (69%)
Slide pos<5,000/µl	405	33 (8.1)	60.6% (55.9-65.4)	52.7% (47.8-57.6)	10%	94%	9.8	48.4%	4/5 (80%)
Slide pos 5000-50,000/µl	917	31 (3.4)	61.3% (58.1-64.4)	57.9% (54.7-61.1)	5%	98%	20.6	42.7%	2/3 (67%)
Slide pos >50,000/µl	873	36 (4.1)	47.2% (43.9–50.5)	52.9% (49.6-56.2)	4%	96%	24.2	47.1%	3/5 (60%)

Table ERROR! MAIN DOCUMENT ONLY. Sensitivity and specificity of current WHO guidelines for antibiotic use in identifying children with falciparum malaria at different rates of parasitaemia

* Number of children with WHO criteria for empirical antibiotics needed to treat to treat 1 child with invasive bacterial disease
† Proportion of children/fatal cases with slide results treated with antibiotics as per WHO guidelines
‡ 56 children were RDT negative slide positive, their results are included in the slide positive data (sensitivity of RDT 97.45%)

§ Positive HRP-2 based RDT and negative slide likely to indicate recent malaria

		Negative blood slides		
Characteristic	IBD prevalence n/N (%)	AOR	95% CI	P
Cough or difficulty breathing	190 (18.2%)	1.51	1.08 – 2.11	0.016
Fever > 7days	24/122 (19.7%)	1.26	0.78 – 2.02	0.345
Respiratory distress*	47/205 (22.9%)	1.62	1.12 – 2.34	0.01
Signs of circulatory shock†	55/277 (19.9%)	1.29	0.92 – 1.82	0.145
Prostration or coma‡	30/102 (29.4%)	2.26	1.44 – 3.55	<0.001
Lethargic	52/144 (36.1%)	3.32	2.28 – 4.82	<0.001
Meningism§	19/35 (54.3%)	6.66	3.34 – 13.26	<0.001
Crepitations in chest	79/343 (23.0%)	1.74	1.28 – 2.36	<0.001
Jaundice	5/23 (21.7%)	1.44	0.52 – 4.03	0.486
Hepatomegaly**	36/109 (33.0%)	2.96	1.91 – 4.58	<0.001
Splenomegaly††	53/185 (28.6%)	2.38	1.65 – 3.41	<0.001
Signs of malnutrition‡‡	24/80 (30.0%)	2.30	1.39 – 3.80	0.001
Hypoxia (PaO2 < 90%)	13/39 (33.3%)	2.59	1.31 – 5.11	0.006
Severe anaemia (Hb <5g/dl)	39/241 (23.9%)	1.69	1.14 – 2.50	0.009
Hypoglycaemia (glucose<2.5mmol/l)	13/32 (40.6%)	3.60	1.75 – 7.41	0.001
Hyperlactataemia (Lactate >5mmol/l)	29/91 (31.9%)	2.50	1.57 – 4.00	<0.001
WBC > 15	99/491 (20.2%)	1.57	1.15 – 2.15	0.005
HIV positive	23/94 (24.5%)	1.70	1.04 – 2.78	0.036
Malaria RDT positive	98/501 (19.6%)	1.38	1.04 – 1.85	0.028

Table 29. Age adjusted odds ratios for invasive bacterial disease in slide negative children.

* Chest indrawing or deep breathing

† Systolic blood pressure <70-mmHg, capillary refill <3 sec or cool peripheries

‡ Blantyre coma score <3 or unable to sit/drink

§ Neck stiffness or bulging fontanelle

** Liver edge >2cm below costal margin

†† Splenic tip > 2cm below costal margin

‡‡ Weight for height Z score <-3, visible severe wasting or bilateral pedal oedema

		Positive blood slides		
Characteristic	IBD prevalence n/N (%)	AOR	95% CI	P
Cough or difficulty breathing	70/1318 (5.3%)	1.47	0.95 – 2.29	0.084
Fever > 7days	5/58 (8.6%)	2.10	0.81 – 5.41	0.125
Respiratory distress*	178/239 (7.1%)	1.56	0.91 – 2.69	0.109
Signs of circulatory shock†	36/548 (6.6%)	1.59	1.02 – 2.46	0.040
Prostration or coma‡	20/308 (6.5%)	1.72	1.03 – 2.87	0.038
Lethargic	23/363 (6.3%)	1.61	0.99 – 2.62	0.053
Meningism§	1/19 (5.3%)	1.25	0.16 – 9.65	0.833
Crepitations in chest	12/259 (4.6%)	0.90	0.48 – 1.68	0.741
Jaundice	4/40 (10.0%)	3.16	1.07 – 9.29	0.037
Hepatomegaly**	24/282 (8.5%)	2.13	1.32 – 3.43	0.002
Splenomegaly††	32/560 (5.7%)	1.32	0.86 – 2.04	0.209
Signs of malnutrition‡‡	1/30 (3.3%)	0.76	0.10 – 5.66	0.786
Hypoxia (PaO2 < 90%)	3/39 (7.7%)	1.79	0.54 – 5.93	0.343
Severe anaemia (Hb <5g/dl)	31/413 (7.5%)	1.89	1.21 – 2.93	0.005
Hypoglycaemia (glucose<2.5mmol/l)	9/85 (10.6%)	2.50	1.21 – 5.18	0.014
Hyperlactataemia (Lactate >5mmol/l)	27/338 (8.0%)	1.78	1.11 – 2.83	0.016
WBC > 15	34/400 (8.5%)	2.64	1.64 – 4.28	<0.005
HIV positive	4/48 (8.3%)	2.72	0.94 – 7.92	0.066

Table 30. Age adjusted odds ratios for invasive bacterial disease in slide positive children.

*Chest indrawing or deep breathing

† Systolic blood pressure <70-mmHg, capillary refill <3 sec or cool peripheries

‡ Blantyre coma score <3 or unable to sit/drink
§Neck stiffness or bulging fontanelle
**Liver edge >2cm below costal margin
†† Splenic tip > 2cm below costal margin
‡‡ Weight for height Z score <-3, visible severe wasting or bilateral pedal oedema

Characteristic	AOR	95% CI	P
Negative blood slide			
Meningism	6.91	3.14 – 15.21	<0.001
Lethargic	2.79	1.77 – 4.38	<0.001
Splenomegaly	2.11	1.31 – 3.38	0.002
Signs of malnutrition	2.06	1.10 – 3.85	0.024
Hepatomegaly	1.92	1.11 – 3.32	0.02
Crepitations in chest	1.57	1.08 – 2.29	0.019
Positive blood slide			
Hypoglycaemia	2.75	1.21 – 6.24	0.015
WBC > 15	2.52	1.51 – 4.19	<0.001
Signs of circulatory shock	1.73	1.05 – 2.85	0.032

Table 31. Multivariate analysis of risk factors for invasive bacterial disease in children with and without malaria parasitaemia

WHO severe malaria

Asexual *P.falciparum* parasitaemia with any of:

- >2 seizures/24hrs
- BCS < 3
- resp distress *
- prostration†
- jaundice
- severe anaemia (Hb<5g/dL)
- hypoglycaemia (glucose <2.5mmol/L)
- hyperlactataemia (lactate >5mmol/L)
- hyper-parasitaemia (≥20% of red cells infected)‡

Box 3. WHO definition of severe malaria.[1]

* Respiratory distress defined as chest indrawing and/or deep breathing

† Prostration defined as inability to sit after the age of 9 months, inability to drink prior to that age

‡ Hyperparasitaemia excluded from definition of severe malaria syndrome in these studies as not readily available or reliable in most district hospitals.

	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV	NNT*	% all children treated†	Proportion of fatal IBD cases treated[2] (%)
Blood slide positive for *P. falciparum*							
WHO criteria	56.0% (53.9-58.1)	55.0% (52.9-57.1)	5.6%	96.3%	17.9	45.5%	9/13 (69.2%)
***Plus* Severe anaemia**	66.0% (64.0–68.0)	47.3% (45.2-49.3)	5.6%	96.7%	17.7	53.3%	11/13 (84.6%)
***Plus* Severe anaemia *or* prostration**	70.0% (68.1-71.9)	45.3% (43.2-47.3)	5.8%	96.9%	17.4	55.4%	12/13 (92.3%)
***Plus* Severe anaemia *or* prostration *or* HIV positive**	71.0% (69.1-72.9)	44.5% (42.4-46.6)	5.8%	97.0%	17.4	56.2%	12/13 (92.3%)
***Plus* any features of severe malaria *or* HIV positive**	71.0% (69.1-72.9)	42.6% (40.5-44.6)	5.6%	96.9%	17.9	58.0%	12/13 (92.3%)
Blood slide negative for *P. falciparum*							
WHO criteria	69.7% (67.3-72.1)	50.1% (47.5-52.7)	21.9%	89.2%	4.6	53.2%	38/35 (84.4%)
***Plus* Severe anaemia**	73.9% (71.6-76.1)	45.4% (42.8-47.9)	21.3%	89.7%	4.7	57.8%	39/45 (86.7%)
***Plus* Severe anaemia *or* prostration**	74.7% (72.5-76.9)	44.4% (41.8-47.0)	21.2%	89.7%	4.7	58.8%	41/45 (91.1%)
***Plus* Severe anaemia *or* prostration *or* HIV positive**	75.5% (73.3-77.7)	43.1% (40.5-45.6)	21.0%	89.8%	4.8	60.0%	41/45 (91.1%)
***Plus* any features of severe malaria *or* HIV positive**	76.8% (74.6-78.9)	40.1% (39.2-43.3)	20.6%	89.7%	4.9	60.2%	43/45 (95.6%)

Table 32. Sensitivity, specificity & test characteristics of selected additions to WHO criteria for the presumptive treatment of invasive bacterial disease (IBD) to detect IBD in children with and without *P. falciparum* parasitaemia.
*Number of children with WHO criteria for empirical antibiotics needed to treat to treat 1 child with invasive bacterial disease
†Proportion of children/fatal cases with slide results treated with antibiotics as per WHO guidelines

		NTS	*S Pneumoniae*	*H influenzae*	Other Gram neg	Other Gram pos
WHO Criteria	Slide Negative	41/108 (38.0%)	7/45(15.6%)	5/35 (14.3%)	15/39 (35.9%)	5/14 (35.7%)
	Slide Positive	27/52 (51.9%)	3/11 (27.3%)	0/4 (0%)	10/22 (45.5%)	4/11 (36.4%)
***Plus* anaemia**	Slide Negative	34/108 (31.5%)	7/45(15.6%)	5/35 (14.3%)	14/39 (35.9%)	3/14 (21.4%)
	Slide Positive	18/52 (34.6%)	3/11 (27.3%)	0/4 (0%)	9/22 (40.9%)	4/11 (36.4%)
***Plus* anaemia *or* prostration**	Slide Negative	32/108 (29.6%)	7/45 (15.6%)	5/35 (14.3%)	14/39 (35.9%)	3/14 (21.4%)
	Slide Positive	15/52 (28.8%)	3/11 (27.3%)	0/4 (0%)	9/22 (40.9%)	3/11 (27.3%)

Table 33. Proportion of invasive bacterial isolates 'missed' by WHO guidelines and various amendments of WHO guidelines for empirical treatment by malaria slide result.

4.5 Are clinical signs in children reproducible between different grades of staff?

A total of 439 children were examined by a RCO and then examined a second time by a HN (189), HCO (129) or another RCO (121). Table 35 shows the numbers of repeat examinations undertaken by individuals in each of the staff categories. The median time between examinations was 14.2, 13.4 and 14.2 minutes for RCO-HN, RCO-HCO or RCO-RCO respectively. The mean (median) age of children examined was 25(18) months and 39% cried during the first or second examination; neither age nor crying varied by category of examining staff (p=0.13 and p=0.11 respectively).

Overall, the agreement between RCOs on clinical signs was slightly higher than for HCOs or HNs; the mean (median) Kappa scores for all signs examined were 0.54 (0.57) for RCO-RCO, 0.49 (0.49) for RCO-HCO and 0.50 (0.49) for RCO-HN pairs of examiners, indicating moderate agreement in all groups. (Box 4, Figure 12).

Level of consciousness or prostration are important predictors of outcome in severely ill children and all grades of staff reached 'moderate' or 'substantial' levels of agreement for these. However, agreement on the presence of 'chest wall indrawing' between RCOs or HNs compared to the first RCO examination was 'poor'. (Table 36)

Agreement between examiners, particularly RCOs and HNs, for the presence of 'delayed capillary refill' and 'temperature difference' was 'poor'. Since the ability to see capillary refill depends on a colour change in the nailbed we calculated the overall agreement (irrespective of staff grade) on 'delayed refill' (Kappa=0.14, 95%

CI 0.06-0.22) and then in the 159 children whose haemoglobin levels were <7g/dl (Kappa=0.083, 95% CI -0.04-0.20) compared to repeatability in the 280 children with haemoglobin≥7g/dl (Kappa=0.21, 95% CI 0.15-0.29).

We tested the repeatability of a combination of signs that would indicate the need for parenteral therapy according to IMCI criteria. These corresponded to the IMCI definitions of severe or very severe pneumonia or severe dehydration. The agreement between hospital nurses and research clinical officers for the presence or absence of one of these combinations was 'moderate' (Kappa=0.51, 95% CI 0.39-0.57) for severe or very severe pneumonia and substantial (Kappa=0.61, 95% CI 0.49-0.73) for severe dehydration.

A composite sign of 'severe disease' was constructed from a combination of respiratory signs, reduced consciousness, severe pallor or jaundice. (Table 36) The overall Kappa value (irrespective of staff grade) was 0.61 (95% CI 0.56-0.76) while the weighted mean Kappa of its components was 0.56.

Forty percent of signs in children over 18 months of age reached 'substantial' or better levels of agreement compared to only 10% in children under 18 months of age. Similarly, 38% of elicited signs reached substantial or better agreement in children who did not cry compared to only 25% in children who did cry. Most children (66%) were examined within 15 minutes of the first examination and repeatability of examination results was no better in these than the minority (34%) who were examined with a delay of more than 15 minutes.

Kappa Score	Level of agreement
<0	Less agreement than by chance
0 – 0.2	Slight
0.21 - 0.4	Fair
0.41- 0.6	Moderate
0.61 – 0.8	Substantial
0.81 – 1.00	Almost perfect

Box 4. Classification of Kappa scores according to Landis & Koch[191]

RCO	No. examined	HCO	No. examined	HN	No. examined
1	20	1	44	1	46
2	25	2	28	2	41
3	42	3	27	3	31
4	34	4	30	4	56
				5	3
				6	9
				7	3
Total	121		129		189

Table 34. Number of repeat examinations by individual staff and staff categories.

All children were examined first by a RCO, no child was examined twice by the same RCO.

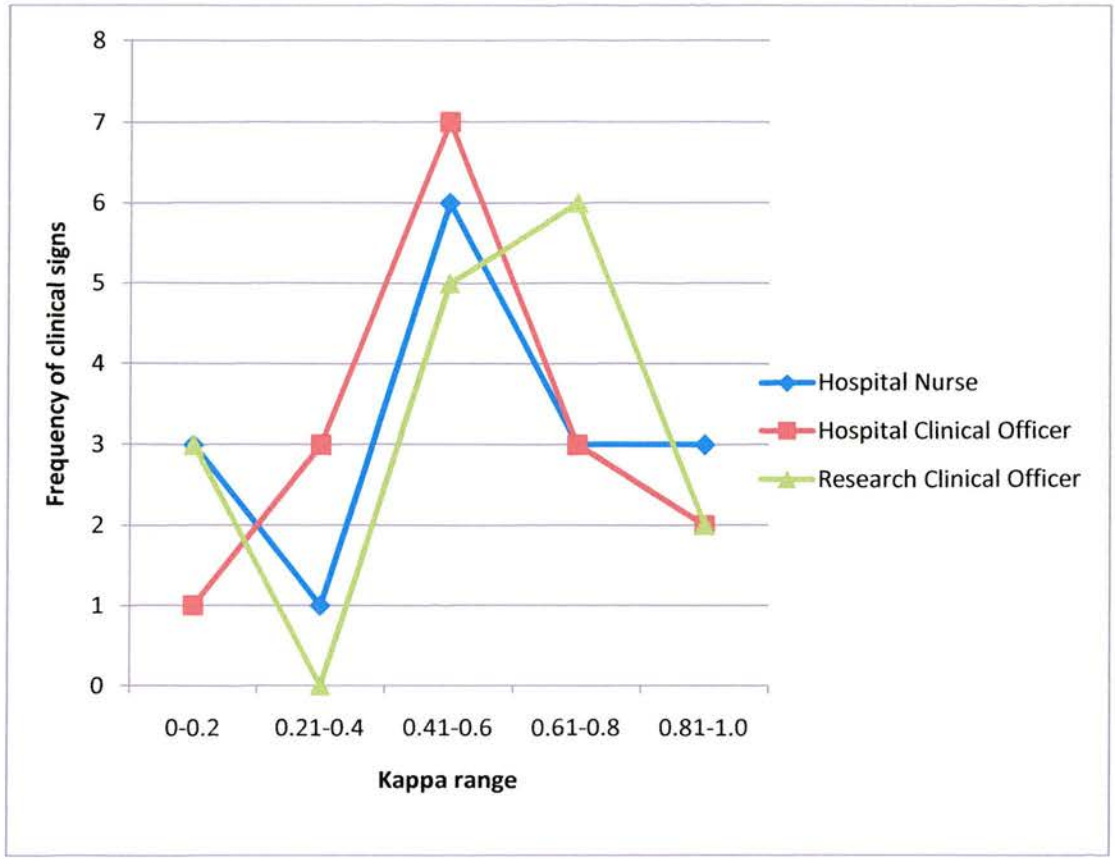


Figure 12. Number of signs with various Kappa values according to second examiner staff category

BLANK PAGE

			HN		HCO		RCO		ALL	
		Prevalence* (%)	Kappa	(95% CI)	Kappa	(95% CI)	Kappa	(95% CI)	Kappa	(95% CI)
Respiratory signs	Raised resp rate for age†	294/416 (71)	0.46	(0.32-0.60)	0.54	(0.36-0.72)	0.46	(0.28-0.64)	0.47	(0.37-0.57)
	Lower chest wall indrawing	60/435 (14)	0.13	(0.01-0.25)	0.63	(0.45-0.81)	0.18	(0.00.0.36)	0.31	(0.21-0.41)
Conscious level	Prostration	64/439 (15)	0.83	(0.69-0.97)	0.83	(0.65-1.01)	0.88	(0.70-1.06)	0.84	(0.74-0.94)
	Unable to sit over 8 months‡	49/436 (11)	0.63	(0.47-0.79)	0.68	(0.50-0.86)	0.85	(0.67-1.03)	0.71	(0.61-0.81)
	Unable to breast feed/drink	22/439 (5)	0.69	(0.55-0.83)	0.56	(0.38-0.74)	0.71	(0.59-0.83)	0.66	(0.58-0.74)
	Lethargy	68/436 (16)	0.84	(0.70-0.98)	0.57	(0.39-0.75)	0.72	(0.54-0.90)	0.63	(0.53-0.74)
	Blantyre score		0.45	(0.35-0.55)	0.35	(0.23-0.47)	0.44	(0.32-0.56)	0.42	(0.34-0.50)
	Eyes	39/439 (9)	0.68	(0.54-0.82)	0.64	(0.46-0.82)	0.71	(0.53-0.89)	0.68	(0.60-0.76)
	Motor	38/439 (9)	0.51	(0.39-0.63)	0.09	(-0.07-0.25)	0.7	(0.54-0.86)	0.55	(0.47-0.63)
	Verbal	46/439 (10)	0.57	(0.45-0.69)	0.45	(0.27-0.63)	0.62	(0.46-0.78)	0.56	(0.48-0.64)
	Any reduced BCS	65/438 (15)	0.67	(0.53-0.81)	0.45	(0.27-0.63)	0.67	(0.49-0.85)	0.61	(0.53-0.69)
	Any reduced consciousness	92/435 (21)	0.63	(0.49-0.77)	0.47	(0.29-0.65)	0.6	(0.42-0.78)	0.47	(0.39-0.55)
Perfusion	Slow Capillary refill	43/438 (10)	0.13	(0.03-0.23)	0.24	(0.12-0.36)	-0.02	(-0.20-0.16)	0.14	(0.06-0.22)
	Temp difference	59/439 (13)	0.11	(-0.03-0.25)	0.48	(0.30-0.66)	0.19	(0.01-0.37)	0.17	(0.09-0.25)
Other	Sunken eyes	24/439 (5)	0.27	(0.15-0.39)	0.48	(0.34-0.62)	0.47	(0.31-0.63)	0.43	(0.33-0.53)
	Oedema of both feet	22/439 (5)	0.89	(0.75-1.03)	0.24	(0.12-0.36)	0.49	(0.31-0.67)	0.47	(0.39-0.55)
	Very slow skin pinch	15/439 (3)	0.49	(0.35-0.63)	0.32	(0.18-0.46)	0.66	(0.48-0.84)	0.48	(0.38-0.58)
	Jaundice	10/439 (2)	0.49	(0.35-0.63)	0.85	(0.67-1.03)	0.49	(0.33-0.65)	0.66	(0.58-0.74)
	Severe Pallor	115/439 (26)	0.48	(0.34-0.62)	0.56	(0.38-0.74)	0.5	(0.32-0.68)	0.5	(0.42-0.58)
	Any sign of severe disease§	226/439 (51)	0.44	(0.30-0.58)	0.5	(0.34-0.68)	0.54	(0.36-0.72)	0.66	(0.56-0.76)

Table 35. Agreement on the presence of clinical signs as elicited by nurses, hospital & research clinical officers compared to first examination by research clinical officers. Footnotes facing page.

5 DISCUSSION

5.1 OVERVIEW

These studies have demonstrated several important findings that will be expanded on below but are summarised here.

The literature on the pattern of bacterial disease in sub-Saharan Africa has been summarised earlier[119 122-126 128-134] (Table 1). Data from the studies contained in this thesis differ from the published studies in that the prevalence of non-Typhi Salmonella and the proportion of mortality attributable to this organism were much higher in this study. This thesis also importantly 'adds to the map' of studies describing the aetiology of infection in hospitalised children in sub-Saharan Africa.

There is little published data on the aetiology of disease in children whose presentation is typical of malaria but who test negative for *P falciparum*. This thesis has described a high prevalence of invasive bacterial disease in children that tested negative for malaria (16.7%) with a high proportion of bacterial infections due to non-Typhi Salmonella in this group.(Table 18, Figures 8, 9)

This is the first study to test WHO guidelines on the presumptive use of antibiotics in children admitted to hospital with infection[62] in a high malaria transmission setting. The data show that the guidelines had poor sensitivity in detecting children with invasive bacterial disease and positive blood slides for falciparum malaria. (Table 29)

In children with severe malaria there remains debate as to whether a policy of treating such children with antimalarials alone is safe.[117 136-137] Data from this thesis would support a change of policy to treating all children with severe malaria with broad spectrum antibiotics in addition to antimalarials. (Tables 19, 33)

Few of the studies described earlier are from district hospitals serving the rural communities where most of the morbidity of childhood infection in East Africa lies. Where studies do concern rural communities the hospitals involved in studies have often been established research centers that may no longer represent the 'norm' in terms of admissions. The data from these studies were generated from research conducted in a hospital that had little experience of inpatient research and should be more representative of the distribution of disease encountered in most hospitals in high malaria transmission areas.

5.2 Aetiology of febrile disease

Infectious diseases are the most important cause of child mortality and morbidity in sub-Saharan Africa. This series of studies have demonstrated that, even in a setting with a high level of malaria transmission, invasive bacterial disease is a major cause of mortality. Invasive bacterial disease was associated with almost a third (31.5%) of all paediatric deaths and occurred in almost 1 in 10 children. As discussed previously there have been few studies that have examined all febrile admissions to ascertain the infections associated with death, but the data from this study are similar to that described in studies from 'neighbouring' Kilifi (26%).[119] The prevalence of invasive

bacterial disease is also similar to that quoted from other hospitals serving malaria endemic regions.[131 133 199] Further in keeping with data from Kilifi[119] we found that approximately 1 in 5 deaths in children with bacterial disease had positive blood slides for malaria and 14% of deaths in children with malaria also had invasive bacterial disease.

As discussed earlier both the prevalence of invasive bacterial disease found, and its impact on mortality are likely to be under-estimates owing to the poor sensitivity of a single blood culture,[71-72] with some authors suggesting that the true prevalence of invasive bacterial disease may be up to 50% higher than that predicted by blood culture studies.[116] Several diagnostic strategies would have improved the yield of bacteria from cultures in children with febrile disease. Lung aspiration or sputum culture in pneumonia, urine culture and multiple blood culture should all increase the sensitivity for the detection of invasive bacterial disease.[71-72 200-212] In one study from West Africa percutaneous lung or pleural aspiration increased the yield of bacterial pathogens in 100 children with pneumonia from 18 to 52.[200] However it is an invasive procedure that is operator dependent and requires specific training and expertise.[209] Sputum culture is difficult in children and samples are often more representative of oro-pharyngeal colonization than lung parenchyma infection. Some studies have found promising results using induced sputum,[204 213-215] with one study from Uganda detecting bacterial pathogens in two thirds of children admitted with severe or very severe pneumonia using induced sputum and blood cultures.[215] It can be difficult to obtain multiple blood cultures and delaying treatment in a sick child is not possible. More cultures would also increase the demands on labs that are already

under pressure. Similarly the resources and personnel required to obtain sterile urine samples on a large scale were prohibitive. However it is likely that a high proportion of children sick enough to die from sepsis would have bacteraemia, reducing the impact of restricting microbiological sampling to blood cultures on mortality studies.

An autopsy study from Zambia in 2002 showed a high rate of tuberculosis in HIV negative children that died with respiratory illnesses and a high rate of tuberculosis, *Pneumocystis jiroveci* and cytomegalovirus in HIV positive children.[216] The absence of any mycobacterial culture, virology, sputum cytology or chest radiography in this study makes it difficult to produce data on these infections. It is worth noting that the Zambian study involved autopsies on only 16% of the children that died and 68% of these were HIV positive. One might expect that the care-givers of children in whom a diagnosis was not apparent might be more likely to give consent to autopsy, possibly biasing the results to diagnoses that are more difficult to make in a resource poor environment.

There were also likely to have been benefits, in terms of antibiotic prescribing, in having on site microbiological facilities. This allowed children who may not have initially received the appropriate antimicrobials to be given them when results of blood cultures became known, potentially reducing the mortality associated with the infection.

The most common bacterial isolates were non-typhi Salmonella, found in almost half of all admissions, followed by *Strep. pneumoniae* and *Haemophilus influenzae* b (Table 17). There are substantial differences in the literature between sites in the relative frequency of different bacterial species.[119 122-126 128-134] The data from this

study are similar to the studies from Malawi,[122-123] Mozambique[133] and the Democratic Republic of Congo[131] in that non-Typhi Salmonella were the most common invasive isolates and contrast with data from The Gambia,[126 128] where pneumococci appeared the most prevalent cause of invasive bacteria disease. Given the data suggesting a link between recent malaria and non-typhi Salmonella sepsis[118 217] it is tempting to postulate that the level of transmission of falciparum malaria may account for the differences in the spectrum of bacterial isolates observed. However, whilst the prevalence of malaria in hospitalised children was similar in the study by Siguaque *et al.* in Mozambique[133] to that in this study another study reporting similar malaria prevalence, in Nigeria,[129] reported only a few cases of invasive non-typhi salmonellosis and many studies did not report on the prevalence of malaria amongst children admitted. The paucity of data makes it difficult to ascertain a pattern; without more data it is difficult to know to what degree the differences observed represent temporal rather than geographical variation and additionally how much variation there may be within countries as well as between them.

A further problem for those planning interventions is understanding to what extent data from inpatients (admissions) to hospitals is representative of the distribution of bacterial disease in the community. It is possible that more severe disease, perhaps due to more virulent pathogens, may be underestimated in some hospital settings as children living in remote areas may not survive to present to hospital. It has been suggested that this is the reason for the absence of epiglottitis (usually associated with *Haemophilus influenzae* b) in any of the reported literature despite *Haemophilus* being a common cause of pneumonia and meningitis in areas where there is little or

no vaccine coverage.[119 122 126 133] Prior antibiotic use can also render cultures negative underestimating the burden of bacterial disease.

Perhaps unsurprisingly in this setting malaria was the most common infection associated with paediatric inpatient mortality in this hospital, being implicated in around half of all paediatric deaths. (Fig 7) This is over twice the proportion that was recorded by researchers in Kilifi, Kenya[119] despite the two hospitals being less than 400km apart and at similar altitudes. The case fatality rates for malaria found in this study (0.6% and 10.6% in non-severe and severe disease respectively) compare well with data from other sites including Kenya[65 67-68 70 152-157] (Table 6) and the high proportion of deaths due to malaria may simply represent the high caseload of severe malaria seen.

Malaria infection appears to have had an influence on the epidemiology of bacterial disease. Children with co-infection had similar case fatalities to those with bacterial disease alone, yet those with co-infection appeared to die at a speed that was more in keeping with children with malaria alone than those with bacterial disease alone. (Table 15, 16) Recent or current malaria also had a strong effect on the spectrum of bacteria isolated with a higher proportion of non-typhi Salmonellae seen particularly in children with recent, but not current malaria whilst those without any evidence for malaria suffered a higher frequency of more traditional pathogens such as *Strep pneumoniae* and *Haemophilus influenzae* b (Fig 9 & Table 18). Clinicians and policy makers need to be aware that the majority (58%) of invasive bacterial disease was found in children who had a positive rapid diagnostic test for malaria, indicating current or recent falciparum malaria. (Table 18) Clearly in this region many young

children will have a malaria parasitaemia, even when 'well'. However recent malaria was associated with an increased risk of invasive bacterial disease over other slide negative children (Table 30). Malaria transmission may thus be a major factor in the differences observed in the spectra of invasive bacterial isolates described in this and other studies (Tables 1 & 17) and be a major predisposing factor in invasive bacterial disease. It will be enlightening to see the effect of malaria controls strategies on the spectrum and incidence of invasive bacterial disease over the coming years. There is also some operational significance to this finding. Should health centers and hospitals move to using HRP-2 based rapid diagnostic tests for falciparum malaria, many children with bacterial disease are likely to present with a positive malaria test due to recent, but not current malaria. This may result in further misdiagnosis, unnecessary malaria treatments and the potentially life threatening consequences of missing invasive bacterial disease.

Although invasive non-typhi Salmonellae infections had the lowest case fatality rate of any bacteria isolates, they were also the most common isolates associated with inpatient death (Table 17). Despite an epidemiological link between invasive non-typhi Salmonellae and malaria being known for over 20 years[217], there is still no clear pathological link. The high incidence of invasive non-typhi Salmonellosis in patients with sickle cell disease[218] and some recent data in animal models[219] point to a possible role for haemolysis. Dendritic cell function has been found to be abnormal in murine models following malaria infection with impairment of their oxidative burst, delayed maturation of monocytes laden with haemozoin (a by-product of haemoglobin found in malaria) and impaired T-cell activation by these dendritic

cells.[220] It remains unclear why such impairments should select for invasive salmonellosis.

This study has also demonstrated that concomitant malaria may change the clinical presentation of bacterial disease with more bacteraemic, parasite positive, children presenting with symptoms and signs that are not typical of the recognized WHO clinical syndromes (Tables 29 & 34). The reasons for this change in presentation are not clear but may involve the changes in immune function associated with malaria outlined above. These children may be presenting with primary septicaemia, with the host unable to contain infection due to impaired immune function. This hypothesis could be explored by a study looking at the proportion of children with radiographic changes associated with pneumococcal bacteraemia in children with and without concurrent or recent malaria. Nonetheless it is clear that children co-infected with invasive bacteria and falciparum malaria are at an increased risk of dying compared non-bacteraemic children.

Although neonates were not enrolled in the studies described in this thesis, the incidence and case fatality rate of invasive bacterial disease among hospitalised children was highest in children under 1 year of age, as in studies from elsewhere[119] [133] (Tables 15 & 17), indicating that infants and not just neonates were at increased risk. This is unlikely to relate to vaccination as none of the pathogens identified were being vaccinated for at the time of the study. There were no cases of malaria-bacterial co-infection in children over 5 years age, though there were relatively few children in this category (Table 15).

The prevalence of HIV in children admitted to hospital was 3.9%, in children that died in hospital it was 9.8%. In contrast to HIV negative children the most common bacterial isolate in HIV positive children was *Strep. pneumoniae*, accounting for 48% of isolates. (Table 21) In in contrast with data from Kenya and Malawi [118-119 137 221] invasive non-typhi salmonellosis was not associated with positive HIV status, possibly due to any effect being swamped by the impact of malaria. Invasive bacterial disease was more common and falciparum malaria less common in children that were HIV positive compared with HIV negative children. Study children with previously diagnosed HIV may have had access to interventions that would have reduced the risk of malaria (such as insecticide treated nets) though other interventions available would have reduced the risk of both bacterial disease and malaria (such as cotrimoxazole).[222-223] A minority (11%) of children's care givers reported that the child had been given cotrimoxazole in the 48hrs prior to admission, though we did not record whether children were known to have HIV prior to admission.

5.3 Severe Malaria

The issue of whether children with severe malaria should be prescribed broad spectrum antibiotics is a contentious one. In this study children aged less than 5 years with severe malaria by WHO criteria[1] had around a 1 in 20 chance of invasive bacterial disease, with a threefold increase in their risk of dying despite a hospital policy of broad-spectrum antibiotics in this group. This is a similar prevalence as found in studies on severe malaria in Kenya,[117] Malawi[137] and Ghana[136] discussed earlier. Despite showing similar rates of bacterial co-infection, these other studies

have found varying statistical significance for its impact on mortality. (Table3) The finding that by changing the case definition of severe malaria the magnitude and statistical significance of the effect of invasive bacterial disease on mortality can be altered is important (Table 19). There are many possible reasons for this discrepancy. The role of invasive bacterial disease in severe malaria may differ according to local factors, such as the intensity of malaria transmission, the prevalence of HIV infection, local conditions affecting the epidemiology of bacterial disease itself (such as vaccination, provision of water and sanitation *etc.*) It is also possible that other studies may have inadvertently selected for a group of children with severe malaria in whom bacterial disease has less impact. Additionally the authors of the study from Ghana[136] commented that *Strep. pneumoniae* was difficult to culture in their lab and postulated a high level of antibiotic use prior to admission as a cause for this. This may have blunted the effect of bacteraemia on mortality which together with the small number of children involved (182 children with slide proven severe malaria) may account for the findings.

The finding that the prevalence of invasive bacterial disease appeared highest in children with severe malarial anaemia (Fig 10) is consistent with at least one other study,[137] though the mortality in children with severe malarial anaemia alone is low in this (Fig 11) and other studies.[65 70 152 158] In research settings it is likely that these children are picked up and treated for their infections quickly, however bloodstream infection could give rise to significant mortality in this group if not promptly managed.

These data would support the assertion by others[116-117] that all young children with severe malaria should be treated with broad spectrum antibiotics. Doing so increased the proportion of bacterial disease treated in slide-positive children from 56% to 71% (Table 33) with no change in the number of children being given antibiotics for each child with invasive bacterial disease captured. Where malaria is being over-diagnosed, or if HRP-2 based rapid diagnostic tests are being used in severely ill inpatients, such an approach is even more important if the burden of invasive bacterial disease is to be effectively treated. Ideally questions concerning the management of severe malaria would be addressed by a randomised clinical trial but given the data outlined in this thesis it seems likely that it would be unethical to recruit children into the placebo arm of such a trial. A large proportion of inpatient deaths in febrile children occur within hours of admission and it is possible that these outcomes will not be altered by antibiotic therapy.

5.4 Identifying children at risk of invasive bacterial disease and death

In settings where resources and personnel are few it is clearly important to target those children in most need. In keeping with earlier studies[65 67 70 157-158] (Table 6), we found that WHO criteria of severe disease[1] predicted over 90% of inpatient deaths in children with malaria. Children who had non-severe malaria had a low (<1%) risk of dying in this setting. Mortality in children with severe anaemia without evidence of acidosis also had a low mortality (Fig 19). It has been suggested that a short stay ward would help in the management of children in a resource poor setting.[224] Such a

unit with a slimmed down staffing might help save resources whilst allowing children with non-severe malaria to be reassessed for signs of developing severe disease.

It was more difficult to identify children with negative slides at increased risk of death. The WHO severe malaria criteria, applied to slide negative children, selected children at risk of death with similar sensitivity to a set of criteria set up de-novo. However both criteria missed a quarter of deaths. Clinicians need to be aware of the difficulties in predicting children at risk of death who have negative malaria slides and act with extra caution in these admissions. As in a previous study from East Africa,[120] the sensitivity of WHO guidelines for detecting invasive bacterial disease were greater in slide negative than slide positive children (Table 29), but in this study could be improved by the addition of severe anaemia or prostration to the criteria for antibiotic therapy (Table 33). Other signs that might cause clinicians to consider antibiotic therapy were splenomegaly or hepatomegaly and crepitations audible on auscultation of the chest – all associated with invasive bacterial disease on multivariate analysis (Table 32). Both prostration and severe anaemia are fairly straight forward signs to elicit. Prostration had a 'near perfect' Kappa score for agreement between observers when repeat examinations were conducted as part of these studies (Table 36) and 'substantial' in another study from Tanzania.[193] Prostration is also well validated as a predictor of mortality in other studies of severe malaria[65 147] and was associated with death in both children with and without malaria in this study (Table 24, Table 25). Severe anaemia requires some means to measure blood haemoglobin or haematocrit, but this is usually available in most district

hospitals. Where it is not, severe pallor, a clinical sign, picked up 79% of the children with a blood hemoglobin of less than 5g/dl and 85% of the invasive bacterial disease associated with severe anaemia. Agreement on severe pallor was 'moderate' in this study and 'moderate' or better in two other East African studies.[193 225] Clearly any such new criteria should be tested prospectively and in different settings if they are to be deployed more widely.

The data on the reproducibility of clinical data showed adequate agreement, and were similar to those obtained by other studies.[193 195-196] Yet in some areas, such as capillary refill and temperature gradient, the agreement was only slight. The reasons for this are not clear, but they may reflect the little attention given to emergency fluid resuscitation in this setting.[226] There is some evidence that the interpretation of clinical signs can differ substantial if the signs are subtle.[192] It would then be expected that disagreement would be less pronounced in the more severely ill children where clinical signs are more likely to be exaggerated. Unfortunately there is no data to support this assertion. Most clinicians would agree that some objective data, such as blood lactate and oxygen saturation, is useful to help triage children. These data support this, with improved sensitivities and specificities of algorithms for the detection of life threatening illness and bacterial disease when such test results were incorporated into the models. Unfortunately these laboratory or bedside tests are not available to most clinicians practicing in this setting. The results of these studies would support the more widespread distribution of such technology to enable more accurate triage and more appropriate use of scarce resources.

5.5 Critique of methodology & Limitations

5.5.1 Overview

These studies are limited in that they are of an observational nature and from a single site in Tanzania, indeed these data have shown important differences from data from other centers. However no study data can automatically be generalized to other sites, and it is important to have a range of sites providing data to allow an understanding of the variation in findings. Similarly these data only describe what was found at the time of the study. Emerging infectious diseases, changes to vaccination programmes and developments in vector control, education, housing and sanitation may all have influences on these data over time.

An observational study such as this can only provide evidence of association rather than cause. Whilst it may be a fair assumption that the finding of pathogenic bacteria in the cerebrospinal fluid of a febrile child are likely to be the cause of death if that child goes on to die in the next few hours or days the same cannot be said of finding malaria parasites in the blood of children in an area endemic for this infection.

Some infections, including most viral infections but also mycobacterial infections, were not looked for in these studies. Although this may have limited the findings the treatment of most viral infections are out-with the capability of most East African district hospitals; whilst these data would have been important for discussion on the provision and need for vaccines they are less so for the clinical management of admissions. It is worth noting that at the time of these studies human influenza A H1N1 ('Swine 'flu') had not been described. Infections that were uncommon at the

time of the study may also not have been picked up due to the limited number of cases described.

5.5.2 Enrolment and un-enrolled patients

The level of refusal of consent for the study (1.3%) was extremely low, as was the loss of enrolled children due to incomplete data (1.9%); both a reflection of the hard work and diligence of the research team. However some criticism of the enrolment procedures can be made. Due to staffing constraints, relating to funding and providing a clinical service, we were unable to recruit patients 24 hours a days, 7 days a week. Consequently the selection of study children was incomplete, and only 67% of children admitted over the year were assessed for inclusion in the study. The most scientifically robust response to the staffing constraints would have been to randomly select days over which the study would recruit (randomly selecting patients would have required staff being present at all times). Although this approach might have been taken, I considered that it was important to have a degree of permanence about the opening times of the assessment unit, to avoid confusion amongst hospital staff. This allowed the unit to become a recognized element of hospital care, ensuring that all children being admitted were sent directly to the assessment unit. Sampling of 'out of hours' admissions was achieved by changing the enrollment times to recruit children admitted over weekend for part of the study period. There is some evidence that children admitted outside of working times have a poorer prognosis,[227] though there is no reason for such children to have a different spectrum of infections to those admitted within working times. In this study mortality in children admitted out of study hours (187/2136, 8.8; 95% CI 7.6 – 10.0%) was significantly higher than

that of those admitted within study hours (259/4334, 6.0%; 95% CI 5.3 – 6.7%) (Fig 5).

However it is important to mention several issues regarding the out of study hours data. Children admitted into the study had accurate tracking of their progress and discharge (or death). However the data was reliant on standard hospital records for the outcomes of children not admitted into the study. These records were very poor. The 39 children who died with 'incomplete screening data' are an example of this (Fig 5). These children were recorded in the hospital records as being admitted during hospital hours, yet there was no record of them having been seen by study staff. It seems unlikely that children admitted severely ill would have bypassed what had become the hospitals resuscitation service (though it is possible that these children were already dead on arrival at the hospital). The quality of the out of hours data could have been improved by a closer monitoring of the hospital data during the study period. There was an attempt to establish which of the 'out of hours' admissions would have been eligible for admission and to collect improve data on them. This was dependent on hospital staff (as opposed to research staff) filling out forms on all out of study hours admissions and was poorly taken up and consequently abandoned. The quality of routine hospital data was improved towards the end of the study as part of other ongoing attempts to improve paediatric care in the region.

Another group of children worthy of consideration are children that may have had infection but presented without fever or history of fever. Although it is usual for both bacterial disease and malaria to present with fever certain groups of children,

particularly those with malnutrition, may present without fever despite severe and life threatening infection. These children may not have met the inclusion criteria and thus our study may not represent a complete picture of the role of infection or malnutrition in inpatient mortality.

5.5.3 THE AETIOLOGY OF INFECTIONS

The study was limited by cost and scope to a single blood culture taken at admission. More intensive investigation using multiple blood cultures, mycobacterial culture, urine and sputum culture and systematic radiological examination could have improved the sensitivity of study investigations to detect invasive bacterial disease as discussed previously. Acute and convalescent serology samples were taken from children with severe febrile disease, but these have not yet been analysed for cost reasons. Clearly autopsy data, if complete, would have been the gold standard test for the aetiology of terminal cases. This was not available.

5.5.4 PREDICTING INVASIVE BACTERIAL DISEASE

The improved sensitivity in algorithms for the detection of children with invasive bacterial disease obtained by making hypothetical changes to the antibiotic prescribing guidelines were tested on the same group of patients that they were developed in. This is flawed and a preferable approach would be to apply the revised guidelines suggested (treating all children with severe malaria and all children with negative slides but severe anaemia or prostration with antibiotics) to a new cohort of children (for example the subsequent years hospital admissions) and seeing how they performed. Unfortunately other commitments prevented me from collecting this data and analyzing it.

5.5.5 Medical management of study children

Common sense, and some data, suggest that outcomes of paediatric admissions are improved by ensuring guidelines are followed.[228] As cited above the mortality of children admitted during study hours was significantly lower than that for children admitted when the study was not running. This may be in part due to improved early management. It is impossible to say what interventions by study staff were responsible and how they affected the course of different infections. I strongly suspect that the study children received more aggressive fluid resuscitation and antibiotic therapy than children admitted outside of the study. This may have disproportionately lessened the impact of invasive bacterial disease.

Additionally, as a result of the study, there were considerable improvements in the provision of supplies, from oxygen through to antibiotics and anticonvulsants. As part of running the assessment unit stocks of oxygen essential drugs and other important consumables were always checked and replaced through a system independent of the hospital supply chain. Again common sense and some evidence suggest that ensuring supplies of essentials, such as oxygen for the treatment of pneumonia,[229] improves outcomes. Consequently diseases associated with hypoxia, such as pneumonia, bronchiolitis and Pneumocystis may have had an improvement in their outcomes due to this greater availability of oxygen, potentially downplaying their importance as a cause of death and enhancing that of other illnesses where hypoxia is less commonly a feature (such as malaria). My feeling is that such an effect would have been small.

It should be emphasised that none of the treatments or management provided to study children were outside of what could, and indeed should, be available to children admitted to any district hospital in East Africa.

5.6 The impact of ethical considerations

The aim of this section is to draw attention to certain areas of study design and practice that were strongly influenced by perceived ethical considerations (largely by the author). However this section is not intended to be a review of the ethical considerations of research in the developing world. Ensuring that research involving patients is of the highest possible quality is an essential component in maintaining high ethical standards, yet I found that in several areas what I felt was our duty as clinicians to ensure the best possible care for patients conflicted with the research agenda to collect valid and complete research data.

5.6.1 The acute assessment unit & research staff roles.

At conception of the study it was planned that the research staff would examine children and obtain research samples, but not contribute to the care of children other than by providing results to the hospital clinicians. In the intervening time between initial study design and study start-up there had been a sustained drop in hospital staff numbers, probably as a result of uncompetitive wages, expansion of the state healthcare system (drawing staff away from Mission Hospitals) and dissatisfaction with hospital management. This had adversely affected the clinical care provided. Children were not adequately assessed in outpatients and there were not the staff to assess children again on admission. It was not uncommon to find children in septic

shock, suffering from hypoglycaemic coma or with clear meningitis without an appropriate management plan. This situation was not unique to this hospital, and indeed may be the norm throughout the region.[31] Although all new admissions were reviewed by the on call physician in an evening round, this left many children waiting, severely ill, for up to 12 hours. With upwards of 50 children to review on occasion, and other duties in the hospital, the on call physician would struggle.

The Declaration of Helsinki suggests that in interventional studies even the control arm should be given "the best universal standard of care",[230] this has been interpreted by some to mean that the best care available anywhere in the world for that condition must be provided.[231] Others have argued that this is unreasonable and potentially harmful given that the 'best universal standard' for many severe illnesses that afflict those in the developing world would involve supportive treatments (intubation, ventilation renal and cardiovascular support for example) that is not available.[232-233] However the ethical requirements of an observational study in this situation are not clear, though the General Medical Council of Great Britain advises that doctors involved in clinical research should have the patient's priorities uppermost in their considerations.[234]

In view of the above I changed the study protocol such that all admissions should come to one ward for assessment, resuscitation and initial doses of treatments. These duties would be primarily performed by the research staff recruited and trained specifically for the study. Hospital staff would be encouraged to come to the ward for education and skills training. An Acute Assessment Unit (AAU) was constructed at the entrance to one of the wards, next to the paediatric high dependency unit (a room

with provision of oxygen via oxygen concentrators). This unit was fitted with extra equipment to provide urgent resuscitation to severely unwell children (short of intubation and ventilation). Staff were trained by myself and colleagues in paediatric resuscitation in addition to data collecting and consent procedures. A triage system according to WHO emergency triage and assessment guidelines (ETAT) was established at the outpatients department. Staff there gave red or orange cards to children who had emergency or priority signs (Appendix 3). Children were encouraged to go to the AAU urgently and present the cards. Sick children were thus readily identified by the research staff and prioritized for resuscitation, consent and ongoing management.

These changes did result in a more comfortable and calm space for the standardised examinations than would otherwise have been possible but the changes in hospital care may have had an impact on mortality and even risk factors for death as considered above. In addition the increased workload, due to involvement of research staff in the resuscitation and treatment of children, resulted in more staff being needed on each shift. This limited the ability to recruit children as described below.

5.6.2 Enrolment hours

As discussed above, to avoid any possibility of bias, it would be desirable to screen every child that was admitted and enroll children around the clock, seven days per week. However as a direct consequence of providing sufficient staff to resuscitate and treat children, whilst collecting data, we were unable to collect data over the full week, 24 hours a day. Faced with this an ideal alternative would be to select days that the unit would be operating at random and work for 24 hours on those days. I

considered this option but rejected it as I considered that both the hospital and research staff would have difficulty coping with such a provision of service. A compromise solution that involved splitting the study enrollment days such that for 5 months children were recruited at weekends was instituted to enable some representation of children admitted outside of working hours.

5.6.3 Treatment decisions

As a result of the new admissions process, research staff became involved in making treatment and management decisions concerning children. National and international guidelines on treatment of severe malaria do not mandate the use of broad spectrum antibiotics in children with severe malaria. Hospital policy on my arrival did not stipulate this either, nor were antibiotics commonly prescribed for that syndrome. Data from a nearby research centre in Kenya suggested that there were significant rates of bacteraemia in children with severe malaria and a significant mortality associated with bacterial co-infection.[117] These data had informed their policy which was to give broad spectrum antibiotics to all young children with severe malaria. I decided that we should adopt a similar policy for the children being treated in the Assessment Unit, as to do otherwise would not be providing the best available care that was known to me at the time. Clearly this may have resulted in this study underestimating the real mortality risk of bacterial disease in these children as they will have been given early antibiotics that children in other units would not. Data from these studies must be interpreted in this context.

5.6.4 EXCLUSIONS & NON-CONSENTING CHILDREN

Setting up a resuscitation service as part of the study creates some issues concerning those that refuse consent or are considered ineligible. It is clear that all children should be entitled to the resuscitation facilities that were available as a result of the study and did not discriminate between those consenting and those preferring not to be involved. This also increased the workload for the research staff, but thankfully the low rate of refusal of consent meant this was not a major problem.

6 CONCLUSIONS

This series of studies have shown that a large part of the burden of infectious disease in East Africa is due to invasive bacterial disease, in addition to falciparum malaria and that current guidelines do not adequately predict, nor adequately treat invasive bacterial disease. These data are supported by published studies from several centers. The key bacterial organisms involved are Gram negative organisms, in particular non-typhi Salmonellae and *Haemophilus influenzae* b, and also *Strep. pneumoniae*. (Table 1 and this study)

There are two important ramifications of this. Firstly, given that effective vaccines are available against *Haemophilus influenzae* b and *Strep. pneumoniae* it is imperative that these are deployed rapidly.[138 235-243] Data from this study was presented to the Tanzanian Ministry for Health in 2007 and undoubtedly had a role in the decision to adopt Hib vaccination into the EPI programme for Tanzania.[244] Secondly current guidelines for treatment of febrile disease in children[62 161] advise the use of inadequate antibiotics in many situations. Penicillin – considered the antibiotic of choice in severe pneumonia – has no activity against most Gram negative organisms.[245] Additionally non-typhi Salmonellae are often resistant to commonly available antibiotics such as ampicillin, chloramphenicol and cotrimoxazole.[124 127 139 246-247] It has been suggested that there should be greater use of third generation cephalosporins, such as ceftriaxone, in severely ill children in this setting.[139] The optimum antibiotic choice must take into account local sensitivities, cost and potential effect on resistance. Clearly there is a need to expand the network

for bacterial surveillance across Africa to cater for this need. It is unlikely that there would be a dramatic effect on resistance from the use of a third generation cephalosporin in a select group of children with severe febrile disease however such a change in policy should ideally be supported by randomized control trial evidence and enhanced surveillance.

These studies have shown that current guidelines on the criteria for antibiotic use in children with febrile disease are insufficiently sensitive at identifying invasive bacterial disease in areas of high malaria transmission. These data would suggest that all children under 5 years of age with severe malaria should receive broad spectrum antibiotics. In addition children with severe anaemia or prostration without malaria should also be treated with broad spectrum antibiotics. Ideally these revised criteria should be prospectively evaluated in other high transmission settings.

Clinical examination, in this research setting, was adequate at predicting poor outcome. However abnormalities in bedside laboratory tests for oxygen saturation, blood haemoglobin, lactate and glucose were all significantly associated with death and improved the sensitivity for the prediction of death in children admitted with febrile disease. Further work to explore the use of these tests in other resource poor settings is indicated.

APPENDIX 1 – CONSENT, ELIGIBILITY & CASE RECORD FORMS

(Actual eligibility forms and case record forms were in a format for scanning, precise layout therefore differed from these reproductions).

Watafiti STUDY IDNO: |___|___|___|___|___|

Utafiti huu unafanywa chini ya Daktari Rajabu Malahiyo wa Hospitali ya Teule, Prof. Raimos Olomi wa Kilimanjaro Christian Medical College (KCMC) na Daktari Hugh reyburn wa London School of Hygiene and Tropical Medicine kama sehemu ya Joint Malaria Programme hapa Tanzania.

Sababu ya utafiti

Thumuni ya kufanya utafiti huu ni kufahamu mengi kuhusu magonjwa ambayo yanatokea mara kwa mara katika eneo hili na kutafuta sababu zinazowafanya wagonjwa wengine kuugua magonjwa makali na wakati wengine hawaugui. Taarifa hii itasaidia kuwatibu watoto walio na magonjwa makali hapo baadaye.

Utaratibu wa utafiti
Kama utakubali kushiriki kwenye utafiti huu utaulizwa maswali kadhaa kuhusu ugonjwa ambao mwanao anaugua hivi sasa. Mwanao atafanyiwa uchunguzi na sampuli ya damu itachukuliwa (kiasi kisichozidi kijiko kimoja na nusu cha chai). Damu hiyo itatumwa kwenye uchunguzi wa kuotesha ili kuchunguza maambukizi ya vijimea, damu ya malaria, kiasi cha damu, kipimo cha sukari na acidi kwenye damu, na vipimo vya kinga kwenye magonjwa ya maambukizi. Kipimo cha mkojo kinaweza kuchukuliwa kama dalili za maambukizi. Majibu haya yatapewa daktari ambaye anakuhudumia wakati tu zitakapokuwa tayari. Pia, timu ya wauguzi wa hospitali wanaweza kupendekeza kama wewe na mwanao mnahitaji kupigwa picha ya kifua au kutolewa maji ya uti wa mgongo; kama itakuwa hivyo, tungependa kunakili/recodi majibu ya matokeo.

Kama sehemu ya utafiti huu tungependa kukupatia mawaidha kabla ya kufanyiwa uchunguzi halafu tumpime mwanao kama ana virusi vya ukimwi. Hii inapendekezwa kama desturi ya huduma Hospitali ya Teule; majibu yatapewa daktari anayemhudumia mwanao kwa njia ya siri. Kama itahitajika, mawaidha ya namna mbalimbali, vipimo vya maaabara na matibabu ya virusi vya ukimwi yanapatikana sasa bure katika hospital hii.

Kiwango kidogo cha damu ambayo imechukuliwa itahifadhiwa kwa vipimo vya baadaye ili kuchunguza maambukizi mengine na hatari ya magonjwa ya maambukizi; baadhi ya hivi vipimo vitafanyiwa maabara nje ya Tanzania. Majibu hayatapatikana kwa muda ufaao ili kuchangia kwenye huduma ya mwanao na kwa hiyo hautapewa majibu hayo.

Huenda tukamwangalia mwanao hapa Hospitali ya Teule mwezi mmoja baada ya yeye kuruhusiwa kutoka hospitali hii ili kuona kama amepona vizuri, na kumwangalia kama ana upungufu wa damu na pia kuchukua damu takriban kijiko kimoja cha chai ili kuangalia kinga yake kwenye maambukizi.

Hatari na faida

Kuchukua kipimo cha damu kinaweza kusababisha kidonda au maumivu lakini madhhara mengine huenda yasitokee na tutatoa matibabu kama itahitajika. Kama taarifa ya siri ya mwanao itagundulika, haya yanaweza kuleta majonzi lakini taarifa yote itakayokusanywa kwenye utafiti huu yatawekwa kwa siri na kwa njia dhabiti.

Uchunguzi wote ambao utafanywa kwa watoto kwenye utafiti huu utakuwa bure lakini huduma ya matibabu na uangalizi utalipiwa kwa njia ya kawaida. Matokeo ya uchunguzi ama taarifa nyingine ambayo tumekusanya hayatashirikishwa na mtu yeyote isipokuwa wahudumu wa hospitali wanaomuuguza mwanao na timu ya watafiti. Faili za compyuta zitapewa namba ya siri na faili za karatasi zitafungiwa na kuhifadhiwa kwa usalama na wafanyakazi wanaoruhusiwa tu ndio watakao kuwa na idhini ya kuziangalia.

Uhuru wa kukataa au kijiondoa
Kama utaamua kutoshiriki kwenye utafiti huu vipimo hivi vitapatikana kama utaomba timu ya wauguzi na matibabu yako hayataadhirika kwa njia yeyote. Uko huru kukataa kushiriki kwenye utafiti huu au anaweza kuondoa idhini yako wakati wowote, bila kutoa sababu, na hii haitaadhiri matibabu na huduma ambayo utapatiwa
.

Karatasi ya taarifa " Utafiti wa sababu za homa kali" nimesomewa/nimesoma na nimeelewa karatasi ya taarifa. Nimejibiwa maswali kuhusu utafiti huu na mfanyakazi wa utafiti.

Ninakubali mwanangu ashirikishwe kwenye utafiti huu

Jina la Mzazi .....

Jina la Mtoto .....

Sahihi/ sahihi ya kidole gumba ya mzazi ..... Tarehe / /

Yahusu mtafiti msaidizi
Nimemsomea/namemuelezea mzazi aliyetajwa hapa chini kuhusu utafiti huu kwa kutumia lugha ambayo anaelewa na ameelewa taarifa hii na anaruhusu kiwango kidogo cha damu kutolewa kwa hisani yake mwenyewe.

Jina la mtafiti msaidizi .....

Sahihi ya mtafiti msaidizi ..... Tarehe...../...../20.....

Consent form – English version

This study is being conducted under Dr Rajabu Malahiyo of Teule Hospital, Prof. Raimos Olomi of Kilimanjaro Christian Medical College (KCMC) and Dr Hugh Reyburn of the London School of Hygiene and Tropical Medicine as part of the Joint Malaria Programme in Tanzania.

The reason for doing the study is to find out more about what diseases are common in this area and to find the reasons that some children suffer from severe illness while others do not. This information will help to treat children will severe illness in the future.

If you agree to take part you will be asked some questions about your social situation and your child's current illness. Your child will then be examined and a venous blood sample will be taken (about one tea-spoon). Blood will be sent for culture (to test for bacterial infection), a blood slide for malaria, a full blood count, and testing for sugar and acid in the blood. These results will be given to the doctors looking after your child as soon as they are available. In addition the medical team looking after your child may recommend that your child needs a chest x-ray or lumbar puncture; if so we would like to record the result.

As part of this study we would like to provide you with pre-test counselling and then to test your child for HIV. This is now recommended as part of routine care in Teule hospital; the result will allow better treatment of your child both now and during his/her recovery. If needed HIV treatment is now available free in Teule Hospital.

Some blood will be stored for future tests to look for other infections and risk factors for illness in children, including genetic (DNA) studies; some of these tests will be done in laboratories outside Tanzania. These results will not be available in time to contribute to the care of your child so will not be given back to you.

All the investigations done for children in the study will be free but hospital care and treatments will be paid for in the usual way. Results of investigations or other information that we collect will not be shared with anyone other than the medical staff looking after your child and authorised members of the research team.

We would like to check your child 1 month after their discharge from hospital to see if they have made a good recovery, to check them for anaemia and to take

For further information or if you have any complaint relating to your involvement in

this study you may contact:

Dr Andrew Kitua, Director, National Institute for Medical Research,

Ocean Road, Dar es Salaam.

approximately 1 teaspoon of blood for evidence of your child's immune response to infection.

If you decide not to allow your child to be included in the study the same tests will be available on the request of the medical team and your treatment will not be affected in any way. You are free to withdraw your consent at any time, without giving reasons and this will not affect the care and treatment that is provided.

The purpose of this study and its procedures and risks and benefits have been explained to me by.....

I agree for my child to participate in this study.....

Eligibility form

Child's first name

Child's last name

Hospital number

Subject ID

Date of admission ☐☐ / ☐☐ / ☐☐☐☐ Reported age (yr/mm) ☐☐ / ☐☐

Sex O Male O Female

1.0 SCREENING QUESTIONS FOR ELIGIBITY

1.1 Has this child had a fever in the last 48 hours ***or*** does the child have an axillary temperature of ≥37.5 ^{0}C or a rectal temperature of ≥38 ^{0}C ?

☐ No — Do not collect this patient's data

Yes

1.2 Is the patient aged between 2 months and 13 years?

☐ No — Do not collect this patient's data

Yes

1.3 Was the patient admitted with known malignancy, renal failure, hepatic failure, marrow aplasia, congenital abnormality, or for trauma or surgery?

☐ Yes — Do not collect this patient's data

No

1.4 Is the child part of the MOMS cohort study?

☐ Yes — Do not collect this patient's data

No

The child is eligible to participate in the SFI study. If he or she is willing to provide assent and her or his parent/guardian/legal representative is willing to provide consent, please document consent according to the study SOP and proceed. Keep the screening form for all subjects consented. Record all subjects screened for the study in the screening log book.

IDNO

Patient first name

Patient last name

Hospital number

2.0 PATIENT DETAILS

Date of admission / / Time of admission (24hr clock) .

Date of study recruitment / /

Time of study recruitment .

Date of birth / /

Or (if month or year missing from DOB)

Reported age (yr/mm) /

Village name

village code (JMP village list)

Sex (choose only one) O Male O Female

Tribe- Mother O Chagga O Pare O Msambara O Wabondei O Other (spec).....

Tribe- Father O Chagga O Pare O Msambara O Wabondei O Other(spec).....

Mother education level O None O<standard 7 O standard 7 O Higher

3.0 MEDICAL HISTORY

Referred from other inpatient facility O Yes O No Referred from Mawenzi O Yes O No

Number of days ill days

Diarrhoea 3 or more times in the last 24hrs O Yes O No

Fever in last 48hrs O Yes O No Diarrhoea lasting more than 14day O Yes O No

Fever lasting more than 7 days O Yes O No Diarrhoea with blood O Yes O No

Cough O Yes O No Vomit 3 or more times in the last 24hrs O Yes O No

Breathing difficulty O Yes O No

Vomit everything O Yes O No

Convulsion in the last 48hrs O Yes O No

Type of convulsion O Focal O General

Convulsion in the last 1hr O Yes O No

Anticonvulsant in the last 6hrs O Yes O No

Number of convulsions last 24hrs ☐☐

Medications

Oral antimalarial in last 48 hrs O Yes O No

Antibiotic in last 48 hrs O Yes O No

Parenteral antimalarial in the last 48 hrs O Yes O No

If So: O Amoxcillin O Cotrimox O Other

(Spec).....

Birth weight ☐☐ . ☐ Kgs

Severe congenital abnormality (inc. ss) O Yes O No

Known Chronic renal or liver disease O Yes O No

Specify congenital abnormality ☐

code congenital abnormality ☐☐

Specify other relevant Information (inc. known HIV status) ☐

code other relevant information ☐☐

4.0 PHYSICAL EXAMINATION

Weight of child |___|___|.|___| Kg **OR** Weight of child + adult |___|___|.|___| Kg

Weight of adult alone |___|___|.|___| Kg

Rectal temperature ☐☐ . ☐ °C **OR** Axillary temperature ☐☐ . ☐ °C

MUAC |___|___|.|___| cm

Severe pallor O Yes O No

Lymphadenopathy: >1cm in >2 areas O Yes O No
Pitting oedema of both feet O Yes O No
Visible severe wasting O Yes O No Jaundice O Yes O No
Oral candidiasis O Yes O No
Temp. different between hand and chest? O Yes O No
Skin pinch > 2secs O Yes O No Sunken eyes O Yes O No
Capillary refill > 3secs O Yes O No Lethargy O Yes O No

Intercostal recession O Yes O No Low chest in-drawing O Yes O No
Central cyanosis O Yes O No Deep breathing O Yes O No

Inspiratory stridor O Yes O No Sustained nasal flaring O Yes O No
Wheeze in chest O Yes O No Respiratory grunting O Yes O No
Crepitations in chest O Yes O No

Respiratory [ ][ ][ ] breaths per minute [ ][ ][ ] per minute

Oxygen saturation [ ][ ][ ] % Blood pressure [ ][ ][ ] / [ ][ ][ ] mmHg

Liver [ ][ ] cm below costal margin Spleen [ ][ ] cm below costal margin
Able to sit if>9months (observed) O Yes O No
Able to breastfeed or drink O Yes O No
Bulging fontanelle O Yes O No Stiff neck O Yes O No
Blantyre score Eyes: O Directed O Not Directed

Verbal: O Normal O Abnormal O None

Motor: O Location O No location O None
Indication to repeat Blantyre O Yes O No
(Blantyre score < 5 **and:** Blood sugar < 2.5 mmol/L **or** conv<1hr **or** anticonv<6hr)

Repeat Blantyre score Eyes: O Directed O Not Directed

Verbal: O Normal O Abnormal O None

Motor: O Location O No location O None
Unable to repeat Blantyre O

Admission blood glucose [ ][ ] . [ ] mmol/L Admission Hb [ ][ ] . [ ] g/dL
Lactate [ ][ ] . [ ] mmol/L Paracheck: O Positive O Negative O Unsure

Paracheck Control line O Present O Not present

5.0 ADMISSION SUMMARY

Admission diagnosis code 1 [ ][ ][ ] Admission diagnosis 1 [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]
Admission diagnosis code 2 [ ][ ][ ] Admission diagnosis 2 [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]
Admission diagnosis code 3 [ ][ ][ ] Admission diagnosis 3 [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

ID Number

6.0 DISCHARGE DETAILS

Did the patient die during admission? O Yes O No

Date of discharge or death ☐☐ / ☐☐ / ☐☐☐☐

Time of death (24hr clock) ☐☐ . ☐☐ hrs

Treatment during admission

Quinine O

Convulsion after admission O Yes O No

Any anticonvulsant during admission O Yes O No
Glucose given for hypoglycaemia O Yes O No
If so, did glucose resolve coma O Yes O No

Antibiotics (Check as many as given):

Ceftriaxone	O
Chloramphenicol	O
Ampicillin/Amoxycillin	O
Gentamycin	O
Cloxacillin	O
Cotrimoxazole	O

O Blood transfusion

O Other *Specify other*

Code other

Record **additional** information (i.e. not already on the form), including results of ultrasound or Xray performed after admission and give details if it was not possible to measure Blantyre. It is not necessary to give results of lab tests.

APPENDIX 2 – CLINICAL STANDARD OPERATING PROCEDURES (SOPS)

Standard Operating Procedure for study eligibility and consent.

Pre-eligibility procedure: registration on arrival for admission.

Pre-registration and recruitment times

Patients are seen in the MCH clinic where admission decisions are made. Children waiting to be seen are triaged according to IMCI guidelines and those with priority or danger signs will be given essential treatment, where required and available, and then sent to the ward with either a yellow (priority) or red (danger) card indicating the need for prompt attention on arrival at the ward.

Patients will be identified on arrival to the registration at the entrance to Hills ward during study recruitment times.

These times are: 8am to 6pm Monday to Friday inclusive.

During certain months these times will be 8am-6pm Thursday to Monday inclusive.

At other times patients will be dealt with in the usual way by Teule Hospital staff. MCH and Outpatient staff will be kept aware as to the recruiting times of the study by verbal instruction and posted rostas.

The following SOP refers to the procedure to be followed during study recruitment times and does not apply to admissions outside these times.

Secondary triage

Patients arriving will be identified by the SFI research assistant. If they have a red ('danger sign') card, medical staff will be alerted immediately to assess the need for immediate treatment. Once this is provided, study recruitment can be followed.

Registration

All patients presenting for admission will be recorded in the study register. Details to be recorded are the name, age, date and time. This record constitutes the 'Eligibility log' for the study and also provides registration details for routine Teule hospital use.

Standard Operating Procedure for study eligibility and consent.

Elligibility

Following registration of all new patients the Eligibility Form will be completed. This includes

A pre-printed study IDN, Name, age, and date.

A reported history of fever within the previous 48 hours

- *Answer Yes = eligible*

The child is aged between 2 months and 13 years (i.e. including children who are exactly 2months or 13 years of age).

- *Answer Yes = eligible*

Absence of a known diagnosis of severe chronic or severe congenital disease. If there is any doubt about whether a reported condition qualifies in this category the patient should be judged eligible and details of the condition will later be recorded on the CRF. Sickle cell and known HIV positive patients ARE eligible. The details of there disease (CD4 count etc) will be noted on the CRF after consent.

- *Answer No = eligible*

Prior recruitment into the MOMS cohort study, either as reported by the parent/guardian or through inspection of a MOMS study ID card. Enrolment in the Diana Centre for HIV care does NOT constitute a barrier to eligibility, however treatment from the Diana Centre for malignancy (Burkitts, lymphoma etc) is an exclusion (under the question above).

- *Answer No = eligible*

In order to be eligible for the study the parent must have given the 'eligible' response to ALL questions.

Standard Operating Procedure for study eligibility and consent.

Children who are not eligible.

Children will have their heights/lengths determined by measuring against a cm scale either standing or lying, with feet flat to the foot-stop and head looking straight ahead. They will be weighed on specified scales situated in the admission area; the child with minimal clothing will be lifted by the caretaker and weight calculated by subtracting the holders own weight. Older children who are able to stand may be weighed on their own. The scales shall be calibrated daily against a standard weight.

The child and parent/guardian should then be conducted to the ward nursing station.

The eligibility form should be placed in the appropriate box file in the data-entry area of the ward.

Children who are not eligible but require emergency resuscitation should still be managed by the admission team.

Children who are eligible.

A study CRF should be part-completed to include the date and time, the child's name and age, and the child's weight and height (or length). The study IDN on the eligibility form should be transcribed onto all pages of the CRF, the severe disease checklist (along with the patient's name), and onto the consent form.

Children's heights/lengths and weights will be recorded as described above onto the CRF.

The village name and code should be established and entered.

The consent form should be part-completed to include the name of the mother or guardian and the date.

Eligible patients will be asked to wait and the forms for study recruitment placed in the 'Awaiting consent slot' for study consenting and completion of the CRF in the assessment area of the ward.

Standard Operating Procedure for study eligibility and consent.

The forms to be place in the 'Awaiting consent slot' consist of:

- The completed Eligibility form
- The consent form with name of parent/guardian, date and study IDN
- The SFI CRF with name of the child, age or d.o.b. of the child, the height and weight of the child and village name and code.
- The severe disease checklist with name and study No.

Standard Operating Procedure for study eligibility and consent.

Informed consent procedure

The consent form is to be completed by staff who have undergone training in medical ethics as applied to children and who are have also undergone training in 'Voluntary Counselling and Testing' for HIV.

Consenting should take place in a pre-defined area that is screened for privacy. If the child is over 12 years of age and conscious he/she must accompany the parent/guardian. If not, the presence of the child is optional.

The consent form should be shown and given to the parent/guardian who should be asked if they would prefer to read it themselves or have it read to them. As much time as the parent/guardian requires should then be allowed for this to happen. The parent/guardian should then be asked if they have understood the content of the form and if they have any additional questions, and these should then be answered. The parent/guardian should then be offered additional time to discuss with any other adult of their choice (spouse, friend etc).

If the child is aged 12 years or more the content of the consent form should be explained to them and they should be given the opportunity to ask questions. They should then be asked if they assent to be included in the study. If they do not assent they should not be included.

If the parent/guardian is willing to provide consent the consent form should be signed first by the research assistant and then by the parent/guardian. Where the parent/guardian is unable to provide a signature they may provide a thumb-print.

Following informed consent the CRF should be filled; CRF SOP

The Consent form, completed eligibility form and CRF should be stored in the relevant box file in the data area after inputing of the CRF by the Data Entry staff.

The completion of the admission form will be undertaken when 5 preconditions are met:

1. That the child has been assessed for his/her need for emergency treatment according to hospital guidelines for ETAT and that, if needed, such treatment has been provided.
2. That the parent or guardian has provided verbal consent to provide information to determine eligibility for the study.
3. That the child is eligible for inclusion in the study.
4. That the parent or guardian has undergone consenting procedures and has provided written consent to participate.
5. That the completion of the form will not compromise the provision of essential treatment or the completion of routine hospital procedures.

The study admission form

Materials needed

1. The form
2. Weighing scales
3. Height/length backboard
4. Watch/ clock
5. Black fine-tipped roller-ball pen
6. Digital axillary thermometer..
7. Oxygen saturation meter
8. Automated (electronic) sphygmomanometer
9. Stethoscope
10. Measuring tape marked in centimetres

The following notes specify definitions or give a brief explanation of each question in the form. If it is not possible to fill a question in, it should be left blank and an attempt should be made later to fill in that category. If it is not possible at a later stage the reason should be written in the free text box and '99' should be inserted if the question has a number box or the circle should be left blank for yes/no questions. **Where a correction is made the correct answer must be written clearly in pen, the entry initialled and dated.**

Q No.	PATIENT IDENTIFICATION
Name	The patients name will conform to the name given for hospital admission.
Study Id	This will run sequentially from 0001. The IDN should have been transcribed onto all pages of the form; this should be checked before filling the CRF.
Hospital Number	This will be transcribed from the hospital case file.
1	Date admitted in European format (dd/mm/yyyy)
2	Time admitted will be according to the 24 hour clock (not Swahili time), East African local time. There will be a wall mounted clock in the ward.
3	Date of recruitment will be recorded to detect delays between admission and recruitment.
4	Time of recruitment will be recorded for the same reason.
5	Date of birth will be asked for all subjects. If only month or year are available these will recorded with date or month left blank.
6	If either month or year of birth is unknown, the age in years and months reported by the caretaker will be recorded.
7	The caretaker will be asked their village of normal residence. This will be checked against a list of villages that conform to the Tanzanian National Census 2003, most of which have been geo-positioned.
8	Unique codes will be filled from a list that conforms to the Tanzanian Census 2003 that will be kept in the admission area.
9	Sex will be asked, not assumed
10	The caretaker will be asked the tribe of the child's mother. Tribe can be confirmed by language group if there is doubt.

11	The caretaker will be asked the tribe of the child's father
12	SOCIO-ECONOMIC INDICATORS
13	The Child's mother's education level is asked of the caretaker defined as completion and certification of Standard 7.
	MEDICAL HISTORY
14	Referral from any other health facility will be noted.
15	The caretaker's estimate of the number of days ill will be noted; the response of the caretaker will be accepted but the interviewer will ask if this was the same illness and there had not been recovery during the reported time.
16	Diarrhoea is defined as loose or watery stools at least 3 times per day
17	Fever in the last 48 hours; the caretaker response will be accepted as long as the child felt hot (i.e. the potential confusion in Kiswahili that 'homa' can mean fever and illness will be clarified at the time of data collection).
18	Diarrhoea for 14 days is defined as diarrhoea that has been present on every day for the last 14 days.Fever for more than 7 days is defined as a palpable fever for some part of every day for the last 7 days.
19	Fever for more than 7 days is defined as a palpable fever for some part of every day for the last 7 days .
20	Diarrhoea with blood in stool will be asked
21	Presence of coughing in the last 48 hours will include a cough sufficient to concern the caretaker but exclude an occasional or unsustained cough
22	Vomiting 3 or more times in the last 24 hours will be taken as a guide for potential dehydration and electrolyte imbalance
23	Breathing difficulty includes any breathing problem sufficient to concern the caretaker
24	'Vomiting everything' is defined as vomiting within 30 minutes of any feed (solid food or fluid) over the last 24 hours. This is a general danger sign in IMCI
25	A convulsion in the study is defined as abnormal movements with altered consciousness. Research assistants will ask the respondent to mimic the abnormal movement. The WHO training video defines categories of convulsion.
26	Evidence of focal convulsions (a potential indication for lumbar puncture) will be sought as above, i.e. whether all limbs were involved from the description of the respondent. This question should be left blank if there is no history of convulsions.
27	A convulsion less than 1 hour prior to the interview.

28	Anticonvulsant medication within the last 6 hours will be used to exclude a post-ictal state or medication as a cause of altered consciousness and thus the Blantyre Coma Scale (BCS). If either of these is positive the examination for coma will be repeated after one hour if the BCS is <5 (<4 if age <9Mo). If BC=5 (or 4 if age <9 Mo) it will not be repeated.
29	The number of convulsions as described above, in the last 24 hours will be recorded. **If the response to question 24 was no then this question can be left blank**. If the answer was yes but there have been no convulsions in the last 24hrs then this question should be filled '00'.
29, 30	Reported use of medication in the previous 48 hours. **If the answer to question 29 is no then questions 30-34 can be left blank.** Traditional and non-traditional medicines will be accepted as 'yes' and oral rehydration solution (ORS); these should be specified under 'other'
31	Reported use of antimalarial therapy will be used as a possible explanation for malaria with a negative blood slide for malaria parasites. The name of the antimalarial will not be recorded.
32	Reported use of antimicrobial therapy will be used as a possible risk factor for negative blood culture results. The name of the antibiotic will be recorded in Q 43.
33	The use of parenteral (injection) antimalarial within the last 48hrs will be noted here.
34	The name of antibiotic will be recorded here. The question should be left blank if no antibiotic has been taken.
35.	Reported birth weight will be recorded in Kg. The birth weight will be checked against the child health card if available and this source preferred to the caretaker report. If it is not known this question should be marked 'X' in all 3 boxes.
36	The presence of a severe congenital abnormality as reported by the caretaker will be recorded as a check against inclusion of children with severe birth defects. Wherever possible this report will be validated by reference to the medical notes. Birth defects associated with poor childhood survival (egg. cardiac or neurological defects, biliary atresia, sickle cell disease) will be included in severe congenital abnormality. Congenital defects not associated with poor childhood survival will be eligible for the study. (egg. Most dermatological disorders, squint etc). If research assistants are in doubt, children with congenital defects will be included and the

	details of the defect noted.
37	The presence of chronic disease such as malignancy or marrow aplasia will be excluded from the study. If in doubt these children will be included and a note made of the specific disease. Children with known HIV-AIDS will be included.
38	The details of any reported congenital or chronic illness will be recorded and later verified by reference to the case notes.
39	Any additional details available at the time of interview about the current illness or pre-existing conditions likely to affect the outcome will be noted. Known HIV disease should be noted here.
	EXAMINATION
40	Children's weights will be recorded on a scale situated in a fixed position on concrete flooring in the ward. The child with minimal clothing will be lifted by the research assistant or caretaker and weight calculated by subtraction of the holder's own weight. Scales will be checked for calibration using a standard weight daily. Alternatively the child may be weighed on it's own (for older children)
41	Height/length will be measured by placing the child against the measuring board with feet flat to the base, heels against the back board and knees straight. The length from the base to the top of the child's head in cm while looking forward will be recorded.
42	Axillary temperature will be measured in degrees centigrade with a digital thermometer, leaving the bulb in the axilla for 2 bleep cycles.
43	Mid-upper arm circumference. The circumference in cm around the mid-point of the child's left arm will be measured using a soft measuring tape pulled tight to the arm.
44	Pallor is noted by examination of the conjunctiva and palms. Only severe pallor need be noted.
45	The neck (anterior and posterior), both axillae and groins should be examined for adenopathy. Significantly enlarged lymph nodes in 3 or more sites should be noted.
46	The dorsum of the feet should be examined for pitting oedema. A finger should be pressed firmly on the surface of each foot for 3 seconds. If an imprint is made the swelling is defined as pitting oedema. It must be present in both feet for this question to be marked 'yes', oedema in one foot should be marked 'no'.
47	The child should be inspected for severe wasting over the buttocks and scapulae.
48	Whilst examining for pallor (q 44) the sclera should be examined for

	signs of jaundice (yellow discolouration).
49	The oral cavity will be examined for the presence of candida (white plaques
50	One of the forearms will be felt and its temperature compared to the temperature felt on laying the hand flat on the chest wall. This is best done with the back of the hand. Any palpable difference will be recorded as a sign of reduced peripheral circulation
51	Hydration status will be assessed using the skin pinch test. A full thickness of skin midway between the umbilicus and the side of the body in the vertical plane (i.e. up and down the body) will be held firmly and then released. A return to normal in 3 seconds or more indicates significant dehydration.
52	A subjective assessent of the presence of sunken eyes will be made and recorded.
53	Capillary refill is assessed by pressing the nail for 3 seconds to blanche (make white) the area, when the pressure is released the time taken until the area becomes pink is measured. If this is over 3 seconds the result is noted as 'yes'
54	Lethargy is defined as a child who does not take any interest in his/her surroundings or does not respond normally to sound or movement. It is an IMCI danger sign. A child who is not awake is considered to show lethargy.
	Respiratory function – This should be examined when the child is calm and undisturbed. This will often be at the beginning of the examination. A crying child, a child that has just been crying, or is about to cry will not have a valid respiratory examination. If the child is crying it can often be calmed by the mother breastfeeding but the respiratory rate must be measured in a non-feeding child.
55	Intercostal indrawing will be assessed by inspection of the chest for inward movement of the area between adjacent ribs.
56	Lower chest wall in-drawing will be assessed by inspection of the chest for an inward movement of the lower chest wall on inspiration. The WHO training video defines this.
57	Central cyanosis will be assessed by inspection of oral mucosa.
58	Deep breathing will be assessed by close observation of the child lying flat. A subjective judgement will be made of abnormally deep respiration, sometimes referred to as 'air hunger' or 'Kusmaul respiration'. The WHO training video defines this.
59	The inspiratory sound of stridor will be listened for and recorded.

60	Nasal flaring should be assessed by inspection whilst counting the respiratory rate. It should be sustained throughout the minute.
61	The chest should be auscultated with a stethoscope to listen for wheeze (an expiratory, musical noise).
62	Respiratory grunting can be heard with each breath at expiration.
63	The chest should be auscultated with a stethoscope for crepitations (also known as crackles). The chest should be auscultated in 3 sites at on each side of the back, 2 sites (including the apices) on each side of the front of the chest and both axilla. Crackles in any of these positions qualifies as 'yes'.
64	The respiratory rate will be measured by counting respirations at the lower chest over the course of 1 minute in a non-feeding child at rest and who is not crying.
65	The heart rate and blood pressure will be simultaneously measured using an appropriately sized cuff and digital sphygmomanometer. The heart rate should be inserted as displayed on the sphygmomanometer. If this does not display a pulse it can be read from the nonin portable meter (see q 66) or recorded manually from the femoral artery n(counted over 30 seconds and multiplied by 2) in that order of preference. If it is not possible to record a blood pressure after 2 attempts the field should be entered 'XXX/XX'
66	Oxygen saturation will be measured using a Nonin 9550 portable meter placed over the finger tip, toe or ear lobe. The result will be interpreted in conjunction with a palpable difference in temperature between a hand and the chest. The appropriately sized attachment should be left in place for at least 10 seconds. The reading should include a pulse AND saturation, it should be repeated at another site if one is missing. The result should be looked at carefully and in the context of the clinical situation. If no good trace (signified by regular rhythmic flashing of the LED with a saturation and pulse rate) can be obtained the field should be marked XXX. The heart rate obtained should be ignored unless the heart rate cannot be obtained from the sphygmomanometer (see q 65).
67	The edge of the liver will be sought by moving the leading edge of the hand progressively up from the abdomen to the right costal margin. When the edge is felt it will be marked with a ball point pen and the distance between this and the costal margin in the mid-clavicular line recorded in centimetres using the ruler/tape-measure.
68	The spleen will be palpated by moving the leading edge of the hand from the umbilicus towards the left costal margin. When the edge of

	the spleen is felt its lowest tip will be marked with a ball point pen mark on the skin and the distance between this mark and the nearest point on the costal margin will be recorded in centimetres using the ruler/tape-measure.
69	Ability to sit will be assessed in children aged 9 months or more by supporting the child from behind to sit and then letting go while keeping the hands close to catch a child unable to sit. Inability to sit defines prostration in this age range**. Children aged under 9 months do not need this documented and it can be left blank.**
70	Ability to drink will be assessed by the caretaker offering a drink or the breast in the way that is usual for the child. The research assistant should observe that the child actually drinks rather than just accepting fluid or the nipple in his/her mouth. Inability to drink defines prostration in a child <9 months old**. In children older than 9 months this can be left blank.**
71	The anterior fontanelle in children less than 1 year old will be assessed by close inspection for evidence that the skin over the fontanelle bulges forward compared to nearby skin over the bony cranium**. If the fontanelle is closed this should be left blank.**
72	Neck stiffness will be assessed by cradling the head of a prone child in one hand and gently flexing the neck. As the neck flexes reflex stiffness (positive neck stiffness) prevents the chin touching the chest. In some children it may be necessary to observe for neck stiffness. A child that voluntarily flexes/extends its neck to look up/down does not have a stiff neck.
	CNS
	Blantyre Score – much of this can be gathered from observing the child during the preceding examination. For example a child that grabs for the stethoscope as it is put on its chest is localising pain. A child that tracks the examiner as he/she moves around them is showing normal eye movements. The following descriptions are given for children whose behaviour is not easily classified.
73	Eye movements will be assessed by holding the child's face at 12 inches from the interviewers face and observing if eye movements follow movements of the face (directed) or not.
74	The verbal response will be assessed to be normal or abnormal by the nature of the child's cry (subjectively judged to be high-pitched or feeble) or, in a child over the age of 2 years, incoherent or moaning speech. Absence of verbal response is coded as 0.
75	The response to pain will be assessed from the response to firm

	rubbing over the sternum with the knuckles. A normal (score 2) response is to attempt to push the pressurising hand away, an impaired response (score 1) is to flex any limb, lack of motor response is scored 0. A child aged less than 9 months may not have developed the ability to localise pain (i.e. a 'normal' score in this group is 1 or 2.
76	The indications to repeat the Blantyre scale are shown on the form. If a repeat is needed the CRF should be placed in the 'Repeat Blantyre ' tray and the timer set for 1 hour. The examination should be repeated at that time.
77	If it is not possible to repeat the Blantyre score this should be noted and the reason given in the 'additional comments' text box.
78	Blood glucose (Hemocue) will measured on admission on a fingerprick or venous sample on all children. See bedside tests SOP
79	Haemoglobin (Hemocue) will measured on admission on a fingerprick or venous sample on all children. See bedside tests SOP
80	Blood lactate will be measured on admission on a fingerprick or venous sample in all children. See bedside tests SOP
81	Blood for HRP-2 (Paracheck) will measured on admission on a fingerprick or venous sample on all children. See bedside tests SOP
82	Admission diagnoses based on the examination findings and bedside tests will be entered.
83	Second admission diagnosis
84	Third admission diagnosis
85	The responsible staff for each form will record their initials clearly.

Venepuncture will be undertaken as soon as possible after the study admission form is completed. The child will have been weighed in filling the questionnaire and this will be used to calculate the target volume of blood. (2ml/kg to a maximum of 8ml)

Hand washing

Before starting, the blood-taker will wash her/his hands thoroughly with chlorhexidine hand washing solution using a scrubbing brush as for theatre procedures.

Latex gloves should be used.

The following equipment should be laid out:

23 and 25 g Butterfly cannulae- at least 4 of each

19 gauge needle

10ml disposable syringe

Skin cleaning swabs, alcohol and iodine solutions (specify..)

Tourniquet

New microscope slide

Sample tubes

Bactalert blood culture bottles, paediatric aerobic

2 ml heparinised tube

2ml EDTA tube

2ml plain tube

The above will be place on a stainless steel-topped clinical trolley. The surface will be cleaned with chlorhexidine after and again immediately before use.

Staff required

1 qualified staff and an assistant should undertake the procedure with the caretaker.

Positioning the child

The child will be prone and in a bed accompanied by his/her caretaker, preferable mother, who should sit with the child's head in her lap or lying across her lap (depending on preference and size of child) and the assistant should restrain the child.

Venepuncture

A suitable vein will be found in the arm or dorsum of the hand. If a vein is not available in the upper limbs, the lower limbs and then the scalp will be inspected in that order. The skin should be well cleaned with iodine followed by alcohol. A 23-25g 'Butterfly' canula will be unsheathed immediately before insertion and inserted into the vein.

If more than one attempt is made to gain access to the vein the needle should be changed between attempts. Canulae contacting any other surface before venepuncture will be discarded and replaced.

When blood is seen to flow back, the plastic hub will be replaced by a 10 ml syringe and the target volume of blood collected.

On withdrawing the canula the hub will be changed for a 19g needle and the Butterfly discarded into the sharps container.

Filling sample tubes.

Sample tubes will be filled in the following order:

BC, EDTA, heparinised, clotted, drop on a slide for ward tests and malaria slide.

5ml BC, 1ml EDTA, 1-2 ml plain, 0.5ml heparinised + 1 drop on microscope slide

Where less than 8ml blood is obtained an attempt should be made to get at least 2ml of blood for BC in addition to the other samples.

EDTA and heparinised tubes should be rolled slowly between the hands to ensure good mixing.

Labelling and transport of specimens to the lab

Each tube will be marked as follows

Name

Date

Study IDN

A blood sample form should be completed with the same information as above and with the time the samples were taken.

The tubes and the form should be placed in a lidded plastic container and kept in the specified tray (room temperature) for collection. One patient per plastic container. This should be transported to the lab reception by the study assistant as soon as possible and within 2 hrs as a maximum delay.

This SOP refers to bedside measures and testing but excludes taking of samples for lab analysis

The following tests are done at the bedside:

1. Hemocue measurement of blood haemoglobin (Hb)
2. Hemocue measurement of blood glucose
3. Measurement of blood lactate
4. Paracheck for P falciparum
5. HIV tests –
    a. Capillus
    b. Determine
6. Generation of 'Hospital slide' for malaria

The above may be tested on venous blood obtained at venepuncture (see Blood Taking SOP) or capillary blood obtained by finger prick with a lancet. The above order is the order in which it is preferable to use venous blood over finger prick blood – that is if there is limited venous blood it should be used for Hb rather than paracheck (and finger prick blood used for the latter).

For venous blood bedside tests the blood should be put onto a hydrophobic (water repelling) surface such as the wrapper of the syringe or butterfly. **Blood that has clotted should not be used for any of these tests.**

For finger prick blood it is always important to squeeze out a sufficiently large drop that the test device (Paracheck loop or Hemocue cuvette) is able to be completely filled. It is also important that the first drop of blood is wiped away as this drop will be diluted by alcohol/interstitial fluid.

Be aware that the Hemocue Haemoglobin analyser and its microcuvettes looks very similar to the Hemocue glucose analyser and its microcuvettes. Always ensure that you are using the correct cuvettes and machine for your test.

1/ **Hemocue haemoglobin measurement**

Equipment needed

1. Hemocue Haemoglobin analyser model no.
2. Hemocue haemoglobin cuvettes
3. Blood on hydrophobic surface *or*
4. Droplet of blood on skin of finger/toe/heel.
5. A pen and study form
6. Approved sharps disposal container

Procedure

1. Turn on the Hemocue analyser
2. Open the cuvette holder to the loading position, the display should show three flashing dashes.
3. Remove a Hemocue microcuvette from the container and close immediately.
4. Touch the tip of the Hemocue cuvette to the blood, filling it completely in one continuous process– do not refill cuvettes.
5. Ensure that there is no excess blood on the outside of the cuvette, wiping blood off if necessary. **Make sure that no blood is drawn out from the cuvette sample in this process**.
6. Look for bubbles in the filled cuvette, if present discard into the sharps bin and take a new sample. Small bubbles around the edge of the cuvette (outside the marked circle) can be ignored.
7. Place the cuvette in the holder – this should be done immediately.

8. Push the cuvette to the measuring position.
9. During measurement the '⌛' symbol will be displayed.
10. After 15 – 60 seconds the haemoglobin (Hb) result will be displayed
11. Write the result down on the patient case record sheet (using '0' as the first digit if the result is <10)
12. Open the cuvette holder and dispose of the cuvette in the sharps bin.

Calibration and maintenance

1. Every morning Nurse 1 should ensure that the Hemocue Hb machine is working and that there are spare batteries in the cabinet.
2. The cuvette holder should be cleaned every morning with an alcohol swab.
3. If the cuvette holder becomes dirty (with blood) it should be cleaned at that time by Nurse 1 using an alcohol swab.
4. If the machine shows an error consult the manual. The most common errors are E01- E05. The machine can be cleaned by following the guidance in the manual and substituting a 'cotton bud' wetted with alcohol for the 'Hemocue cleaner'. Full description on page 28 of the manual.
5. Every Monday the accuracy should be checked with the first sample drawn. For this sample the EDTA sample should be used and the resulting hemocue HB measurement written on the form with the letters QC. The lab will then compare this result to their own.

2/ **Blood glucose measurement**

Equipment needed

1. Hemocue blood sugar analyser.
2. Hemocue blood sugar cuvettes (stored in refrigerator)
3. Blood on hydrophobic surface *or*
4. Droplet of blood on skin of finger/toe/heel.
5. A pen and study form
6. Approved sharps disposal container

Procedure

1. Turn on the Hemocue analyser
2. Open the cuvette holder to the loading position, the display should show three flashing dashes.
3. Remove the Hemocue microcuvette container from the fridge.
4. Remove a Hemocue microcuvette from the container and close immediately.
5. Replace the Hemocue microcuvette container in the refrigerator.
6. Touch the tip of the Hemocue cuvette to the blood, filling it completely in one continuous process– do not refill cuvettes.
7. Ensure that there is no excess blood on the outside of the cuvette, wiping blood off if necessary. **Make sure that no blood is drawn out from the cuvette sample in this process**.

8. Look for bubbles in the filled cuvette, if present discard into the sharps bin and take a new sample. Small bubbles around the edge of the cuvette (outside the marked circle) can be ignored.
9. Place the cuvette in the holder – this should be done immediately.
10. Push the cuvette to the measuring position.
11. During measurement the '⌛' symbol will be displayed.
12. After 15 – 60 seconds the glucose result will be displayed
13. Write the result down on the patient case record sheet (using '0' as the first digit if the result is <10)
14. Open the cuvette holder and dispose of the cuvette in the sharps bin.

Calibration and maintenance

1. Every morning Nurse 1 should ensure that the Hemocue Hb machine is working and that there are spare batteries in the cabinet.
2. The cuvette holder should be cleaned every morning with an alcohol swab.
3. If the cuvette holder becomes dirty (with blood) it should be cleaned at that time by Nurse 1 using an alcohol swab.
4. If the machine shows an error consult the manual. The most common errors are E01- E05. The machine can be cleaned by following the guidance in the manual and substituting a 'cotton bud' wetted with alcohol for the 'Hemocue cleaner'. Full description on page 28 of the manual.

3/ **Blood Lactate Measurement**

Equipment needed

1. Lactate Pro blood lactate analyser.
2. Lactate Pro test strips
3. Blood on hydrophobic surface *or*
4. Droplet of blood on skin of finger/toe/heel.
5. A pen and study form
6. Approved sharps disposal container

Procedure

1. If you are opening a new box of lactate test strips the meter must be calibrated using the calibration strip contained in each box. To do this insert the strip into the inlet. The function no (F-0 to F-12) will flash on the display. This should match the number on the box (and on all the test strips).
2. Peel back the foil packet of the test strip to the line and insert the strip into the inlet.
3. The function number and last measured result will blink alternately. The displayed function number should match that on the test strip.
4. Touch the tip of the test strip to the blood and hold there until the machine 'bleeps'.
5. The readout will show a countdown from 60 seconds. After 60 seconds the blood lactate result will be shown.
6. Having recorded the lactate measurement in the CRF remove the strip and discard it.

Calibration and maintenance

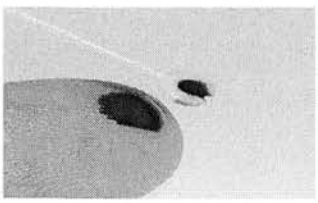
1. Every morning Nurse 1 should ensure that the ProLactate machine is working and that there are spare batteries in the cabinet.
2. Every morning the machine should be tested with the control test strip. If the reading is outside the range given for the control strip the meter should not be used and the fault reported to the Project Leader.

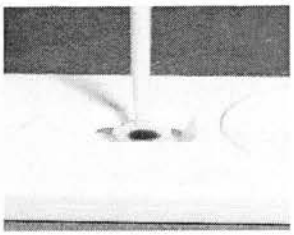
4/ **Rapid test for P.falciparum (Paracheck)**

Equipment needed

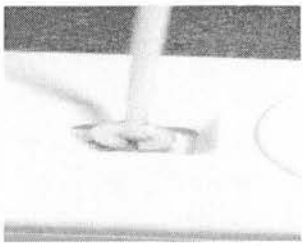
1. Paracheck individual test packet.
2. Paracheck buffer bottle.
3. Anticoagulated blood (EDTA) *or*
4. Blood on hydrophobic surface *or*
5. Drop of blood on skin of finger/toe/heel.
6. Timer with alarm.
7. A pen and study form
8. Approved sharps disposal container

Procedure

1. Open Paracheck packet and remove contents.
2. Check silicagel is blue, if not discard and open new paracheck packet.
3. Touch the loop of the blood collector to the blood.
4. Check that a full drop of blood has been picked up (5μL)
5. 
6. Apply the loop to the filter paper in the sample well of the paracheck device by holding it vertically straight with the loop parallel to the side of the device:

7. 

8. Blood will then be released into the sample well pad

9. 

10. Ensure that the entire specimen is released to the test pad.

11. Discard the loop into the sharps container

12. Deliver 6 drops of buffer into the buffer well with the bottle held vertically 2cm above the well.

13. Label the Paracheck device with the patient's name and the time.

14. Set the timer for 15 minutes and place it with the paracheck device.

15. When the alarm sounds examine the device under good light.

16. If there is no control line discard the test and repeat.

17. If there is only a control line the test is negative – record this in the patient's case record form

18. If there are two lines (control and test) the test is positive – record this in the patient's case record form.

19. It is acceptable to record a positive result before 15 minutes if both lines are clearly visible

20. **It is never acceptable to record a result as negative before 15 minutes**.
21. If it is not possible to read the Paracheck, record unsure. The child should be treated according to the clinical scenario – if severely unwell treat for malaria, if not await B/S result.
22. Always record whether the control line is seen.
23. Dispose of the paracheck device into the sharps container.

5/ **HIV Test**

a) Capillus

The Capillus test is a test for antibodies to HIV 1 and 2. It is the screening test to be used on all patients who consent after councelling. Patients who wish to have anonymous testing will not be tested at the bedside, but rather blood will be sent for testing there (see anonymous testing SOP).

The test employs latex beads coated with proteins that are the targets of HIV antibodies. When these a solution of these beads is mixed with antibody the beads clump together; this is the basis of a positive test.

Positive and negative control samples should be run at least once every day. The results should be documented at the top of every day sheet in the HIV/VCT book. If a new box is started during the day, the controls should be repeated for that box. If controls do not give the required result the box should be discarded and a new set used. This should be reported to the project supervisor.

Materials required:

1. Blood (may be EDTA or blood on hydrophobic surface **NOT** direct from fingerprick) or control.
2. 10µL pipette and disposable tip (both supplied in capillus boxed set)
3. Latex reagent
4. Capillus test slides
5. Black background strip.
6. Disposable gloves
7. Sharps container

Procedure:

1. Remove the capillus tests from the fridge. It is permitted to have the box out of the fridge during the day for testing. It should be returned to the fridge at the end of the day.
2. Remove test slide and lay flat.
3. Allow to come to room temperature.
4. Check patient has consented for VCT.
5. Label with name on specimen bottle.
6. Ensure that you are wearing disposable gloves throughout the procedure.
7. Invert capped reagent bottle to mix latex particles.
8. Draw latex up and down into dropper a few times to further mix.
9. Draw up latex reagent into dropper up to the marked line.
10. Dispense latex reagent into mixing well of slide, away from the capillary channel.
11. Using the pipette dispense 10µL of blood/control into the mixing well. Dispense directly into the latex reagent. Using the pipette mix the sample by pumping the mixture in and out of the tip three times and stirring in a circular motion at least 5 times.
12. Continue to use the pipette to move the sample to the opening of the channel until capillary flow down the channel occurs.
13. Dispose of the pipette tip in an approved sharps container.
14. Allow the mixture to flow through the entire capillary channel into the viewing chamber before interpreting the result.

15. Do not tip the slide upright – this will cause the solution to flow more quickly and may result in false negative tests.

16. Read the slide against a black background.

17. A positive or reactive test is indicated by aggregation of latex particles. All positive tests should be confirmed using a determine test (see 7b in this SOP). The result should be documented in the HIV/VCT log book and entered on the lab request form.

18. Negative tests should be recorded in the HIV/VCT log book and on the laboratory request form. The result should also be entered into the patient hospital notes and post test counselling performed.

19. The slide should be disposed of in an approved sharps container.

b) Determine

The Determine test is a test for antibodies for HIV 1 and 2. The test is used in the study to confirm a positive Capillus test. The test should therefore be done only on patients who have tested positive by Capillus unless there is other good reason (discussed with Project Supervisor).

Materials required:

1. Blood (may be EDTA or blood on hydrophobic surface **NOT** direct from fingerprick).
2. 50µL pipette and disposable tip.
3. Determine test kit
4. Electronic timer
5. Chaser buffer
6. Disposable gloves
7. Sharps container

Procedure

1. Remove one Determine test strip from the packaging and immediately reseal the bag.
2. Peel away the strip cover and lay test strip flat.
3. Label with the name on the sample bottle.
4. Transfer 50µL of blood using the pipette to the end of the test strip.
5. Set the timer for 15 minutes.
6. Dispose of pipette tip in sharps container.

7. If the blood stops moving up the strip, add a single drop of chaser buffer.
8. Read the strip at 15 minutes.
9. The strip must have a control line to be valid, if there is no control line and the blood has not progressed fully up the strip add a drop of chaser buffer and leave for a further 15 minutes (use timer). If the blood has progressed up the strip, but there is no control line, the test should be discarded and repeated.
10. If there is a line in test window the test is positive. If there is no line in this window the test is negative.
11. It is often possible to read a positive before this time if both lines are clearly present.
12. Record both the test result and the presence of a control line in the HIV/VCT book.
13. Record the test result and the presence of a control line on the lab request form.
14. Dispose of the strip in the sharps bin.

Blood slide blood specimen collection

Definition
This SOP describes how to collect a thick blood film. Malaria blood smears are best prepared using finger/heel prick capillary blood and can also be made from blood collected in EDTA tubes or on a hydrophobic surface.

Thick blood film is about 30 times more sensitive compared to thin film (detecting about 20 parasites/μl) a thick film is therefore the most suitable for the rapid detection of malaria parasites, particularly when they are few. In thick films the blood is not fixed. The red cells are lyzed during staining, allowing parasites and white blood cells to be seen in much larger volume of blood.

For the study two slides will be stored. One will be prepared from the EDTA sample in the lab. The other is prepared on the ward – the procedure is outlined here.

Procedure for collecting blood slide from finger/heel prick

Materials required:
* Dry and wet swabs soaked in methylated spirit (70% Ethanol)
* Swab containers
* Disposable sterile blood lancet
* Glass slide
* Permanent marker pen or pencil
* Sharps box
* Clean plastic disposable gloves

Procedure:
Prepare the equipment making sure sharps are safe.
Using a maker pen/pencil, label the microscope slide with the patient name, study identification number and date on the (e.g. 90012, Juma Athumani, 18/08/06). Explaining the slide of patient called Juma Athumani collected on 18th August 2004, from Teule site with study number 90012 – **this is for the 'hospital' slide.**
Explain to the parent/carer the procedure you are about to do and reason why.
Wear a clean pair of disposable gloves
Disinfect thumb, finger or heel with a swab moistened with methylated spirit and blot dry using a dry swab
Using a sterile blood lancet, prick the finger or heel.

Wipe away the first few drops of blood.
Squeeze gently to obtain drop of blood.
Discard used lancets directly into the sharps disposal container
Using a completely clean *grease-free* microscope slide collect blood directly onto slide (a large drop in the centre of microscope slide).

Procedure for making blood slide from venous blood

* Dry and wet swabs soaked in methylated spirit (70% Ethanol)
* Swab containers
* Sterile syringe containing patients blood (see Blood taking SOP)
* Glass slide
* Slide box
* Permanent marker pen or pencil
* Sharps box
* Clean plastic disposable gloves

Procedure:

Prepare the equipment making sure sharps are safe
Using a completely clean *grease-free* microscope slide collect blood directly onto slide (a large drop in the centre of microscope slide).
Using a maker pen/pencil, label the microscope slide with the patient name, study identification number and date on the (e.g. 90012, Juma Athumani, 18/08/06). Explaining the slide of patient called Juma Athumani collected on 18th August 2004, from Teule site with study number 90012 – **this is for the 'hospital' slide.**
Discard syringe unless blood is required for other tests.

APPENDIX 3 – TRIAGE CARDS

EMERGENCY PATIENT

- **Obstructed breathing**
- **Central cyanosis**
- **Severe respiratory distress**
- **Cool hands *and* weak, fast pulse *and* capillary refill > 3 seconds**
- **Coma**
- **Convulsing now**
- **Diarrhoea and 2 of**
    - **Lethargy**
    - **Sunken eyes**
    - **Very slow skin pinch**

PRIORITY PATIENT

- **Any respiratory Distress**
- **Visible severe wasting**
- **Oedema of both feet**
- **Severe Palmar Pallor**
- **Lethargy**
- **Continually restless and irritable**
- **Less than 2 months old**
- **Major Burn**
- **Urgent Referral from another facility**

APPENDIX 4 – REFERENCES

1. Severe falciparum malaria. World Health Organization, Communicable Diseases Cluster. *Trans R Soc Trop Med Hyg* 2000;94 Suppl 1:S1-90.
2. Snow RW, Armstrong JR, Forster D, Winstanley MT, Marsh VM, Newton CR, et al. Childhood deaths in Africa: uses and limitations of verbal autopsies. *Lancet* 1992;340(8815):351-5.
3. Morris SS, Black RE, Tomaskovic L. Predicting the distribution of under-five deaths by cause in countries without adequate vital registration systems. *Int J Epidemiol* 2003;32(6):1041-51.
4. Bryce J, Boschi-Pinto C, Shibuya K, Black RE. WHO estimates of the causes of death in children. *Lancet* 2005;365(9465):1147-52.
5. Sacarlal J, Nhacolo AQ, Sigauque B, Nhalungo DA, Abacassamo F, Sacoor CN, et al. A 10 year study of the cause of death in children under 15 years in Manhica, Mozambique. *BMC Public Health* 2009;9:67.
6. Setel PW, Whiting DR, Hemed Y, Chandramohan D, Wolfson LJ, Alberti KG, et al. Validity of verbal autopsy procedures for determining cause of death in Tanzania. *Trop Med Int Health* 2006;11(5):681-96.
7. Binka FN, Kubaje A, Adjuik M, Williams LA, Lengeler C, Maude GH, et al. Impact of permethrin impregnated bednets on child mortality in Kassena-Nankana district, Ghana: a randomized controlled trial. *Trop Med Int Health* 1996;1(2):147-54.
8. Adjuik M, Smith T, Clark S, Todd J, Garrib A, Kinfu Y, et al. Cause-specific mortality rates in sub-Saharan Africa and Bangladesh. *Bull World Health Organ* 2006;84(3):181-8.
9. D'Alessandro U, Olaleye BO, McGuire W, Langerock P, Bennett S, Aikins MK, et al. Mortality and morbidity from malaria in Gambian children after introduction of an impregnated bednet programme. *Lancet* 1995;345(8948):479-83.

10. De Francisco A, Hall AJ, Schellenberg JR, Greenwood AM, Greenwood BM. The pattern of infant and childhood mortality in Upper River Division, The Gambia. *Ann Trop Paediatr* 1993;13(4):345-52.
11. Delaunay V, Etard JF, Preziosi MP, Marra A, Simondon F. Decline of infant and child mortality rates in rural Senegal over a 37-year period (1963-1999). *Int J Epidemiol* 2001;30(6):1286-93; discussion 94-5.
12. Greenwood BM, Greenwood AM, Bradley AK, Tulloch S, Hayes R, Oldfield FS. Deaths in infancy and early childhood in a well-vaccinated, rural, West African population. *Ann Trop Paediatr* 1987;7(2):91-9.
13. Ibrahim MM, Omar HM, Persson LA, Wall S. Child mortality in a collapsing African society. *Bull World Health Organ* 1996;74(5):547-52.
14. Jaffar S, Leach A, Greenwood AM, Jepson A, Muller O, Ota MO, et al. Changes in the pattern of infant and childhood mortality in upper river division, The Gambia, from 1989 to 1993. *Trop Med Int Health* 1997;2(1):28-37.
15. Kahn K, Tollman SM, Garenne M, Gear JS. Who dies from what? Determining cause of death in South Africa's rural north-east. *Trop Med Int Health* 1999;4(6):433-41.
16. Mtango FD, Neuvians D, Broome CV, Hightower AW, Pio A. Risk factors for deaths in children under 5 years old in Bagamoyo district, Tanzania. *Trop Med Parasitol* 1992;43(4):229-33.
17. Shamebo D, Muhe L, Sandstrom A, Wall S. The Butajira rural health project in Ethiopia: mortality pattern of the under fives. *J Trop Pediatr* 1991;37(5):254-61.
18. Jones G, Steketee RW, Black RE, Bhutta ZA, Morris SS. How many child deaths can we prevent this year? *Lancet* 2003;362(9377):65-71.
19. United Nations Millennium Declaration, 2000.
20. Bryce J, Terreri N, Victora CG, Mason E, Daelmans B, Bhutta ZA, et al. Countdown to 2015: tracking intervention coverage for child survival. *Lancet* 2006;368(9541):1067-76.
21. UNICEF. The state of Africa's children, 2008.
22. Brewster DR, Greenwood BM. Seasonal variation of paediatric diseases in The Gambia, west Africa. *Ann Trop Paediatr* 1993;13(2):133-46.

23. Campbell JD, Sow SO, Levine MM, Kotloff KL. The causes of hospital admission and death among children in Bamako, Mali. *J Trop Pediatr* 2004;50(3):158-63.

24. Gernaat HB, Dechering WH, Voorhoeve HW. Clinical epidemiology of paediatric disease at Nchelenge, north-east Zambia. *Ann Trop Paediatr* 1998;18(2):129-38.

25. Kakembo AS, Walker BF, Walker AR. Causes of admission of African patients to Gelukspan Hospital, North West Province, South Africa. *East Afr Med J* 1996;73(11):746-51.

26. Menge I, Esamai F, van Reken D, Anabwani G. Paediatric morbidity and mortality at the Eldoret District Hospital, Kenya. *East Afr Med J* 1995;72(3):165-9.

27. Zwi KJ, Pettifor JM, Soderlund N. Paediatric hospital admissions at a South African urban regional hospital: the impact of HIV, 1992-1997. *Ann Trop Paediatr* 1999;19(2):135-42.

28. Campbell H, Duke T, Weber M, English M, Carai S, Tamburlini G. Global initiatives for improving hospital care for children: state of the art and future prospects. *Pediatrics* 2008;121(4):e984-92.

29. English M, Esamai F, Wasunna A, Were F, Ogutu B, Wamae A, et al. Assessment of inpatient paediatric care in first referral level hospitals in 13 districts in Kenya. *Lancet* 2004;363(9425):1948-53.

30. Nolan T, Angos P, Cunha AJ, Muhe L, Qazi S, Simoes EA, et al. Quality of hospital care for seriously ill children in less-developed countries. *Lancet* 2001;357(9250):106-10.

31. Reyburn H, Mwakasungula E, Chonya S, Mtei F, Bygbjerg I, Poulsen A, et al. Clinical assessment and treatment in paediatric wards in the north-east of the United Republic of Tanzania. *Bull World Health Organ* 2008;86(2):132-9.

32. Robertson MA, Molyneux EM. Triage in the developing world--can it be done? *Arch Dis Child* 2001;85(3):208-13.

33. Snow RW, Mung'ala VO, Foster D, Marsh K. The role of the district hospital in child survival at the Kenyan Coast. *Afr J Health Sci* 1994;1(2):71-75.

34. Barnes KI, Durrheim DN, Little F, Jackson A, Mehta U, Allen E, et al. Effect of artemether-lumefantrine policy and improved vector control on malaria burden in KwaZulu-Natal, South Africa. *PLoS Med* 2005;2(11):e330.

35. Bhattarai A, Ali AS, Kachur SP, Martensson A, Abbas AK, Khatib R, et al. Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. *PLoS Med* 2007;4(11):e309.

36. Ceesay SJ, Casals-Pascual C, Erskine J, Anya SE, Duah NO, Fulford AJ, et al. Changes in malaria indices between 1999 and 2007 in The Gambia: a retrospective analysis. *Lancet* 2008;372(9649):1545-54.

37. Moyou-Somo R, Lehman LG, Awahmukalah S, Ayuk Enyong P. Deltamethrin impregnated bednets for the control of urban malaria in Kumba Town, South-West Province of Cameroon. *J Trop Med Hyg* 1995;98(5):319-24.

38. Chanda P, Hamainza B, Mulenga S, Chalwe V, Msiska C, Chizema-Kawesha E. Early results of integrated malaria control and implications for the management of fever in under-five children at a peripheral health facility: a case study of Chongwe rural health centre in Zambia. *Malar J* 2009;8:49.

39. Chuma J, Gilson L, Molyneux C. Treatment-seeking behaviour, cost burdens and coping strategies among rural and urban households in Coastal Kenya: an equity analysis. *Trop Med Int Health* 2007;12(5):673-86.

40. Foster S. Treatment of malaria outside the formal health services. *J Trop Med Hyg* 1995;98(1):29-34.

41. Molyneux CS, Mung'Ala-Odera V, Harpham T, Snow RW. Maternal responses to childhood fevers: a comparison of rural and urban residents in coastal Kenya. *Trop Med Int Health* 1999;4(12):836-45.

42. Nyamongo IK. Health care switching behaviour of malaria patients in a Kenyan rural community. *Soc Sci Med* 2002;54(3):377-86.

43. Onwujekwe O, Ojukwu J, Uzochukwu B, Dike N, Ikeme A, Shu E. Where do people from different socio-economic groups receive diagnosis and treatment for presumptive malaria, in south-eastern Nigeria? *Ann Trop Med Parasitol* 2005;99(5):473-81.

44. Ruebush TK, Kern MK, Campbell CC, Oloo AJ. Self-treatment of malaria in a rural area of western Kenya. *Bull World Health Organ* 1995;73(2):229-36.

45. Abuya TO, Mutemi W, Karisa B, Ochola SA, Fegan G, Marsh V. Use of over-the-counter malaria medicines in children and adults in three districts in Kenya: implications for private medicine retailer interventions. *Malar J* 2007;6:57.

46. Amin AA, Kokwaro GO. Antimalarial drug quality in Africa. *J Clin Pharm Ther* 2007;32(5):429-40.

47. Nsimba SE, Rimoy GH. Self-medication with chloroquine in a rural district of Tanzania: a therapeutic challenge for any future malaria treatment policy change in the country. *J Clin Pharm Ther* 2005;30(6):515-9.

48. Onwujekwe O, Kaur H, Dike N, Shu E, Uzochukwu B, Hanson K, et al. Quality of anti-malarial drugs provided by public and private healthcare providers in south-east Nigeria. *Malar J* 2009;8:22.

49. Amin AA, Snow RW, Kokwaro GO. The quality of sulphadoxine-pyrimethamine and amodiaquine products in the Kenyan retail sector. *J Clin Pharm Ther* 2005;30(6):559-65.

50. Minzi OM, Moshi MJ, Hipolite D, Massele AY, Tomson G, Ericsson O, et al. Evaluation of the quality of amodiaquine and sulphadoxine/pyrimethamine tablets sold by private wholesale pharmacies in Dar Es Salaam Tanzania. *J Clin Pharm Ther* 2003;28(2):117-22.

51. Kaur H, Goodman C, Thompson E, Thompson KA, Masanja I, Kachur SP, et al. A nationwide survey of the quality of antimalarials in retail outlets in Tanzania. *PLoS ONE* 2008;3(10):e3403.

52. Gove S. Integrated management of childhood illness by outpatient health workers: technical basis and overview. The WHO Working Group on Guidelines for Integrated Management of the Sick Child. *Bull World Health Organ* 1997;75 Suppl 1:7-24.

53. Gouws E, Bryce J, Habicht JP, Amaral J, Pariyo G, Schellenberg JA, et al. Improving antimicrobial use among health workers in first-level facilities: results from the multi-country evaluation of the Integrated Management of Childhood Illness strategy. *Bull World Health Organ* 2004;82(7):509-15.

54. El Arifeen S, Blum LS, Hoque DM, Chowdhury EK, Khan R, Black RE, et al. Integrated Management of Childhood Illness (IMCI) in Bangladesh: early findings from a cluster-randomised study. *Lancet* 2004;364(9445):1595-602.

55. Bryce J, Gouws E, Adam T, Black RE, Schellenberg JA, Manzi F, et al. Improving quality and efficiency of facility-based child health care through Integrated Management of Childhood Illness in Tanzania. *Health Policy Plan* 2005;20 Suppl 1:i69-i76.

56. Bryce J, Victora CG, Habicht JP, Black RE, Scherpbier RW. Programmatic pathways to child survival: results of a multi-country evaluation of Integrated Management of Childhood Illness. *Health Policy Plan* 2005;20 Suppl 1:i5-i17.

57. Naimoli JF, Rowe AK, Lyaghfouri A, Larbi R, Lamrani LA. Effect of the Integrated Management of Childhood Illness strategy on health care quality in Morocco. *Int J Qual Health Care* 2006;18(2):134-44.

58. Armstrong Schellenberg JR, Adam T, Mshinda H, Masanja H, Kabadi G, Mukasa O, et al. Effectiveness and cost of facility-based Integrated Management of Childhood Illness (IMCI) in Tanzania. *Lancet* 2004;364(9445):1583-94.

59. Arifeen SE, Hoque DM, Akter T, Rahman M, Hoque ME, Begum K, et al. Effect of the Integrated Management of Childhood Illness strategy on childhood mortality and nutrition in a rural area in Bangladesh: a cluster randomised trial. *Lancet* 2009;374(9687):393-403.

60. Gove S, Tamburlini G, Molyneux E, Whitesell P, Campbell H. Development and technical basis of simplified guidelines for emergency triage assessment and treatment in developing countries. WHO Integrated Management of Childhood Illness (IMCI) Referral Care Project. *Arch Dis Child* 1999;81(6):473-7.

61. Molyneux E, Ahmad S, Robertson A. Improved triage and emergency care for children reduces inpatient mortality in a resource-constrained setting. *Bull World Health Organ* 2006;84(4):314-9.

62. WHO. *Management of the child with a serious infection or severe malnutrition: guidelines for care at the first level of referral in developing countries.* Geneva: Department of child and adolescent health, WHO, 2000.

63. Duke T, Kelly J, Weber M, English M, Campbell H. Hospital care for children in developing countries: clinical guidelines and the need for evidence. *J Trop Pediatr* 2006;52(1):1-2.

64. English M, Scott JA. What is the future for global case management guidelines for common childhood diseases? *PLoS Med* 2008;5(12):e241.

65. Marsh K, Forster D, Waruiru C, Mwangi I, Winstanley M, Marsh V, et al. Indicators of life-threatening malaria in African children. *N Engl J Med* 1995;332(21):1399-404.

66. Berkley JA, Ross A, Mwangi I, Osier FH, Mohammed M, Shebbe M, et al. Prognostic indicators of early and late death in children admitted to district hospital in Kenya: cohort study. *BMJ* 2003;326(7385):361.

67. Bassat Q, Guinovart C, Sigauque B, Aide P, Sacarlal J, Nhampossa T, et al. Malaria in rural Mozambique. Part II: children admitted to hospital. *Malar J* 2008;7:37.

68. Dzeing-Ella A, Nze Obiang PC, Tchoua R, Planche T, Mboza B, Mbounja M, et al. Severe falciparum malaria in Gabonese children: clinical and laboratory features. *Malar J* 2005;4:1.

69. Krishna S, Waller DW, ter Kuile F, Kwiatkowski D, Crawley J, Craddock CF, et al. Lactic acidosis and hypoglycaemia in children with severe malaria: pathophysiological and prognostic significance. *Trans R Soc Trop Med Hyg* 1994;88(1):67-73.

70. Schellenberg D, Menendez C, Kahigwa E, Font F, Galindo C, Acosta C, et al. African children with malaria in an area of intense Plasmodium falciparum transmission: features on admission to the hospital and risk factors for death. *Am J Trop Med Hyg* 1999;61(3):431-8.

71. Cockerill FR, 3rd, Wilson JW, Vetter EA, Goodman KM, Torgerson CA, Harmsen WS, et al. Optimal testing parameters for blood cultures. *Clin Infect Dis* 2004;38(12):1724-30.

72. Lee A, Mirrett S, Reller LB, Weinstein MP. Detection of bloodstream infections in adults: how many blood cultures are needed? *J Clin Microbiol* 2007;45(11):3546-8.

73. Njama-Meya D, Clark TD, Nzarubara B, Staedke S, Kamya MR, Dorsey G. Treatment of malaria restricted to laboratory-confirmed cases: a prospective cohort study in Ugandan children. *Malar J* 2007;6:7.

74. Chandler CI, Drakeley CJ, Reyburn H, Carneiro I. The effect of altitude on parasite density case definitions for malaria in northeastern Tanzania. *Trop Med Int Health* 2006;11(8):1178-84.

75. John CC, McHugh MM, Moormann AM, Sumba PO, Ofulla AV. Low prevalence of Plasmodium falciparum infection among asymptomatic individuals in a highland area of Kenya. *Trans R Soc Trop Med Hyg* 2005;99(10):780-6.

76. Ademowo OG, Falusi AG, Mewoyeka OO. Prevalence of asymptomatic parasitaemia in an urban and rural community in south western Nigeria. *Cent Afr J Med* 1995;41(1):18-21.

77. Baliraine FN, Afrane YA, Amenya DA, Bonizzoni M, Menge DM, Zhou G, et al. High Prevalence of Asymptomatic Plasmodium falciparum Infections in a Highland Area of Western Kenya: A Cohort Study. *J Infect Dis* 2009;200(1):66-74.

78. Greenwood BM. Asymptomatic malaria infections--do they matter? *Parasitol Today* 1987;3(7):206-14.

79. Kimbi HK, Nformi D, Ndamukong KJ. Prevalence of asymptomatic malaria among school children in an urban and rural area in the Mount Cameroon region. *Cent Afr J Med* 2005;51(1-2):5-10.

80. Mabunda S, Aponte JJ, Tiago A, Alonso P. A country-wide malaria survey in Mozambique. II. Malaria attributable proportion of fever and establishment of malaria case definition in children across different epidemiological settings. *Malar J* 2009;8:74.

81. Salako LA, Ajayi FO, Sowunmi A, Walker O. Malaria in Nigeria: a revisit. *Ann Trop Med Parasitol* 1990;84(5):435-45.

82. Schellenberg JR, Smith T, Alonso PL, Hayes RJ. What is clinical malaria? Finding case definitions for field research in highly endemic areas. *Parasitol Today* 1994;10(11):439-42.

83. Wagner G, Koram K, McGuinness D, Bennett S, Nkrumah F, Riley E. High incidence of asymptomatic malara infections in a birth cohort of children less than one year of age in Ghana, detected by multicopy gene polymerase chain reaction. *Am J Trop Med Hyg* 1998;59(1):115-23.

84. Perneger TV, Szeless T, Rougemont A. Utility of the detection of Plasmodium parasites for the diagnosis of malaria in endemic areas. *BMC Infect Dis* 2006;6:81.

85. Koram KA, Molyneux ME. When is "malaria" malaria? The different burdens of malaria infection, malaria disease, and malaria-like illnesses. *Am J Trop Med Hyg* 2007;77(6 Suppl):1-5.

86. Njama-Meya D, Kamya MR, Dorsey G. Asymptomatic parasitaemia as a risk factor for symptomatic malaria in a cohort of Ugandan children. *Trop Med Int Health* 2004;9(8):862-8.

87. Nsobya SL, Parikh S, Kironde F, Lubega G, Kamya MR, Rosenthal PJ, et al. Molecular evaluation of the natural history of asymptomatic parasitemia in Ugandan children. *J Infect Dis* 2004;189(12):2220-6.

88. Farnert A, Snounou G, Rooth I, Bjorkman A. Daily dynamics of Plasmodium falciparum subpopulations in asymptomatic children in a holoendemic area. *Am J Trop Med Hyg* 1997;56(5):538-47.

89. Jafari-Guemouri S, Boudin C, Fievet N, Ndiaye P, Deloron P. Plasmodium falciparum genotype population dynamics in asymptomatic children from Senegal. *Microbes Infect* 2006;8(7):1663-70.

90. Farnert A, Lebbad M, Faraja L, Rooth I. Extensive dynamics of Plasmodium falciparum densities, stages and genotyping profiles. *Malar J* 2008;7:241.

91. Magesa SM, Mdira KY, Babiker HA, Alifrangis M, Farnert A, Simonsen PE, et al. Diversity of Plasmodium falciparum clones infecting children living in a holoendemic area in north-eastern Tanzania. *Acta Trop* 2002;84(2):83-92.

92. Daubersies P, Sallenave-Sales S, Magne S, Trape JF, Contamin H, Fandeur T, et al. Rapid turnover of Plasmodium falciparum populations in asymptomatic

individuals living in a high transmission area. *Am J Trop Med Hyg* 1996;54(1):18-26.

93. Smith T, Schellenberg JA, Hayes R. Attributable fraction estimates and case definitions for malaria in endemic areas. *Stat Med* 1994;13(22):2345-58.
94. Bejon P, Berkley JA, Mwangi T, Ogada E, Mwangi I, Maitland K, et al. Defining childhood severe falciparum malaria for intervention studies. *PLoS Med* 2007;4(8):e251.
95. Gatton ML, Cheng Q. Evaluation of the pyrogenic threshold for Plasmodium falciparum malaria in naive individuals. *Am J Trop Med Hyg* 2002;66(5):467-73.
96. Boisier P, Jambou R, Raharimalala L, Roux J. Relationship between parasite density and fever risk in a community exposed to a low level of malaria transmission in Madagascar highlands. *Am J Trop Med Hyg* 2002;67(2):137-40.
97. Dicko A, Mantel C, Kouriba B, Sagara I, Thera MA, Doumbia S, et al. Season, fever prevalence and pyrogenic threshold for malaria disease definition in an endemic area of Mali. *Trop Med Int Health* 2005;10(6):550-6.
98. Murray CK, Gasser RA, Jr., Magill AJ, Miller RS. Update on rapid diagnostic testing for malaria. *Clin Microbiol Rev* 2008;21(1):97-110.
99. Beadle C, Long GW, Weiss WR, McElroy PD, Maret SM, Oloo AJ, et al. Diagnosis of malaria by detection of Plasmodium falciparum HRP-2 antigen with a rapid dipstick antigen-capture assay. *Lancet* 1994;343(8897):564-8.
100. Bechem NN, Leke RF, Tietche F, Taylor DW. Evaluation of a rapid test for histidine rich protein 2 for diagnosis of Plasmodium falciparum infection in Cameroonian children. *Trans R Soc Trop Med Hyg* 1999;93(1):46.
101. Cheng A, Bell D. Evidence behind the WHO guidelines: hospital care for children: what is the precision of rapid diagnostic tests for malaria? *J Trop Pediatr* 2006;52(6):386-9.
102. Craig MH, Bredenkamp BL, Williams CH, Rossouw EJ, Kelly VJ, Kleinschmidt I, et al. Field and laboratory comparative evaluation of ten rapid malaria diagnostic tests. *Trans R Soc Trop Med Hyg* 2002;96(3):258-65.

103. Grobusch MP, Hanscheid T, Gobels K, Slevogt H, Zoller T, Rogler G, et al. Comparison of three antigen detection tests for diagnosis and follow-up of falciparum malaria in travellers returning to Berlin, Germany. *Parasitol Res* 2003;89(5):354-7.

104. Guthmann JP, Ruiz A, Priotto G, Kiguli J, Bonte L, Legros D. Validity, reliability and ease of use in the field of five rapid tests for the diagnosis of Plasmodium falciparum malaria in Uganda. *Trans R Soc Trop Med Hyg* 2002;96(3):254-7.

105. Iqbal J, Hira PR, Sher A, Al-Enezi AA. Diagnosis of imported malaria by Plasmodium lactate dehydrogenase (pLDH) and histidine-rich protein 2 (PfHRP-2)-based immunocapture assays. *Am J Trop Med Hyg* 2001;64(1-2):20-3.

106. Iqbal J, Khalid N, Hira PR. Comparison of two commercial assays with expert microscopy for confirmation of symptomatically diagnosed malaria. *J Clin Microbiol* 2002;40(12):4675-8.

107. Jelinek T, Grobusch MP, Schwenke S, Steidl S, von Sonnenburg F, Nothdurft HD, et al. Sensitivity and specificity of dipstick tests for rapid diagnosis of malaria in nonimmune travelers. *J Clin Microbiol* 1999;37(3):721-3.

108. Mason DP, Kawamoto F, Lin K, Laoboonchai A, Wongsrichanalai C. A comparison of two rapid field immunochromatographic tests to expert microscopy in the diagnosis of malaria. *Acta Trop* 2002;82(1):51-9.

109. Moody A. Rapid diagnostic tests for malaria parasites. *Clin Microbiol Rev* 2002;15(1):66-78.

110. Palmer CJ, Bonilla JA, Bruckner DA, Barnett ED, Miller NS, Haseeb MA, et al. Multicenter study to evaluate the OptiMAL test for rapid diagnosis of malaria in U.S. hospitals. *J Clin Microbiol* 2003;41(11):5178-82.

111. Chiodini PL, Bowers K, Jorgensen P, Barnwell JW, Grady KK, Luchavez J, et al. The heat stability of Plasmodium lactate dehydrogenase-based and histidine-rich protein 2-based malaria rapid diagnostic tests. *Trans R Soc Trop Med Hyg* 2007;101(4):331-7.

112. Kyabayinze DJ, Tibenderana JK, Odong GW, Rwakimari JB, Counihan H. Operational accuracy and comparative persistent antigenicity of HRP2 rapid

diagnostic tests for Plasmodium falciparum malaria in a hyperendemic region of Uganda. *Malar J* 2008;7:221.

113. Singh N, Shukla MM. Short report: Field evaluation of posttreatment sensitivity for monitoring parasite clearance of Plasmodium falciparum malaria by use of the Determine Malaria pf test in central India. *Am J Trop Med Hyg* 2002;66(3):314-6.

114. Swarthout TD, Counihan H, Senga RK, van den Broek I. Paracheck-Pf accuracy and recently treated Plasmodium falciparum infections: is there a risk of over-diagnosis? *Malar J* 2007;6:58.

115. Tjitra E, Suprianto S, McBroom J, Currie BJ, Anstey NM. Persistent ICT malaria P.f/P.v panmalarial and HRP2 antigen reactivity after treatment of Plasmodium falciparum malaria is associated with gametocytemia and results in false-positive diagnoses of Plasmodium vivax in convalescence. *J Clin Microbiol* 2001;39(3):1025-31.

116. Gwer S, Newton CR, Berkley JA. Over-diagnosis and co-morbidity of severe malaria in African children: a guide for clinicians. *Am J Trop Med Hyg* 2007;77(6 Suppl):6-13.

117. Berkley J, Mwarumba S, Bramham K, Lowe B, Marsh K. Bacteraemia complicating severe malaria in children. *Trans R Soc Trop Med Hyg* 1999;93(3):283-6.

118. Brent AJ, Oundo JO, Mwangi I, Ochola L, Lowe B, Berkley JA. Salmonella bacteremia in Kenyan children. *Pediatr Infect Dis J* 2006;25(3):230-6.

119. Berkley JA, Lowe BS, Mwangi I, Williams T, Bauni E, Mwarumba S, et al. Bacteremia among children admitted to a rural hospital in Kenya. *N Engl J Med* 2005;352(1):39-47.

120. Berkley JA, Maitland K, Mwangi I, Ngetsa C, Mwarumba S, Lowe BS, et al. Use of clinical syndromes to target antibiotic prescribing in seriously ill children in malaria endemic area: observational study. *BMJ* 2005;330(7498):995.

121. Brent AJ, Ahmed I, Ndiritu M, Lewa P, Ngetsa C, Lowe B, et al. Incidence of clinically significant bacteraemia in children who present to hospital in Kenya: community-based observational study. *Lancet* 2006;367(9509):482-8.

122. Walsh AL, Phiri AJ, Graham SM, Molyneux EM, Molyneux ME. Bacteremia in febrile Malawian children: clinical and microbiologic features. *Pediatr Infect Dis J* 2000;19(4):312-8.

123. Archibald LK, McDonald LC, Nwanyanwu O, Kazembe P, Dobbie H, Tokars J, et al. A hospital-based prevalence survey of bloodstream infections in febrile patients in Malawi: implications for diagnosis and therapy. *J Infect Dis* 2000;181(4):1414-20.

124. Blomberg B, Manji KP, Urassa WK, Tamim BS, Mwakagile DS, Jureen R, et al. Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: a prospective cohort study. *BMC Infect Dis* 2007;7:43.

125. Ghiorghis B, Geyid A, Haile M. Bacteraemia in febrile out-patient children. *East Afr Med J* 1992;69(2):74-7.

126. O'Dempsey TJ, McArdle TF, Lloyd-Evans N, Baldeh I, Laurence BE, Secka O, et al. Importance of enteric bacteria as a cause of pneumonia, meningitis and septicemia among children in a rural community in The Gambia, West Africa. *Pediatr Infect Dis J* 1994;13(2):122-8.

127. Gordon MA, Graham SM, Walsh AL, Wilson L, Phiri A, Molyneux E, et al. Epidemics of invasive Salmonella enterica serovar enteritidis and S. enterica Serovar typhimurium infection associated with multidrug resistance among adults and children in Malawi. *Clin Infect Dis* 2008;46(7):963-9.

128. Hill PC, Onyeama CO, Ikumapayi UN, Secka O, Ameyaw S, Simmonds N, et al. Bacteraemia in patients admitted to an urban hospital in West Africa. *BMC Infect Dis* 2007;7:2.

129. Akpede GO, Abiodun PO, Sykes RM. Relative contribution of bacteraemia and malaria to acute fever without localizing signs of infection in under-five children. *J Trop Pediatr* 1992;38(6):295-8.

130. Meremikwu MM, Nwachukwu CE, Asuquo AE, Okebe JU, Utsalo SJ. Bacterial isolates from blood cultures of children with suspected septicaemia in Calabar, Nigeria. *BMC Infect Dis* 2005;5:110.

131. Bahwere P, Levy J, Hennart P, Donnen P, Lomoyo W, Dramaix-Wilmet M, et al. Community-acquired bacteremia among hospitalized children in rural central Africa. *Int J Infect Dis* 2001;5(4):180-8.

132. Lepage P, Bogaerts J, Van Goethem C, Ntahorutaba M, Nsengumuremyi F, Hitimana DG, et al. Community-acquired bacteraemia in African children. *Lancet* 1987;1(8548):1458-61.

133. Sigauque B, Roca A, Mandomando I, Morais L, Quinto L, Sacarlal J, et al. Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. *Pediatr Infect Dis J* 2009;28(2):108-13.

134. Cotton MF, Burger PJ, Bodenstein WJ. Bacteraemia in children in the south-western Cape. A hospital-based survey. *S Afr Med J* 1992;81(2):87-90.

135. Molyneux ME, Taylor TE, Wirima JJ, Borgstein A. Clinical features and prognostic indicators in paediatric cerebral malaria: a study of 131 comatose Malawian children. *Q J Med* 1989;71(265):441-59.

136. Evans JA, Adusei A, Timmann C, May J, Mack D, Agbenyega T, et al. High mortality of infant bacteraemia clinically indistinguishable from severe malaria. *Qjm* 2004;97(9):591-7.

137. Bronzan RN, Taylor TE, Mwenechanya J, Tembo M, Kayira K, Bwanaisa L, et al. Bacteremia in Malawian children with severe malaria: prevalence, etiology, HIV coinfection, and outcome. *J Infect Dis* 2007;195(6):895-904.

138. Cowgill KD, Ndiritu M, Nyiro J, Slack MP, Chiphatsi S, Ismail A, et al. Effectiveness of Haemophilus influenzae type b Conjugate vaccine introduction into routine childhood immunization in Kenya. *JAMA* 2006;296(6):671-8.

139. Graham SM, English M. Non-typhoidal salmonellae: a management challenge for children with community-acquired invasive disease in tropical African countries. *Lancet* 2009;373(9659):267-9.

140. Mulholland K, Hilton S, Adegbola R, Usen S, Oparaugo A, Omosigho C, et al. Randomised trial of Haemophilus influenzae type-b tetanus protein conjugate vaccine [corrected] for prevention of pneumonia and meningitis in Gambian infants. *Lancet* 1997;349(9060):1191-7.

141. Amos B, Kisakye A, Makewa D, Mudhune S, Mwamtemi H, Nansera D, et al. Behind the data: establishing the Network for Surveillance of Pneumococcal Disease in the East African Region. *Clin Infect Dis* 2009;48 Suppl 2:S162-71.

142. WHO. *Guidelines for the Treatment of malaria*. Geneva: WHO, 2006.

143. Taylor TE, Molyneux ME, Wirima JJ, Fletcher KA, Morris K. Blood glucose levels in Malawian children before and during the administration of intravenous quinine for severe falciparum malaria. *N Engl J Med* 1988;319(16):1040-7.

144. White NJ, Miller KD, Brown J, Marsh K, Greenwood B. Prognostic value of CSF lactate in cerebral malaria. *Lancet* 1987;1(8544):1261.

145. White NJ, Miller KD, Marsh K, Berry CD, Turner RC, Williamson DH, et al. Hypoglycaemia in African children with severe malaria. *Lancet* 1987;1(8535):708-11.

146. White NJ, Warrell DA, Looareesuwan S, Chanthavanich P, Phillips RE, Pongpaew P. Pathophysiological and prognostic significance of cerebrospinal-fluid lactate in cerebral malaria. *Lancet* 1985;1(8432):776-8.

147. Severe and complicated malaria. World Health Organization, Division of Control of Tropical Diseases. *Trans R Soc Trop Med Hyg* 1990;84(Suppl 2):1-65.

148. Lackritz EM, Campbell CC, Ruebush TK, 2nd, Hightower AW, Wakube W, Steketee RW, et al. Effect of blood transfusion on survival among children in a Kenyan hospital. *Lancet* 1992;340(8818):524-8.

149. Taylor TE, Borgstein A, Molyneux ME. Acid-base status in paediatric Plasmodium falciparum malaria. *Q J Med* 1993;86(2):99-109.

150. English M, Waruiru C, Amukoye E, Murphy S, Crawley J, Mwangi I, et al. Deep breathing in children with severe malaria: indicator of metabolic acidosis and poor outcome. *Am J Trop Med Hyg* 1996;55(5):521-4.

151. Jaffar S, Van Hensbroek MB, Palmer A, Schneider G, Greenwood B. Predictors of a fatal outcome following childhood cerebral malaria. *Am J Trop Med Hyg* 1997;57(1):20-4.

152. Biemba G, Dolmans D, Thuma PE, Weiss G, Gordeuk VR. Severe anaemia in Zambian children with Plasmodium falciparum malaria. *Trop Med Int Health* 2000;5(1):9-16.

153. Giha HA, Elghazali G, TM AE, IE AE, Eltahir EM, Baraka OZ, et al. Clinical pattern of severe Plasmodium falciparum malaria in Sudan in an area characterized by seasonal and unstable malaria transmission. *Trans R Soc Trop Med Hyg* 2005;99(4):243-51.

154. Idro R, Aloyo J, Mayende L, Bitarakwate E, John CC, Kivumbi GW. Severe malaria in children in areas with low, moderate and high transmission intensity in Uganda. *Trop Med Int Health* 2006;11(1):115-24.

155. Mockenhaupt FP, Ehrhardt S, Burkhardt J, Bosomtwe SY, Laryea S, Anemana SD, et al. Manifestation and outcome of severe malaria in children in northern Ghana. *Am J Trop Med Hyg* 2004;71(2):167-72.

156. Ranque S, Poudiougou B, Traore A, Keita M, Oumar AA, Safeukui I, et al. Life-threatening malaria in African children: a prospective study in a mesoendemic urban setting. *Pediatr Infect Dis J* 2008;27(2):130-5.

157. Taylor T, Olola C, Valim C, Agbenyega T, Kremsner P, Krishna S, et al. Standardized data collection for multi-center clinical studies of severe malaria in African children: establishing the SMAC network. *Trans R Soc Trop Med Hyg* 2006;100(7):615-22.

158. Al-Taiar A, Jaffar S, Assabri A, Al-Habori M, Azazy A, Al-Mahdi N, et al. Severe malaria in children in Yemen: two site observational study. *BMJ* 2006;333(7573):827.

159. Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. *Lancet* 1997;349(9061):1269-76.

160. Wardlaw T, Salama P, Johansson EW, Mason E. Pneumonia: the leading killer of children. *Lancet* 2006;368(9541):1048-50.

161. WHO. *Pocket book of hospital care for children:guidelines for the management of common illnesses with limited resources*. Geneva: WHO, 2005.

162. English M, Punt J, Mwangi I, McHugh K, Marsh K. Clinical overlap between malaria and severe pneumonia in Africa children in hospital. *Trans R Soc Trop Med Hyg* 1996;90(6):658-62.

163. O'Dempsey TJ, McArdle TF, Laurence BE, Lamont AC, Todd JE, Greenwood BM. Overlap in the clinical features of pneumonia and malaria in African children. *Trans R Soc Trop Med Hyg* 1993;87(6):662-5.

164. Redd SC, Bloland PB, Kazembe PN, Patrick E, Tembenu R, Campbell CC. Usefulness of clinical case-definitions in guiding therapy for African children with malaria or pneumonia. *Lancet* 1992;340(8828):1140-3.

165. WHO. Acute respiratory infections in children: case management in small hospitals in developing countries. A manual for doctors and other senior health workers. Genveva: WHO, 1990.

166. Demers AM, Morency P, Mberyo-Yaah F, Jaffar S, Blais C, Somse P, et al. Risk factors for mortality among children hospitalized because of acute respiratory infections in Bangui, Central African Republic. *Pediatr Infect Dis J* 2000;19(5):424-32.

167. Pepin J, Demers AM, Mberyo-Yaah F, Jaffar S, Blais C, Somse P, et al. Acute lower respiratory infections among children hospitalized in Bangui, Central African Republic: toward a new case-management algorithm. *Trans R Soc Trop Med Hyg* 2001;95(4):410-7.

168. McNally LM, Jeena PM, Gajee K, Thula SA, Sturm AW, Cassol S, et al. Effect of age, polymicrobial disease, and maternal HIV status on treatment response and cause of severe pneumonia in South African children: a prospective descriptive study. *Lancet* 2007;369(9571):1440-51.

169. Smyth A, Tong CY, Carty H, Hart CA. Impact of HIV on mortality from acute lower respiratory tract infection in rural Zambia. *Arch Dis Child* 1997;77(3):227-30.

170. Spooner V, Barker J, Tulloch S, Lehmann D, Marshall TF, Kajoi M, et al. Clinical signs and risk factors associated with pneumonia in children admitted to Goroka Hospital, Papua New Guinea. *J Trop Pediatr* 1989;35(6):295-300.

171. Shann F, Barker J, Poore P. Clinical signs that predict death in children with severe pneumonia. *Pediatr Infect Dis J* 1989;8(12):852-5.

172. Djelantik IG, Gessner BD, Sutanto A, Steinhoff M, Linehan M, Moulton LH, et al. Case fatality proportions and predictive factors for mortality among

children hospitalized with severe pneumonia in a rural developing country setting. *J Trop Pediatr* 2003;49(6):327-32.

173. Subhi R, Adamson M, Campbell H, Weber M, Smith K, Duke T. The prevalence of hypoxaemia among ill children in developing countries: a systematic review. *Lancet Infect Dis* 2009;9(4):219-27.
174. Duke T, Mgone J, Frank D. Hypoxaemia in children with severe pneumonia in Papua New Guinea. *Int J Tuberc Lung Dis* 2001;5(6):511-9.
175. Onyango FE, Steinhoff MC, Wafula EM, Wariua S, Musia J, Kitonyi J. Hypoxaemia in young Kenyan children with acute lower respiratory infection. *BMJ* 1993;306(6878):612-5.
176. Usen S, Webert M. Clinical signs of hypoxaemia in children with acute lower respiratory infection: indicators of oxygen therapy. *Int J Tuberc Lung Dis* 2001;5(6):505-10.
177. Campbell H, Byass P, Lamont AC, Forgie IM, O'Neill KP, Lloyd-Evans N, et al. Assessment of clinical criteria for identification of severe acute lower respiratory tract infections in children. *Lancet* 1989;1(8633):297-9.
178. Duke T, Blaschke AJ, Sialis S, Bonkowsky JL. Hypoxaemia in acute respiratory and non-respiratory illnesses in neonates and children in a developing country. *Arch Dis Child* 2002;86(2):108-12.
179. Lundsgaard C, Van Slyke DD. The Quantitative Influences of Certain Factors Involved in the Production of Cyanosis. *Proc Natl Acad Sci U S A* 1922;8(9):280-2.
180. Crawley J. Reducing the burden of anemia in infants and young children in malaria-endemic countries of Africa: from evidence to action. *Am J Trop Med Hyg* 2004;71(2 Suppl):25-34.
181. Schellenberg D, Schellenberg JR, Mushi A, Savigny D, Mgalula L, Mbuya C, et al. The silent burden of anaemia in Tanzanian children: a community-based study. *Bull World Health Organ* 2003;81(8):581-90.
182. Usen S, Weber M, Mulholland K, Jaffar S, Oparaugo A, Omosigho C, et al. Clinical predictors of hypoxaemia in Gambian children with acute lower respiratory tract infection: prospective cohort study. *BMJ* 1999;318(7176):86-91.

183. Reuland DS, Steinhoff MC, Gilman RH, Bara M, Olivares EG, Jabra A, et al. Prevalence and prediction of hypoxemia in children with respiratory infections in the Peruvian Andes. *J Pediatr* 1991;119(6):900-6.

184. Dyke T, Lewis D, Heegaard W, Manary M, Flew S, Rudeen K. Predicting hypoxia in children with acute lower respiratory infection: a study in the highlands of Papua New Guinea. *J Trop Pediatr* 1995;41(4):196-201.

185. Lozano JM, Steinhoff M, Ruiz JG, Mesa ML, Martinez N, Dussan B. Clinical predictors of acute radiological pneumonia and hypoxaemia at high altitude. *Arch Dis Child* 1994;71(4):323-7.

186. Weber MW, Usen S, Palmer A, Jaffar S, Mulholland EK. Predictors of hypoxaemia in hospital admissions with acute lower respiratory tract infection in a developing country. *Arch Dis Child* 1997;76(4):310-4.

187. Smyth A, Carty H, Hart CA. Clinical predictors of hypoxaemia in children with pneumonia. *Ann Trop Paediatr* 1998;18(1):31-40.

188. Lodha R, Bhadauria PS, Kuttikat AV, Puranik M, Gupta S, Pandey RM, et al. Can clinical symptoms or signs accurately predict hypoxemia in children with acute lower respiratory tract infections? *Indian Pediatr* 2004;41(2):129-35.

189. Laman M, Ripa P, Vince J, Tefuarani N. Can clinical signs predict hypoxaemia in Papua New Guinean children with moderate and severe pneumonia? *Ann Trop Paediatr* 2005;25(1):23-7.

190. Gordon MA. Salmonella infections in immunocompromised adults. *J Infect* 2008;56(6):413-22.

191. Landis JR, Koch GG. An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. *Biometrics* 1977;33(2):363-74.

192. Wagai J, Senga J, Fegan G, English M. Examining agreement between clinicians when assessing sick children. *PLoS ONE* 2009;4(2):e4626.

193. Kahigwa E, Schellenberg D, Schellenberg JA, Aponte JJ, Alonso PL, Menendez C. Inter-observer variation in the assessment of clinical signs in sick Tanzanian children. *Trans R Soc Trop Med Hyg* 2002;96(2):162-6.

194. Margolis P, Gadomski A. The rational clinical examination. Does this infant have pneumonia? *JAMA* 1998;279(4):308-13.

195. Otieno H, Were E, Ahmed I, Charo E, Brent A, Maitland K. Are bedside features of shock reproducible between different observers? *Arch Dis Child* 2004;89(10):977-9.

196. Weber MW, Kellingray SD, Palmer A, Jaffar S, Mulholland EK, Greenwood BM. Pallor as a clinical sign of severe anaemia in children: an investigation in the Gambia. *Bull World Health Organ* 1997;75 Suppl 1:113-8.

197. Chandler CI, Nadjm B, Boniface G, Juma K, Reyburn H, Whitty CJ. Assessment of children for acute respiratory infections in hospital outpatients in Tanzania: what drives good practice? *Am J Trop Med Hyg* 2008;79(6):925-32.

198. Maxwell CA, Chambo W, Mwaimu M, Magogo F, Carneiro IA, Curtis CF. Variation of malaria transmission and morbidity with altitude in Tanzania and with introduction of alphacypermethrin treated nets. *Malar J* 2003;2:28.

199. Norton EB, Archibald LK, Nwanyanwu OC, Kazembe PN, Dobbie H, Reller LB, et al. Clinical predictors of bloodstream infections and mortality in hospitalized Malawian children. *Pediatr Infect Dis J* 2004;23(2):145-51; discussion 51-5.

200. Falade AG, Mulholland EK, Adegbola RA, Greenwood BM. Bacterial isolates from blood and lung aspirate cultures in Gambian children with lobar pneumonia. *Ann Trop Paediatr* 1997;17(4):315-9.

201. Bandyopadhyay T, Gerardi DA, Metersky ML. A comparison of induced and expectorated sputum for the microbiological diagnosis of community acquired pneumonia. *Respiration* 2000;67(2):173-6.

202. Barnes DJ, Naraqi S, Igo JD. The role of percutaneous lung aspiration in the bacteriological diagnosis of pneumonia in adults. *Aust N Z J Med* 1988;18(6):754-7.

203. Ishida T, Hashimoto T, Arita M, Osawa M, Tachibana H, Nishioka M, et al. Efficacy of transthoracic needle aspiration in community-acquired pneumonia. *Intern Med* 2001;40(9):873-7.

204. Lahti E, Peltola V, Waris M, Virkki R, Rantakokko-Jalava K, Jalava J, et al. Induced sputum in the diagnosis of childhood community-acquired pneumonia. *Thorax* 2009;64(3):252-7.

205. O'Neill KP, Lloyd-Evans N, Campbell H, Forgie IM, Sabally S, Greenwood BM. Latex agglutination test for diagnosing pneumococcal pneumonia in children in developing countries. *BMJ* 1989;298(6680):1061-4.

206. Shann F, Gratten M, Germer S, Linnemann V, Hazlett D, Payne R. Aetiology of pneumonia in children in Goroka Hospital, Papua New Guinea. *Lancet* 1984;2(8402):537-41.

207. Bella F, Tort J, Morera MA, Espaulella J, Armengol J. Value of bacterial antigen detection in the diagnostic yield of transthoracic needle aspiration in severe community acquired pneumonia. *Thorax* 1993;48(12):1227-9.

208. Vuori-Holopainen E, Salo E, Saxen H, Hedman K, Hyypia T, Lahdenpera R, et al. Etiological diagnosis of childhood pneumonia by use of transthoracic needle aspiration and modern microbiological methods. *Clin Infect Dis* 2002;34(5):583-90.

209. Zalacain R, Llorente JL, Gaztelurrutia L, Pijoan JI, Sobradillo V. Influence of three factors on the diagnostic effectiveness of transthoracic needle aspiration in pneumonia. *Chest* 1995;107(1):96-100.

210. Morton RE, Lawande R. Frequency and clinical features of urinary tract infection in paediatric out-patients in Nigeria. *Ann Trop Paediatr* 1982;2(3):113-7.

211. Okwara FN, Obimbo EM, Wafula EM, Murila FV. Bacteraemia, urinary tract infection and malaria in hospitalised febrile children in Nairobi: is there an association? *East Afr Med J* 2004;81(1):47-51.

212. Kaditis AG, O'Marcaigh AS, Rhodes KH, Weaver AL, Henry NK. Yield of positive blood cultures in pediatric oncology patients by a new method of blood culture collection. *Pediatr Infect Dis J* 1996;15(7):615-20.

213. Zar HJ, Hanslo D, Tannenbaum E, Klein M, Argent A, Eley B, et al. Aetiology and outcome of pneumonia in human immunodeficiency virus-infected children hospitalized in South Africa. *Acta Paediatr* 2001;90(2):119-25.

214. Zar HJ, Tannenbaum E, Hanslo D, Hussey G. Sputum induction as a diagnostic tool for community-acquired pneumonia in infants and young children from a high HIV prevalence area. *Pediatr Pulmonol* 2003;36(1):58-62.

215. Nantanda R, Hildenwall H, Peterson S, Kaddu-Mulindwa D, Kalyesubula I, Tumwine JK. Bacterial aetiology and outcome in children with severe pneumonia in Uganda. *Ann Trop Paediatr* 2008;28(4):253-60.

216. Chintu C, Mudenda V, Lucas S, Nunn A, Lishimpi K, Maswahu D, et al. Lung diseases at necropsy in African children dying from respiratory illnesses: a descriptive necropsy study. *Lancet* 2002;360(9338):985-90.

217. Mabey DC, Brown A, Greenwood BM. Plasmodium falciparum malaria and Salmonella infections in Gambian children. *J Infect Dis* 1987;155(6):1319-21.

218. Onwubalili JK. Sickle cell disease and infection. *J Infect* 1983;7(1):2-20.

219. Roux CM, B.P.Butler, J.Y.Chau, T.A.Paixao, K.W.Cheung, R.L.Santos, et al. Both hemolytic anemia and malaria parasite-specific factors increase susceptibility to non-typhoidal Salmonella infection in mice. *Infection and Immunity* 2009;In revision.

220. Wykes MN, Good MF. What really happens to dendritic cells during malaria? *Nat Rev Microbiol* 2008;6(11):864-70.

221. Graham SM, Walsh AL, Molyneux EM, Phiri AJ, Molyneux ME. Clinical presentation of non-typhoidal Salmonella bacteraemia in Malawian children. *Trans R Soc Trop Med Hyg* 2000;94(3):310-4.

222. Mermin J, Ekwaru JP, Liechty CA, Were W, Downing R, Ransom R, et al. Effect of co-trimoxazole prophylaxis, antiretroviral therapy, and insecticide-treated bednets on the frequency of malaria in HIV-1-infected adults in Uganda: a prospective cohort study. *Lancet* 2006;367(9518):1256-61.

223. Mermin J, Lule J, Ekwaru JP, Malamba S, Downing R, Ransom R, et al. Effect of co-trimoxazole prophylaxis on morbidity, mortality, CD4-cell count, and viral load in HIV infection in rural Uganda. *Lancet* 2004;364(9443):1428-34.

224. Molyneux E. Emergency care for children in resource-constrained countries. *Trans R Soc Trop Med Hyg* 2009;103(1):11-5.

225. Getaneh T, Girma T, Belachew T, Teklemariam S. The utility of pallor detecting anemia in under five years old children. *Ethiop Med J* 2000;38(2):77-84.

226. Molyneux EM, Maitland K. Intravenous fluids--getting the balance right. *N Engl J Med* 2005;353(9):941-4.

227. Berkley JA, Brent A, Mwangi I, English M, Maitland K, Marsh K, et al. Mortality among Kenyan children admitted to a rural district hospital on weekends as compared with weekdays. *Pediatrics* 2004;114(6):1737-8; author reply 38.

228. Biai S, Rodrigues A, Gomes M, Ribeiro I, Sodemann M, Alves F, et al. Reduced in-hospital mortality after improved management of children under 5 years admitted to hospital with malaria: randomised trial. *BMJ* 2007;335(7625):862.

229. Duke T, Wandi F, Jonathan M, Matai S, Kaupa M, Saavu M, et al. Improved oxygen systems for childhood pneumonia: a multihospital effectiveness study in Papua New Guinea. *Lancet* 2008;372(9646):1328-33.

230. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. *Bull World Health Organ* 2001;79(4):373-4.

231. Angell M. Investigators' responsibilities for human subjects in developing countries. *N Engl J Med* 2000;342(13):967-9.

232. The ethics of research related to healthcare in developing countries: Nuffield Council on Bioethics, 2002.

233. McMillan JR, Conlon C. The ethics of research related to health care in developing countries. *J Med Ethics* 2004;30(2):204-6.

234. GMC. Guidance on good practice, 2006.

235. Adegbola RA, Usen SO, Weber M, Lloyd-Evans N, Jobe K, Mulholland K, et al. Haemophilus influenzae type b meningitis in The Gambia after introduction of a conjugate vaccine. *Lancet* 1999;354(9184):1091-2.

236. Cutts FT, Zaman SM, Enwere G, Jaffar S, Levine OS, Okoko JB, et al. Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia: randomised, double-blind, placebo-controlled trial. *Lancet* 2005;365(9465):1139-46.

237. Iriso R, Ocakacon R, Acayo JA, Mawanda MA, Kisayke A. Bacterial meningitis following introduction of Hib conjugate vaccine in northern Uganda. *Ann Trop Paediatr* 2008;28(3):211-6.

238. Klugman KP, Madhi SA, Huebner RE, Kohberger R, Mbelle N, Pierce N. A trial of a 9-valent pneumococcal conjugate vaccine in children with and those without HIV infection. *N Engl J Med* 2003;349(14):1341-8.

239. Madhi SA, Levine OS, Hajjeh R, Mansoor OD, Cherian T. Vaccines to prevent pneumonia and improve child survival. *Bull World Health Organ* 2008;86(5):365-72.

240. Morris SK, Moss WJ, Halsey N. Haemophilus influenzae type b conjugate vaccine use and effectiveness. *Lancet Infect Dis* 2008;8(7):435-43.

241. Muganga N, Uwimana J, Fidele N, Gahimbare L, Gessner BD, Mueller JE, et al. Haemophilus influenzae type b conjugate vaccine impact against purulent meningitis in Rwanda. *Vaccine* 2007;25(39-40):7001-5.

242. Renner LA, Newman MJ, Ahadzie L, Antwi-Agyei KO, Eshetu M. Introduction of Haemophilus influenzae type B conjugate vaccine into routine immunization in Ghana and its impact on bacterial meningitis in children younger than five years. *Pediatr Infect Dis J* 2007;26(4):356-8.

243. Sow SO, Tapia MD, Diallo S, Keita MM, Sylla M, Onwuchekwa U, et al. Haemophilus influenzae Type B conjugate vaccine introduction in Mali: impact on disease burden and serologic correlate of protection. *Am J Trop Med Hyg* 2009;80(6):1033-8.

244. Samarasekera U. Tanzania to introduce vaccines to tackle childhood pneumonia. *Lancet Infect Dis* 2008;8(7):408.

245. *The Sanford guide to antimicrobial therapy 2007*. 37 ed: Antimicrobial therapy, Inc, 2007.

246. Bejon P, Mwangi I, Ngetsa C, Mwarumba S, Berkley JA, Lowe BS, et al. Invasive Gram-negative bacilli are frequently resistant to standard antibiotics for children admitted to hospital in Kilifi, Kenya. *J Antimicrob Chemother* 2005;56(1):232-5.

247. Kariuki S, Revathi G, Kariuki N, Kiiru J, Mwituria J, Muyodi J, et al. Invasive multidrug-resistant non-typhoidal Salmonella infections in Africa: zoonotic or anthroponotic transmission? *J Med Microbiol* 2006;55(Pt 5):585-91.

248. Nadjm B, Amos B, Mtove G, Ostermann J, Chonya S, Wangai H, et al. WHO guidelines for antimicrobial treatment in children admitted to hospital in an area of intense Plasmodium falciparum transmission: prospective study. *BMJ* 2010;340:c1350.

APPENDIX 5 – ALTERNATIVE ANALYSIS – DO CURRENT GUIDELINES ACCURATELY PREDICT INVASIVE BACTERIAL DISEASE?

INTRODUCTION

The analysis presented in chapter XXXX regarding the sensitivity of WHO guidelines for the detection of bacterial disease in this cohort was based on the principle of defining the clinical criteria that might define "septicaemia" (bacterial disease without clear focus). A definition for septicaemia exists in the WHO guidelines, but it performed poorly. An alternative analysis, based on incorporating this definition into the WHO hierarchical classification (Table A1), is presented below and forms the basis of further publication.[248] Antimicrobial sensitivity data was also available at the time of this later analysis and has been incorporated.

RESULTS

Overall, the sensitivity of WHO criteria to detect IBD was little changed at 67.4% (95%CI 65.9 to 69.0) with a specificity of 51.5% (95%CI 49.9 to 53.1); sensitivity was higher among slide-negative (70.5%, 95%CI 68.2 to 72.9) compared to slide-positive children (60.0%, 95%CI 58.0 to 62.1) and specificity was slightly higher among slide-positives (53.5%, 95%CI 51.4 to 55.6) compared to slide-negatives (48.1%, 95%CI 45.6 to 50.7). The sensitivity of WHO criteria declined with increasing *P. falciparum* parasite density and in children with > 50,000 parasites/μl only 19/36 (52.8%, 95%CI 49.5 to 56.1) children with IBD were correctly identified. The organisms associated with different clinical syndromes are shown in Table A2. The new "septicaemia " criterion only identified 28 children with 3/28 (10.7%).

RISK FACTORS FOR IBD

Clinical and laboratory features associated with IBD were assessed using bivariate odds ratios and a multivariable logistic regression model (Table A2). There was a significant association between IBD and axillary temperature >38 °C, severe anaemia (Hb<5g/dl), and hypoglycaemia (blood sugar<2.5mmol/l) after controlling for the presence of any 'WHO criteria, HIV infection, and current or recent malaria. Prostration was also associated with increased odds of IBD among children without current or recent malaria (OR 2.70, 95%CI 1.47 to 4.97, p=0.001).

ANTIMICROBIAL SUSCEPTIBILITY

In vitro susceptibilities of isolates associated with WHO syndromes are shown in Table A3. Just over half (112/211, 53.1%) of isolates were resistant to the recommended antibiotic. The use of co-trimoxazole for non-severe pneumonia and parenteral penicillin for severe pneumonia were associated with the lowest *in vitro* susceptibilities (31.8% and 33.3% respectively). (Table A3)

CONCLUSION

This further analysis also demonstrated the poor sensitivity of WHO guidelines in the detection of invasive bacterial disease. In addition it highlighted the poor coverage of the currently recommended empirical antibiotic therapy in this setting. The clinical correlate of these antimicrobial sensitivity data is not clear. Observational data from Malawi failed to show an improvement in mortality following a change to broader empirical antibiotic therapy. Ideally alternative antibiotic choices should be explored through clinical trials with careful surveillance to address concerns regarding the impact of the use of broader spectrum antibiotics on bacterial resistance patterns.

Footnotes to Table A1.

Where these categories were used in the analysis, children were assigned to one diagnostic category with priority to those higher in the list. Categories in italics were not used in this analysis.

* Blantyre Coma Score

† Malaria with shock or signs of meningitis defined in WHO guidelines.[62] Children with malaria & signs of meningitis will already be included in the category above.

‡Shock defined as capillary refill>3sec or cool peripheries or systolic BP<50 mm Hg (WHO Pocket Book of Hospital Care for Children 6.2.1, 142)

§ Severe respiratory distress was defined as oxygen saturation <90% or respiratory rate =70 breaths/minute

‖Recommended if chloramphenicol is not available

¶Amoxycillin plus gentamicin is recommended if there is hypoglycaemia or hypothermia or if the child appears lethargic or 'sickly'

'Guidelines for care at first-referral level' criteria for presumptive use of antimicrobials	Recommended antimicrobial	Chapter, page of Guidelines for care at first-referral level manual
Sick young infant Any hospitalised infant age<2months (not included in this study)	Ampicillin plus gentamicin IV/IM Or Penicillin plus gentamicin IV/IM	6.1, 75
Meningoencephalopathy BCS*<3 or stiff neck or bulging fontanelle or >2 convulsions in preceding 24hrs	Chloramphenicol plus ampicillin IV/IM Or Chloramphenicol plus penicillin IV/IM	5.2, 62
Malaria with shock[†] Positive slide for malaria plus Shock[‡] or BCS*<3 or bulging fontanelle or >2 convulsions in preceding 24hrs	Chloramphenicol plus penicillin IV/IM	5.1.1, 59†
Very severe pneumonia Cough/difficulty breathing plus Multiple convulsions or coma or lethargy or vomiting everything or inability to drink or cyanosis or severe respiratory distress[§]	Chloramphenicol IV/IM Or Gentamicin plus penicillin IV/IM[‖]	3.1.1, 30
Severe pneumonia Cough/difficulty breathing plus Lower chest indrawing or nasal flaring or grunting	Benzyl penicillin IV/IM	3.1.2, 32
Non-severe pneumonia Cough/difficulty breathing plus Raised respiratory rate for age	Amoxycillin PO Or Co-trimoxazole PO	3.1.3, 32
Septicaemia Negative slide for malaria plus Axillary temperature = 37.5°C plus Inability to drink/feed or lethargy or 2 convulsions in preceding 24hrs or vomiting everything	Chloramphenicol plus penicillin IV/IM	5.4, 67
Severe acute malnutrition Bilateral oedema or severe wasting or weight-for-height z-score <-3	Co-trimoxazole PO Or [¶]Amoxycillin plus gentamicin IM/IV	7.2.5, 84

Table A1. Definitions of 'guidelines for care at first-referral level' indications for antimicrobial treatment on admission to hospital

Guidelines indication	No organism	NTS	*Haemophilus influenzae* b	Other Gram negative*	*Strep. pneumoniae*	Other Gram positive†
Meningo-encephalopathy	216 (6.6)	7 (4.4)	18 (46.2)	5 (8.2)	5 (8.9)	4 (16.0)
Malaria with shock	76 (2.3)	4 (2.5)	0	2 (3.3)	0	0
Very Severe Pneumonia	311 (9.4)	31 (19.4)	6 (15.4)	10 (16.4)	15 (26.8)	5 (20.0)
Severe Pneumonia	171 (5.2)	13 (8.1)	5 (12.8)	2 (3.3)	9 (16.1)	1 (4.0)
Non-severe Pneumonia	766 (23.2)	39 (24.4)	4 (10.3)	15 (24.6)	15 (26.8)	6 (24.0)
Septicaemia	25 (0.8)	1 (0.6)	0	1 (1.6)	1 (1.8)	0
Severe Acute Malnutrition	34 (1.0)	2 (1.3)	1 (2.6)	2 (3.3)	1 (1.8)	0
None	1,699 (52.5)	63 (39.4)	5 (12.8)	24 (39.3)	10 (17.9)	9 (36.0)
Total	3,298 (100)	160 (100)	39 (100)	61 (100)	56 (100)	25 (100)

Table A2. Number (%) of bacterial isolates in the study by 'guidelines for care at first-referral level' indication for antimicrobial treatment
One child in whom *Strep. pneumoniae* was isolated from CSF and non-Typhi *Salmonella* isolated from blood was classified as infected with *Strep. pneumoniae* alone.
* Included 23 *E. coli* and 11 *S.* Typhi.
† Included 17 *Staph. aureus*

	Unadjusted OR (95%CI)	Adjusted OR (95%CI)
WHO criteria	2.202 (1.738 - 2.789)**	1.669 (1.284 - 2.170)**
Meningo-encephalopathy	1.843 (1.284 - 2.643)**	
Malaria with Shock	0.586 (0.344 - 1.000)	
Very severe pneumonia	2.163 (1.668 - 2.805)**	
Severe pneumonia	1.848 (1.246 - 2.742)**	
Non-severe pneumonia	0.981 (0.754 - 1.276)	
Septicaemia	5.581 (3.915 - 7.956)**	
Severe acute malnutrition	1.719 (0.717 - 4.125)	
HIV-positive	2.38 (1.541 - 3.677)**	1.465 (0.914 - 2.347)
Axillary temperature>38°C	1.339 (1.070 - 1.674)*	1.441 (1.131 - 1.837)**
Prostration †	1.417 (1.029 - 1.951)*	1.007 (0.652 - 1.554)
Hb<5g/dL	1.425 (1.078 - 1.885)*	1.558 (1.134 - 2.141)**
Glucose <2.5mmol/L	2.325 (1.442 - 3.749)**	2.458 (1.390 - 4.347)**
Lactate >5mmol/l	1.459 (1.072 - 1.986)*	
Shock	1.138 (0.689 - 1.879)	0.897 (0.503 - 1.600)
Slide-positive	0.238 (0.187 - 0.304)**	
RDT-pos., slide-neg. ‡	2.897 (2.241 - 3.745)**	1.293 (0.954 - 1.752)
Slide-pos.<5,000/µl	0.843 (0.579 - 1.226)	0.548 (0.359 - 0.836)**
Slide-pos. 5000-50,000/µl	0.272 (0.187 - 0.397)**	0.204 (0.132 - 0.314)**
Slide-pos.>50,000/µl	0.347 (0.244 - 0.495)**	0.241 (0.159 - 0.367)**

Table A3. Results of a logistic regression model of factors associated with IBD in all children in the study.

* $p<0.05$
** $p<0.001$
† Inability to sit unsupported or, if age<8months, inability to drink.
‡ RDT-positive and slide-negative assumed to indicate recent infection with *P. falciparum*

	Amp/ chlor	Pen/ chlor.	Chlor	Pen/ gent†	Penicillin	Amox	Co-trim	Amp/ gent†	Ceftriaxone	Pen/ cip
Meningo-encephalopathy	**22/34 (64.7)** ‡	19/34 (55.9)	16/32 (50.0)	22/24 (91.7)	5/38 (13.2)	13/34 (38.2)	6/25 (24.0)	28/29 (96.6)	31/31 (100)	12/13 (92.3)
Malaria+shock/ meningoencephalopthy	2/6 (33.3)	**2/6 (33.3)**	2/6 (33.3)	4/5 (80.0)	0/6 (0)	2/6 (33.3)	3/5 (60.0)	4/5 (80.0)	5/6 (83.3)	5/5 (100)
Very severe pneumonia	32/62 (51.6)	31/63(49.2)	**26/62 (41.9)**	54/60 (90.0)	16/65 (24.6)	26/62 (41.9)	17/58 (29.3)	55/60 (91.7)	60/61 (98.4)	53/53 (100)
Severe pneumonia	20/29 (69.0)	19/30 (63.3)	17/29 (58.6)	29/29 (100)	**10/30 (33.3)**	18/29 (62.1)	9/26 (34.6)	29/29 (100)	30/30 (100)	23/23 (100)
Non-severe Pneumonia	38/69 (55.1)	36/69 (52.2)	30/66 (45.5)	63/72 (87.5)	16/76 (21.1)	**32/71 (45.1)**	21/66 (31.8)	63/72 (87.5)	71/72 (98.6)	67/67 (100)
Septicaemia	1/2 (50%)	**1/2 (50.0)**	1/2 (50)	3/3 (100)	1/3 (33.3)	1/2 (50.0)	1/2 (50.0)	3/3 (100)	2/2 (100)	3/3 (100)
Severe acute malnutrition	5/6 (83.3)	5/6 (83.3)	5/6 (83.3)	5/5 (100)	1/6 (16.7)	4/6 (66.7)	3/5 (60.0)	**6/6 (100)**	6/6 (100)	5/5 (100)

Table A4. Proportion (%) of bacterial isolates susceptible *in vitro* to recommended and other commonly available antimicrobials by WHO guidelines for care at first-referral level criteria for antimicrobial treatment.*

* Susceptibility includes 'full' or 'intermediate' susceptibility, following CLSI guidelines with the exception of *Salmonella* isolates (noted below). Not all isolates were tested for all antibiotics.

† *Salmonella* susceptibilities to gentamicin are shown as actual in-vitro results although CLSI guidelines recommend that for clinical practice all *Salmonella* should be reported as 'not susceptible' due to poor intracellular penetration of gentamicin. ‡ Figures in bold indicate the first-recommendation treatment.

APPENDIX 6– PRE-SUBMISSION PUBLICATIONS

Poster presentations:

5th MIM Pan-African Malaria Conference, Kenya 2009
B. Nadjm, B. Amos, G. Mtove, K. Chonya, H. Mwangai, J. Kimera, W. Msuya, F. Mtei, D. Dekker, R. Malahiyo, R. Olomi, C.J.M.Whitty, H. Reyburn
"Do current WHO guidelines adequately identify children with invasive bacterial disease in a high malaria transmission setting"

7th International Symposium on Invasive Salmonellosis, Kenya 2009
B.Nadjm, George Mtove, Ben Amos, Kini Chonya, Christopher Whitty, Hugh Reyburn.
"The importance of non-typhi Salmonella as a cause of clinical pneumonia in paediatric admissions to a hospital in a high malaria transmission setting"

RSTMH Centenary Exhibition, London 2007
Behzad Nadjm, Ben Amos, George Mtove, Rajabu Malahiyo, Christina Kiemi, Juma Kimera, Kini Chonya, Chris Whitty, Hugh Reyburn
"The causes of febrile illness in children admitted to a district hospital at high intensity of malaria transmission"

Publications based on these data:

G Mtove, **B Nadjm**, B Amos, I Hendriksen, F Muro, H Reyburn.
The use of a HRP2 based Rapid diagnostic test to guide treatment of children admitted to hospital in a malaria endemic area of NE Tanzania. *In Press*; *Trop Med Int Health*

Nadjm Behzad, Ben Amos, George Mtove, Jan Ostermann, Semkini Chonya, Hannah Wangai, Juma Kimera, Mtei Frank, Denise Dekker, Rajabu Mallahiyo, Raimos Olomi, John A Crump, Christopher J Whitty, Hugh Reyburn
WHO guidelines for antimicrobial treatment in children admitted to hospital in an area of intense Plasmodium falciparum transmission: prospective study. BMJ 2010;340: c1818

Nadjm B, Jeffs B, Mtove G, Msuya W, Mndeme L, Mtei F, Chona K, Reyburn H.
Inter-observer variation in paediatric clinical signs between different grades of staff examining children admitted to hospital in Tanzania. *Trop Med Int Health* 2008;13(9):1213-1219

Publications in which these data were used

Subhi R, Adamson M, Campbell H, Weber M, Smith K, Duke T; Hypoxaemia in Developing Countries Study Group. The prevalence of hypoxaemia among ill children in developing countries: a systematic review. *Lancet Infect Dis.* 2009;9(4):219-27

de Mast Q., **Nadjm B.**, Reyburn H., Kemna E.H, Amos B., Laarakkers C.M, Silalye S., Verhoef H., Sauerwein R.W.,Swinkels D.W, van der Ven A.J.Assessment of urinary concentrations of hepcidin provides novel insight into disturbances in iron homeostasis during malaria infection. *J Infect Dis* 2009; 199(2):253-262

Tropical Medicine and International Health doi:10.1111/j.1365-3156.2011.02737.x

VOLUME 16 NO 5 PP 545–550 MAY 2011

Use of an HRP2-based rapid diagnostic test to guide treatment of children admitted to hospital in a malaria-endemic area of north-east Tanzania

George Mtove[1], Behzad Nadjm[2], Ben Amos[3], Ilse C. E. Hendriksen[4], Florida Muro[5] and Hugh Reyburn[2]

1 *National Institute for Medical Research, Amani Centre, Muheza, Tanzania*
2 *London School of Hygiene and Tropical Medicine, London, UK*
3 *Teule Hospital, Muheza, Tanga, Tanzania*
4 *Mahidol-Oxford Research Unit, Bangkok, Thailand*
5 *Kilimanjaro Christian Medical Centre, Moshi, Tanzania*

Summary

OBJECTIVE To compare the performance of the Paracheck™ rapid diagnostic test (RDT) with microscopy for diagnosing malaria in hospitalised children.

METHODS Children aged between 2 months and 13 years with fever were enrolled in the study over 1 year. A standard clinical history and examination were recorded and blood drawn for culture, complete blood count, Paracheck™ RDT and double-read blood slide.

RESULTS Of 3639 children enrolled, 2195 (60.3%) were slide positive. The sensitivity and specificity of Paracheck were 97.5% (95% CI 96.9–98.0) and 65.3% (95% CI 63.8–66.9), respectively. There was an inverse relationship between age-specific prevalence of parasitaemia and Paracheck specificity. In logistic regression model, false-positive Paracheck results were significantly associated with pre-admission use of antimalarial drug (OR 1.44, 95% CI 1.16–1.78), absence of current fever (OR 0.64, 95% CI 0.52–0.79) and non-typhi *Salmonella* bacteraemia (OR 3.89. 95% CI 2.27–6.63). In spite of high sensitivity, 56/2195 (2.6%) of true infections were Paracheck negative and 8/56 (14.3%) were in patients with >50 000 parasites/μl.

CONCLUSIONS Paracheck had poor specificity in diagnosing malaria in severely ill children; this was likely to be due to HRP2 persistence following recent parasite clearance. The combination of positive Paracheck and negative blood slide results identified a group of children at high risk of non-typhi *Salmonella* infection. While Paracheck was highly sensitive, some high-density infections were missed. For children with severe febrile illness, at least two reliable negative parasitological test results should be available to justify withholding antimalarial treatment; the optimal choice of these has yet to be identified.

keywords malaria, severe, rapid diagnostic test, hospital

Introduction

The use of rapid immunochromatographic tests for malaria (RDTs) in primary care facilities has been studied over a number of years, and these tests are now being rolled out on a large scale in Africa as a means to restrict antimalarial drug use to parasitologically confirmed cases, a policy now supported by WHO (WHO 2010). However, the WHO criteria for malaria diagnosis apply irrespective of severity, and it is therefore surprising that as far as we are aware, only one small study has so far been published on the use of RDTs in patients admitted to hospital (Birku *et al.* 1999).

The wider use of RDTs in patients hospitalised with severe febrile illness seems inevitable given the increasing availability of RDTs. Given current evidence of the low accuracy of routinely read slide results, this may represent an improvement over current practice (Reyburn *et al.* 2004; Zurovac *et al.* 2006). However, the performance of HRP2-based RDTs may differ when used for severely ill children compared to use in non-severe illness. First, specificity may be reduced by the persistence of HRP2 for up to 5 weeks following clearance of parasites and this may be a particular concern in patients who are frequently infected or who have recently taken antimalarial drugs,

© 2011 Blackwell Publishing Ltd

both of which may be more likely in hospitalised children in malaria-endemic areas (Wongsrichanalai *et al.* 1999; Swarthout *et al.* 2007). Secondly, it has been suggested that test sensitivity may paradoxically decline at very high parasite densities (more common in severe than non-severe malaria) because of flooding of RDT capture sites (Reyburn *et al.* 2007). Thirdly, antibodies to HRP2 that are acquired with increasing exposure to malaria might result in age-dependent test performance (Biswas *et al.* 2005). And finally, the interpretation of a combination of a positive RDT result and negative blood slide may indicate 'recent malaria' and this has been associated with certain bacterial infections in severely ill children that may provide added diagnostic value if both RDT and blood slide results are available (Brent *et al.* 2006; Nadjm *et al.* 2010).

In this study, we compared results of a commonly used HRP2-based RDT (Paracheck™) with those from double-read research slides in guiding the care of children enrolled in a 1-year study of children admitted to a district hospital for febrile illness in an area of intense malaria transmission. We compare the technical performance of Paracheck with research-quality blood slide results and suggest how HRP-2-based RDT results might contribute to clinical care in African district hospitals.

Methods

Study site and data collection

The study was conducted in a district hospital in north-eastern Tanzania serving a predominantly rural population with childhood mortality that is typical for Tanzania (165 deaths/1000 person years under the age of 5 years). The area is highly endemic for *Plasmodium falciparum* (*P. falciparum*) malaria.

Details of the study have been published elsewhere (Nadjm *et al.* 2010). Briefly, over the course of 1 year, all daytime paediatric admissions were screened for inclusion and were eligible if aged 2 months to 13 years with axillary temperature ≥37.5 °C or a history of fever within the previous 48 h. Children with chronic illness except HIV or admitted with trauma or a surgical condition were excluded.

After consenting procedures, a standard clinical history and examination based on IMCI guidelines were recorded by a study clinician (WHO 2000a). Pulse oximetry was used on a finger or toe and height and weight were measured. Lumbar puncture was undertaken on suspicion of meningitis according to WHO criteria. Venous blood was drawn for point of care (POC) tests of haemoglobin concentration, blood glucose (Hemocue™, Anglholm, Sweden), blood lactate (Lactate-Pro™; Arkray Inc, Kyoto, Japan), HRP-2-based RDT for *P. falciparum* (Paracheck™; Orchid Biomedical, Mumbai, India) and HIV antibody tests (Capillus HIV-1, HIV-2 Test; Trinity Biotech, Ireland and Determine HIV-1/2 Test; Abbott Laboratories, IL, USA). Blood was sent to the laboratory for full blood count (Act/Dif™; Beckman-Coulter) and aerobic blood culture (BactAlert™; Biomerieux, France) with identification of organisms by standard means. Blood slides were stained with Giemsa and independently double-read with discordant results resolved by a third reader. Paracheck tests were stored in a ventilated room out of direct sunlight with temperature documented not to exceed 40 °C as recommended by the manufacturer.

Data management and analysis

Data were scanned using Teleforms (Verity software Inc.) into MS-Access (Microsoft Corp, Redmond, VA, USA) and analysed using STATA-10 (Stata Corp, College Rd, TX, USA). Final blood slide results are considered as the 'reference standard' result in making comparisons with Paracheck results throughout the paper.

Statistical tests used chi-squared for comparison of proportions and *t*-test (for parametric variable) or rank-sum (for non-parametric variable) for comparison of normally or non-normally distributed data, respectively. The logistic model was constructed with 'positive RDT and negative blood slide' as the dependent variable and factors in Table 3 as independent variables. All of the initially chosen independent variables were retained in the final model.

Ethics

The study was approved by the Ethics Committees of the National Institute for Medical Research, Tanzania (NIMR/HQ/R.8a/Vol.IX/392), and the London School of Hygiene and Tropical Medicine, UK (LSHTM Ethics # 2087). Witten informed consent to participate was obtained from the parent or guardian of each child in the study.

Results

After exclusions for missing data, 3639 children were included in the analysis, all of whom had a blood slide and Paracheck result. Overall, 2139 (58.8%) were both *P. falciparum* slide positive and Paracheck positive, and 943 (25.9%) were negative to both tests. Of the 557 discordant results, 501/557 (90%) were Paracheck positive but slide negative and 56/557 (10%) were Paracheck negative and slide positive. Using the blood slide result as the reference standard, there was an inverse relationship

© 2011 Blackwell Publishing Ltd

Table 1 Age-specific sensitivity, specificity and predictive values of Paracheck compared to blood slide results in 3639 children in the study

	Prevalence of slide positive	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)
2–11 months	487/1054 (46.2%)	96.4 (95.3–97.5)	75.5 (72.3–78.1)	77.3 (74.8–79.8)	96.0 (94.9–97.2)
12–23 months	740/1139 (65.0%)	97.7 (96.8–98.6)	62.2 (59.3–65.0)	82.7 (80.5–84.9)	93.6 (92.2–95.0)
24–59 months	811/1134 (71.5%)	98.2 (97.4–99.0)	48.9 (46.0–51.8)	82.8 (80.6–85.0)	91.3 (89.7–93.0)
60+ months	157/312 (50.3%)	96.2 (94.1–98.3)	70.3 (65.3–75.4)	76.7 (72.0–81.3)	94.8 (92.3–97.3)
Total	2195/3639 (60.3%)	97.5 (96.9–98.0)	65.3 (63.8–66.9)	81.0 (79.8–82.3)	94.4 (93.7–95.1)

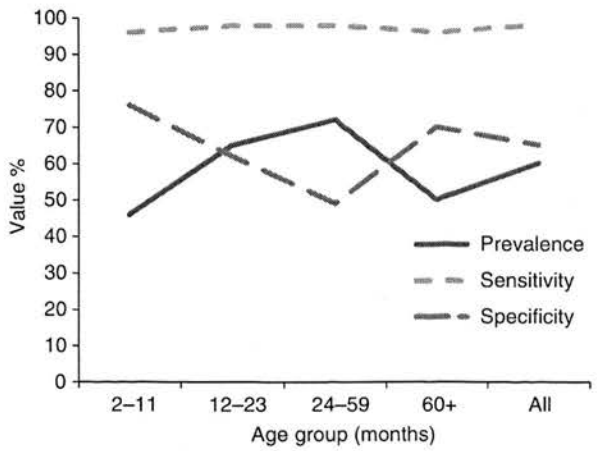


Figure 1 Age-specific sensitivity and specificity of rapid diagnostic test results by prevalence of a positive blood slide of children in the study.

between age-specific prevalence of parasitaemia and the specificity of Paracheck (Table 1, Figure 1).

The sensitivity of Paracheck was above 95% in detecting *P. falciparum* infections with >2000 parasites/μl with no consistent trend with increasing density above this level. The sensitivity of Paracheck was lower in detecting infections with <2000 parasites/μl and especially infections with <200 parasites/μl compared to infections with >2000/μl (Table 2). However, low-density infections were relatively uncommon; only 20/2139 (0.9%) and 240/2, 139 (11.2%) of positive blood slide results were at densities of <200 and <2000 parasites/μl, respectively. More than half of all false-negative Paracheck results (30/56, 53.6%) were in children with a parasite density of >2000 parasites/μl and 8/56 (14.3%) were in children with parasite densities greater than 50 000/μl.

An invasive bacterial infection was isolated in 341/3639 (9.4%) children in the study and these were more common in slide-negative (241/1444, 16.7%) compared to slide-positive children (100/2195, 4.6%, $P < 0.001$). Similarly, bacterial infection was more common in RDT-negatives than in RDT-positives; 194/2640 (7.4%) of RDT-positive and 147/999 (14.7%) of RDT-negative children had invasive bacterial disease ($P < 0.001$). However, of the 501 children who were slide negative but Paracheck positive, 98 (19.6%) had invasive bacterial disease and 67 (69.1%) of these were caused by non-typhi *Salmonella* (NTS) infections (Table 3).

Overall, 597/1850 (32.3%) of children with a true-positive Paracheck result were reported to have taken an antimalarial drug in the 2 days prior to admission compared to 188/443 (42.4%) of children with a false-positive Paracheck result ($P < 0.001$). Factors judged likely to be associated with false-positive Paracheck results were assessed in the logistic regression model in Table 4.

Discussion

The main finding of the study was the low specificity of Paracheck compared to reference blood slides; the overall

Table 2 Sensitivity of Paracheck results by parasite density of reference blood slide results

Parasite density/μl	Prevalence	RDT positive	RDT negative	Sensitivity (95% CI)
1–199	20/1464 (1.4%)	16	4	80.0 (78.0–82.1)
200–1999	220/1664 (13.2%)	198	22	90.0 (88.6–91.4)
2000–4999	165/1609 (10.3%)	159	6	96.4 (95.5–97.3)
5000–49 999	917/2361 (38.8%)	901	16	98.3 (97.7–98.8)
50 000–200 000	693/2137 (32.4%)	689	4	99.4 (99.1–99.7)
>200 000	180/1624 (11.1%)	176	4	97.8 (97.1–98.5)

RDT, rapid diagnostic test.

© 2011 Blackwell Publishing Ltd

Table 3 Pathogenic bacteria isolated from blood or CSF by category of Paracheck and blood slide results

	NTS	Other organism	Total
Slide negative and RDT negative	41 (4%)	102 (11%)	943
Slide negative and RDT positive	67 (13%)	31 (6%)	501
Slide positive, P.f. density <5000/μl	23 (6%)	10 (2%)	405
Slide positive, P.f. density 5000–50 000	13 (1%)	18 (2%)	917
Slide positive, P.f. density 50 000+	16 (2%)	20 (2%)	873
Total	160 (4%)	181 (5%)	3639

NTS, non-typhi *Salmonella*; RDT, rapid diagnostic test.

Table 4 Factors associated with the combination of a negative blood slide and positive Paracheck result

	N (prevalence, %)	Unadjusted OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
Age 12–23 months	1073 (29.5)	1.00 (0.78–1.28)	0.96	1.06 (0.81–1.39)	0.430
Age 24–59 months	2254 (61.9)	1.12 (0.90–1.42)	0.36	1.22 (0.93–1.59)	0.160
Age 60+ months	312 (8.6)	1.14 (0.79–1.63)	0.48	1.35 (0.92–2.00)	0.130
Days ill, OR per day		1.10 (1.01–1.04)	0.002	1.01 (1.00–1.03)	0.113
Antimalarial in last 2 days	1102 (34.8)	1.46 (1.19–1.80)	<0.001	1.44 (1.16–1.78)	0.001
Non-typhi *Salmonella*	169 (4.4)	5.05 (3.63–7.03)	<0.001	3.82 (2.25–6.50)	<0.001
Any invasive bacterial disease	341 (9.4)	2.90 (2.24–3.74)	<0.001	1.52 (0.99–2.33)	0.054
Fever >37.5 °C	2383 (65.6)	0.69 (0.57–0.84)	<0.001	0.64 (0.52–0.79)	<0.001

figure was among the lowest recorded for Paracheck and in children aged between 2 and 5 years, specificity dropped below 50%. The proportion of Paracheck results that were false-positives correlated closely with age-specific parasite prevalence and recent antimalarial drug use but was not independently associated with age.

The rate of false-positive Paracheck results is at least partly a function of the time between parasite clearance and disappearance of HRP2 from blood and this can last for more than 5 weeks (Swarthout *et al.* 2007) and is likely to be associated with recent antimalarial treatment. One would expect this to be particularly common in children who are admitted to a district hospital because this constitutes the first referral level of care, and in our study, we found that over one-third of all children in the study and almost half of the children with a false-positive Paracheck were reported to have taken an antimalarial drug in the 2 days prior to admission. In addition, apparent low specificity may be the result of Paracheck or other RDTs exceeding blood slide results in sensitivity; studies by both Bell *et al.* (2005) and Hopkins *et al.* (2008) have found that a substantial proportion of results that were negative to expert slide reading but positive to Paracheck were positive when tested by polymerase chain reaction (PCR). Without recourse to PCR, we are unable to replicate this result but it seems likely that at least some of the apparent low specificity of Paracheck that we observed was the result of Paracheck detecting submicroscopic parasitaemia.

The influence of antibodies to HRP2 on the accuracy of RDTs is not clear. On the assumption that HRP antibodies accelerate the disappearance of reactive HRP2, one would expect RDT sensitivity and specificity to increase with increasing exposure, for which age is a reasonable proxy in a stable population such as that in our study area. This is supported by the findings of (Fryauff *et al.* 1997) who found a marked difference in sensitivity over and under the age of 10 years among residents of a malaria-endemic area of Irian Jaya, although in our study population, with a much narrower age range, we did not observe age-specific trends in sensitivity. Biswas *et al.* (2005) studied HRP2 levels and antibody titres to HRP2 in 45 subjects in a low-transmission area for up to 6 weeks following infection with *P. falciparum* and found that HRP2 antigen remained elevated for at least 7 days post-treatment, despite the development of HRP2-specific immune responses. Our findings were thus consistent with the conclusions of Biswas *et al.*, that antibody levels to HRP2 are unlikely to exert an important effect on test results in children with severe febrile illness.

The relatively low specificity of Paracheck in our study suggests that its use in a hospital setting will result in significant overuse of antimalarial drugs with the possible neglect of alternative diagnoses. Brent *et al.* (2006) previously described the strong association between a false-positive RDT result and blood stream bacterial infections caused by NTS and other Gram-negative organisms and thus the combination of a negative blood slide result and

© 2011 Blackwell Publishing Ltd

positive Paracheck result should alert clinicians to the possibility of these infections. Given the currently unsatisfactory clinical predictors of bacterial infection in children admitted to hospital in resource-poor settings, this could be a useful diagnostic aid, and a positive HRP2 RDT result should not deter clinicians from providing presumptive treatment with antimicrobials, especially if the blood slide is negative.

By contrast, Paracheck reached very high levels of sensitivity and negative predictive values approached 100%, a finding consistent with other studies (Hopkins *et al.* 2007; Laurent *et al.* 2010). As expected from results of the recent WHO-sponsored evaluation of RDTs, sensitivity dropped below 90% for the detection of low-density infections but otherwise was consistently above the minimum level of recommended level of 95% (WHO 2000a, 2008). The relative importance of low-density infections varies by their prevalence, and low-density infections are more common in low-transmission areas and in asymptomatic individuals; in a community survey in a low-transmission area of the Solomon Islands, Harris *et al.* (2010) found that almost half of all *P. falciparum* infections were with <100 parasites/μl. This is in contrast to hospitalised children in our study where <1% of infections were in this category. Thus, in spite of the high sensitivity of Paracheck in detecting infections above 2000 parasites/μl, more than half of the false-negative Paracheck results in our study were in children with >2000 parasites/μl and almost one in six of the false-negative results was in children with high-density (>50 000 parasites/μl) infections. False-negative RDT results at high density have been described by at least two other studies although the explanation is still not clear (Reyburn *et al.* 2007; Laurent *et al.* 2010). The lack of association with increasing parasite density in our study suggests that flooding of RDT capture sites with excess antigen is an unlikely explanation. Other explanations include mutations in the HRP locus similar to those that have been found in South America (Gamboa *et al.* 2010).

In practice, the small number of false-negative RDT results that we observed suggests that it would be unwise to withhold antimalarial treatment on the basis of a single negative Paracheck result in a severely ill child. Ideally, at least one other parasitological test result should be used and results should ideally be available before starting treatment. This could be a second RDT, preferably based on the detection of alternative antigen to HRP2 such as lactate dehydrogenase (LDH) or a rapidly read quality-controlled blood slide; while the latter is clearly preferable, the limitations on laboratory quality in Africa create serious challenges. More research is needed on what is sufficient evidence to justify withholding antimalarial treatment in a severely ill child admitted to hospital in malaria-endemic areas.

In conclusion, HRP-2 devices in hospitalised children in high-transmission settings suffer from low specificity that is largely dependent on the risk of recently cleared *P. falciparum* infection. Thus, in areas of intense transmission of *P. falciparum*, patients may be overtreated with antimalarial drugs and a positive HRP2 test result should not discourage presumptive treatment with antimicrobial drugs. The combination of a positive HRP2 test with a negative blood slide result may suggest an increased risk of invasive Gram-negative septicaemia. While the Paracheck results in this study reached high levels of sensitivity, a small number of high-density *P. falciparum* infections were recorded as Paracheck-negative, suggesting that at least one more quality-controlled parasitological test should be used before withholding antimalarial treatment in patients with suspected severe malaria. The choice of test or combination of tests to guide treatment of children admitted to hospital with suspected malaria has not so far been defined.

Acknowledgements

We would like to thank the staff and patients of Teule Hospital for their cooperation during study. The following were involved in clinical and laboratory data collection: Aikande Shoo, Halima Mohammed, Charles Mgaya, Celina Wycliffe, Emmanuel Swai, Edward Mtili, Christina Kiemi, Stella Emmanuel, Rosalia Marwa, Simphorosa Silaye, Denise Dekker. Core funding for the study was provided by European Commission (Europaid); BN was supported by grants from the Berkeley Fellowship, Sir Halley Stewart Trust and Pfizer Pharmaceuticals. Pfizer Pharmaceuticals provided equipment and consumables for microbiology. None of the funders had a role in the design, analysis or interpretation of the results.

References

Bell DR, Wilson DW & Martin LB (2005) False-positive results of a *Plasmodium falciparum* histidine-rich protein 2-detecting malaria rapid diagnostic test due to high sensitivity in a community with fluctuating low parasite density. *American Journal of Tropical Medicine and Hygiene* 73, 199–203.

Birku Y, Welday D, Ayele D & Shepherd A (1999) Rapid diagnosis of severe malaria based on the detection of Pf-Hrp-2 antigen. *Ethiopian Medical Journal* 37, 173–179.

Biswas S, Tomar D & Rao DN (2005) Investigation of the kinetics of histidine-rich protein 2 and of the antibody responses to this antigen, in a group of malaria patients from India. *Annals of Tropical Medicine and Parasitology* 99, 553–562.

© 2011 Blackwell Publishing Ltd

Brent AJ, Oundo JO, Mwangi I, Ochola L, Lowe B & Berkley JA (2006) Salmonella bacteremia in Kenyan children. *Pediatric Infectious Disease Journal* **25**, 230–236.

Fryauff DJ, Gomez-Saladin E, Purnomo *et al.* (1997) Comparative performance of the ParaSight F test for detection of *Plasmodium falciparum* in malaria-immune and nonimmune populations in Irian Jaya, Indonesia. *Bulletin of the World Health Organization* **75**, 547–552.

Gamboa D, Ho MF, Bendezu J *et al.* (2010) A large proportion of *P. falciparum* isolates in the Amazon region of Peru lack pfhrp2 and pfhrp3: implications for malaria rapid diagnostic tests. *PLoS ONE* **5**, e8091.

Harris I, Sharrock W, Bain L *et al.* (2010) A large proportion of asymptomatic *Plasmodium* infections with low and sub-microscopic parasite densities in the low transmission setting of Temotu Province, Solomon Islands: challenges for malaria diagnostics in an elimination setting. *Malaria Journal* **9**, 254.

Hopkins H, Kambale W, Kamya MR *et al.* (2007) Comparison of HRP2- and pLDH-based rapid diagnostic tests for malaria with longitudinal follow-up in Kampala, Uganda. *American Journal of Tropical Medicine and Hygiene* **76**, 1092–1097.

Hopkins H, Bebell L, Kambale W, Dokomajilar C, Rosenthal PJ & Dorsey G (2008) Rapid diagnostic tests for malaria at sites of varying transmission intensity in Uganda. *Journal of Infectious Diseases* **197**, 510–518.

Laurent A, Schellenberg J, Shirima K *et al.* (2010) Performance of HRP-2 based rapid diagnostic test for malaria and its variation with age in an area of intense malaria transmission in Tanzania. *Malaria Journal* **9**, 294.

Nadjm B, Amos B, Mtove G *et al.* (2010) WHO guidelines for antimicrobial treatment in children admitted to hospital in an area of intense *Plasmodium falciparum* transmission: prospective study. *BMJ* **340**, c1350.

Reyburn H, Mbatia R, Drakeley C *et al.* (2004) Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. *BMJ* **329**, 1212.

Reyburn H, Mbakiwa H, Mwangi R *et al.* (2007) Rapid diagnostic tests compared with malaria microscopy for guiding outpatient treatment of febrile illness in Tanzania: randomised trial. *BMJ* **334**, 403.

Swarthout T, Counihan DH, Senga RK & Van den Broek I (2007) Paracheck-Pf accuracy and recently treated *Plasmodium falciparum* infections: is there a risk of over-diagnosis? *Malaria Journal* **6**, 58.

WHO (2000a) *Management of the Child with a Serious Infection or Severe Malnutrition*. WHO, Geneva.

WHO (2000b) New perspectives in malaria diagnosis. WHO/CDS/RBM/2000.14.

WHO (2008) *Malaria Rapid Diagnostic Test Performance: results of WHO Product Testing of Malaria RDTs, Round 1*. TDR/WHO. WHO, Geneva.

WHO (2010) Guidelines for malaria diagnosis and treatment. WHO-Roll Back Malaria.

Wongsrichanalai C, Chuanak N, Tulyayon S *et al.* (1999) Comparison of a rapid field immunochromatographic test to expert microscopy for the detection of *Plasmodium falciparum* asexual parasitemia in Thailand. *Acta Tropica* **73**, 263–273.

Zurovac D, Midia B, Ochola SA, English M & Snow RW (2006) Microscopy and outpatient malaria case management among older children and adults in Kenya. *Tropical Medicine and International Health* **11**, 432–440.

Corresponding Author George Mtove, National Institute for Medical Research, Amani Centre, Muheza, Tanzania.
E-mail: mtoveg2002@yahoo.co.uk

© 2011 Blackwell Publishing Ltd

BMJ

RESEARCH

WHO guidelines for antimicrobial treatment in children admitted to hospital in an area of intense *Plasmodium falciparum* transmission: prospective study

Behzad Nadjm, clinical lecturer,[1] Ben Amos, laboratory manager,[2] George Mtove, clinical research scientist,[3] Jan Ostermann, associate professor of health economics,[4] Semkini Chonya, data manager,[5] Hannah Wangai, research nurse,[5] Juma Kimera, research laboratory assistant,[2] Walii Msuya, research clinical officer,[2] Frank Mtei, data manager,[5] Denise Dekker, microbiologist,[5] Rajabu Malahiyo, hospital superintendant,[2] Raimos Olomi, professor of paediatrics,[5] John A Crump, associate professor of clinical microbiology,[6] Christopher J M Whitty, professor of international health,[1] Hugh Reyburn, clinical senior lecturer[1]

[1]London School of Hygiene and Tropical Medicine, London WC1E 7HT

[2]Teule Hospital, Muheza, Tanga, Tanzania

[3]National Institute for Medical Research, Amani Centre, Tanga

[4]Centre for Health Policy, Duke University, Box 90392, Durham, NC 27705, USA

[5]Joint Malaria Programme, Box 2228, Kilimanjaro Christian Medical Centre, Moshi, Tanzania

[6]Division of Infectious Diseases and International Health, Box 102359, Duke University Medical Center, Durham, NC 27710, USA

Correspondence to: H Reyburn
Hugh.reyburn@lshtm.ac.uk

Cite this as: *BMJ* 2010;340:c1350
doi:10.1136/bmj.c1350

ABSTRACT

Objectives To assess the performance of WHO's "Guidelines for care at the first-referral level in developing countries" in an area of intense malaria transmission and identify bacterial infections in children with and without malaria.

Design Prospective study.

Setting District hospital in Muheza, northeast Tanzania.

Participants Children aged 2 months to 13 years admitted to hospital for febrile illness.

Main outcome measures Sensitivity and specificity of WHO guidelines in diagnosing invasive bacterial disease; susceptibility of isolated organisms to recommended antimicrobials.

Results Over one year, 3639 children were enrolled and 184 (5.1%) died; 2195 (60.3%) were blood slide positive for *Plasmodium falciparum*, 341 (9.4%) had invasive bacterial disease, and 142 (3.9%) were seropositive for HIV. The prevalence of invasive bacterial disease was lower in slide positive children (100/2195, 4.6%) than in slide negative children (241/1444, 16.7%). Non-typhi *Salmonella* was the most frequently isolated organism (52/100 (52%) of organisms in slide positive children and 108/241 (45%) in slide negative children). Mortality among children with invasive bacterial disease was significantly higher (58/341, 17%) than in children without invasive bacterial disease (126/3298, 3.8%) (P<0.001), and this was true regardless of the presence of *P falciparum* parasitaemia. The sensitivity and specificity of WHO criteria in identifying invasive bacterial disease in slide positive children were 60.0% (95% confidence interval 58.0% to 62.1%) and 53.5% (51.4% to 55.6%), compared with 70.5% (68.2% to 72.9%) and 48.1% (45.6% to 50.7%) in slide negative children. In children with WHO criteria for invasive bacterial disease, only 99/211(47%) of isolated organisms were susceptible to the first recommended antimicrobial agent.

Conclusions In an area exposed to high transmission of malaria, current WHO guidelines failed to identify almost a third of children with invasive bacterial disease, and more than half of the organisms isolated were not susceptible to currently recommended antimicrobials. Improved diagnosis and treatment of invasive bacterial disease are needed to reduce childhood mortality.

INTRODUCTION

Acute febrile illness is the most common cause of hospital admission and death in African children.[1] The large majority of these illnesses are due to malaria or invasive bacterial disease, but differentiating between these causes is often difficult and can result in missed diagnoses and inappropriate treatment.[2-7] Although *Plasmodium* parasitaemia can be confirmed or excluded by microscopy or rapid diagnostic tests, the diagnosis of invasive bacterial disease is more problematic as blood culture is rarely available and is never in time to guide crucial first treatment decisions. Clinicians therefore often have to rely on the World Health Organization's manual "Management of the child with severe infection or severe malnutrition: guidelines for care at first-referral level in developing countries" (WHO criteria) to guide the presumptive use of anti microbials.[8]

The use of the WHO criteria in an area of moderate transmission of malaria has been shown to identify a high proportion of fatalities due to invasive bacterial disease among children admitted to hospital.[9] However, the criteria have not so far been systematically assessed in an area of intense transmission of *Plasmodium falciparum* where infection with Gram negative organisms, especially non-typhi *Salmonella*, is relatively common and has been associated with severe anaemia, HIV infection, and non-specific clinical features that are not included in the WHO criteria for antimicrobial treatment.[3,5,10-14] In these areas, which include much of rural Africa, clinicians remain uncertain as to which patients to treat and which antimicrobial to use.

We report here the results of a one year prospective study of febrile illness in children admitted to a Tanzanian district hospital serving a population exposed to intense *P falciparum* transmission to assess the ability of the WHO criteria to guide appropriate treatment for invasive bacterial disease.

METHODS

Study site

This was a one year observational study in Teule Hospital, Muheza, northeast Tanzania, a district hospital serving a rural population of approximately 277 000 people with a mortality in children under 5 of 165 per 1000 (Tanzanian census 2002). Transmission of *P falciparum* is intense (50-700 infected bites/person/year) and perennial, with two seasonal peaks.[15] The community prevalence of *P falciparum* in children aged 2-5 years in the study area was recorded as 88.2% in 2002.[15] At the time of the study, routine immunisation did not include vaccination against either *Haemophilus influenzae* or *Streptococcus pneumoniae.* HIV seroprevalence among women attending antenatal clinics was 7.2% in 2007.[16] In 2002 only 5% of paediatric admissions were referred for admission from a primary care facility; the remainer were brought directly to the hospital outpatients department by a parent or caregiver.

Eligibility and enrolment

Consecutive children admitted during the day were given emergency treatment if needed, and then all children were screened for eligibility. Eligible children were aged 2 months to 13 years with a current fever or history of fever within the previous 48 hours. We excluded children with an obvious non-infectious cause for admission such as trauma, surgery, or known malignancy. Children were enrolled over five consecutive days each week. To include children presenting outside normal working hours, we recruited children from Monday to Friday for the first seven months and from Wednesday to Sunday for the next five months. The caregiver of each child in the study gave written informed consent, and pre-test counselling was provided before HIV testing.

Clinical data collection

After consent procedures, clinical officers (a grade of non-physician clinician in Tanzania) assessed children by using a standard medical history and examination based on the WHO criteria (table 1).[8 17] Two study physicians (BN and GM) supervised the collection of clinical data throughout the study. Case management was consistent with WHO guidelines, with the addition that all children with severe malaria were treated with broad spectrum antibiotics consistent with the recommendations of previous studies.[18] Admission outcomes were recorded at discharge or death.

Blood testing and laboratory procedures

After cleaning of the skin, venous blood was drawn for culture (2-5 ml), haemoglobin and glucose concentrations (Hemocue, Angelholm, Sweden), serum lactate (Lactate-Pro, Arkray, Kyoto, Japan), HRP-2 based rapid diagnostic test for *P falciparum* (Paracheck, Orchid Biomedical, Mumbai, India), and a full blood count (Coulter Act/Dif, Beckman-Coulter). We used two rapid tests (Capillus HIV-1, HIV-2 Test, Trinity Biotech, Ireland and Determine HIV-1/2 Test, Abbott Laboratories, IL, USA) in all children to test for HIV antibodies; discordant results were resolved by HIV-1 enzyme linked immunosorbent assay (ELISA) (Vironistika UniForm II Plus-O Test, bioMérieux, NC, USA).[19] Children aged under 18 months with positive

Table 1 | WHO criteria for presumptive antimicrobial treatment in children admitted to hospital

WHO criteria	Recommended antimicrobial	Chapter, page of guidelines for care at first referral level[a]
Sick young infant—any infant aged <2 months admitted to hospital (not included in this study)	Ampicillin plus gentamicin IV/IM or penicillin plus gentamicin IV/IM	6.1, 75
Meningo-encephalopathy—BCS<3 or stiff neck or bulging fontanelle or >2 convulsions in preceding 24 hours	Chloramphenicol plus ampicillin IV/IM or chloramphenicol plus penicillin IV/IM	5.2, 62
Malaria with shock*—positive slide for malaria plus shock† or BCS<3 or bulging fontanelle or >2 convulsions in preceding 24 hours	Chloramphenicol plus penicillin IV/IM	5.1.1, 59†
Very severe pneumonia—cough/difficulty breathing plus multiple convulsions or coma or lethargy or vomiting everything or inability to drink or cyanosis or severe respiratory distress‡	Chloramphenicol IV/IM or gentamicin plus penicillin IV/IM§	3.1.1, 30
Severe pneumonia—cough/difficulty breathing plus lower chest indrawing or nasal flaring or grunting	Benzyl penicillin IV/IM	3.1.2, 32
Non-severe pneumonia—cough/difficulty breathing plus raised respiratory rate for age	Amoxycillin PO or co-trimoxazole PO	3.1.3, 32
Septicaemia—negative slide for malaria plus axillary temperature ≥37.5°C plus inability to drink/feed or lethargy or >2 convulsions in preceding 24 hours or vomiting everything	Chloramphenicol plus penicillin IV/IM	5.4, 67
Severe acute malnutrition—bilateral oedema or severe wasting or weight for height z-score <−3	Co-trimoxazole PO or amoxycillin plus gentamicin¶ IM/IV	7.2.5, 84

Where categories were used in analysis, children were assigned to one diagnostic category with priority to those higher in list.
BCS=Blantyre coma score; IM=intramuscular; IV=intravenous; PO=oral.
*"Malaria with shock or signs of meningitis" defined in WHO guidelines[8]; however, children with malaria and signs of meningitis will already be included in category above.
†Shock defined as capillary refill >3 seconds or cool peripheries or systolic blood pressure <50 mm Hg (WHO Pocket Book of Hospital Care for Children 6.2.1, 142).
‡Defined as oxygen saturation <90% or respiratory rate ≥70 breaths/minute.
§Recommended if chloramphenicol is not available.
¶Recommended if hypoglycaemia or hypothermia present or if child seems lethargic or "sickly."

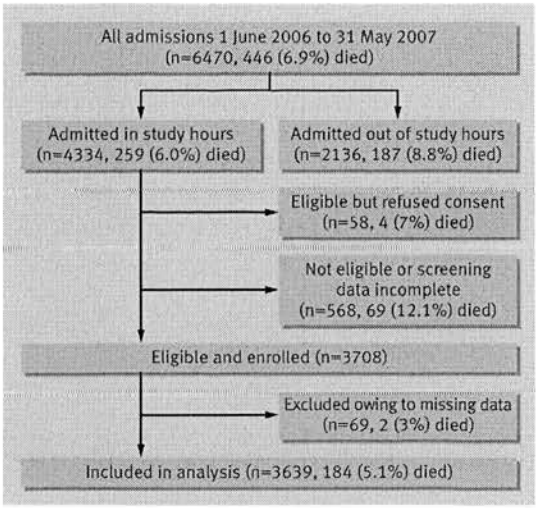


Fig 1 | Admissions, deaths, inclusions, and exclusions during study. 2383/3639 (65.5%) had an axillary temperature ≥37.5 on admission, 1234/3639 (33.9%) were afebrile with a history of fever, and 22/3639 (0.6%) gave a history of fever with no temperature recorded

results were tested for HIV-1 RNA (Abbott Real-Time m2000 System, Abbott Molecular, IL, USA).

Giemsa-stained blood slides were prepared from venous blood and independently double read; discordant results (either positive/negative discordance or greater than twofold density difference above 400 parasites/μl) were resolved by an independent third reading. We calculated parasite densities from the geometric mean of the two closest counts of asexual parasites/200 white blood cells and the actual white blood count or, if missing, 8000 cells/μl. We classified children negative for *P falciparum* on microscopy and with a positive rapid diagnostic test as "recent malaria" because of the persistence of detectable HRP-2 for up to one month after clearance of parasites.[3]

Blood for culture was inoculated into a BactALERT Paediatric-fan bottle (bioMérieux, France) and incubated in the BacT/ALERT 3D automated microbial detection system. We used standard methods to identify cultures that flagged positive. We determined antimicrobial resistance patterns by disc diffusion and E-test by using the interpretive criteria of the Clinical Laboratory Standards Institute.[20] We considered cultures positive for non-cryptococcal yeasts, coagulase negative *Staphylococcus*, *Micrococcus*, *Corynebacterium*, *Bacillus* sp, or viridans group *Streptococci* to be contaminants and classified them as "negative culture" unless a pathogenic organism was also isolated. We considered the following as indications for lumbar puncture, regardless of malaria test result: history of multiple or partial seizures, history of seizures in children aged under 6 months or over 6 years, confusion or reduction in conscious level, bulging fontanelle, or neck stiffness. We examined cerebrospinal fluid by microscopy and cultured it. Bacterial identifications were independently confirmed for *Salmonella* (Queensland Health Forensic and Scientific Services, Brisbane, Australia), *Strep pneumoniae*, or *Haemophilus influenzae* b isolates (Netspear, Kilifi, Kenya). The laboratory participated in a bacteriology external quality assurance programme coordinated by a reference laboratory in Moshi, Tanzania, which participates successfully in external quality assurance programmes of the College of American Pathologists and is monitored regularly by the US National Institutes of Health and its contractors.

Data management and analysis

We used the Cardiff Teleform system (Cardiff, Vista, CA, USA) to scan data into a Microsoft Access database and analysed them in Stata-10. We calculated nutritional z scores from NCHS/WHO reference data in Epi-6 (CDC, Atlanta, USA). We used the χ^2 test to compare proportions.

Table 1 shows the WHO criteria for antimicrobial treatment used in the analysis; these are consistent with the pocket book version of the guideline produced in 2005.[8,21] The guideline has been interpreted to apply irrespective of *P falciparum* parasitaemia; this is consistent with a previous study and justified by the finding that *P falciparum* parasitaemia is common in asymptomatic children.[9,15,22,23] We used logistic regression models to determine bivariable and multivariable

Table 2 | Sensitivity and specificity of WHO "guidelines for care at first-referral level" criteria for antimicrobial treatment in identifying children with invasive bacterial disease (IBD) by rapid diagnostic test (RDT) and blood slide results

	Total cases (No; % died)	No (%) with IBD	Sensitivity—% (95% CI)	Specificity—% (95% CI)	PPV (%)	NPV (%)	NNT*	% fatal cases with IBD treated†
RDT and slide negative‡	943 (56; 5.9)	143 (15.2)	72.7 (69.9 to 75.6)	47.3 (44.1 to 50.4)	19.8	90.6	5.1	88.9
RDT positive, slide negative§	501 (33; 6.6)	98 (19.6)	67.3 (63.2 to 71.5)	49.9 (45.5 to 54.3)	24.6	86.3	4.1	83.3
Slide positive <5000/μl	405 (19; 4.7)	33 (8.1)	60.6 (55.9 to 65.4)	51.9 (47.0 to 56.8)	10.1	93.7	10.1	80.0
Slide positive 5000-50 000/μl	917 (33; 3.6)	31 (3.4)	67.7 (64.7 to 70.8)	55.9 (52.7 to 59.1)	5.1	98.0	19.6	66.7
Slide positive >50 000/μl	873 (43; 4.9)	36 (4.1)	52.8 (49.5 to 56.1)	51.6 (48.3 to 54.9)	4.5	96.2	22.3	60.0

NPV=negative predictive value; PPV=positive predictive value.
*Number needed to treat presumptively with antimicrobials to correctly treat one child with IBD.
†Proportion of all IBD associated fatalities with "guidelines for care at first-referral level" indication for antimicrobial treatment.
‡56 children were RDT negative and blood slide positive and are included in slide positive data (sensitivity of RDT compared with slide reading was 97.4%).
§Assumed to indicate recent infection with *P falciparum*.

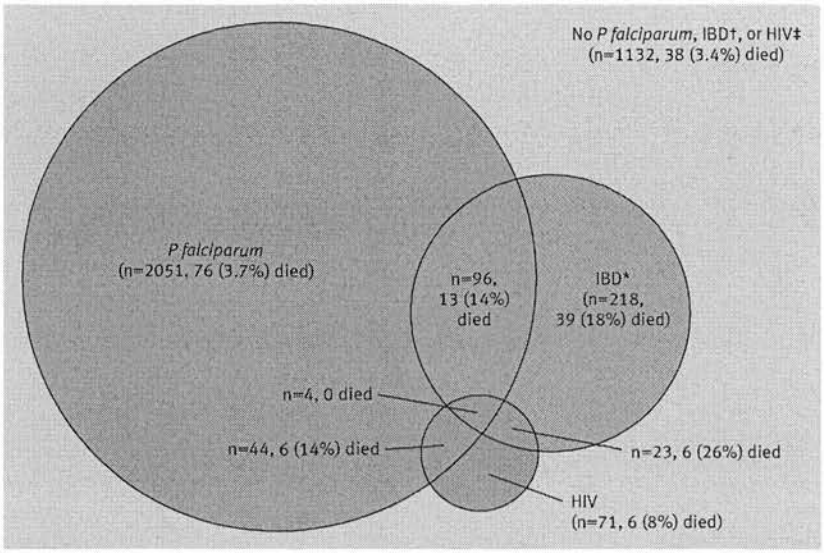


Fig 2 | Numbers and deaths of children infected with *P falciparum* by blood slide, invasive bacterial disease (IBD), or HIV. Areas in Venn diagram approximately to scale. *IBD consisted of 336 children with a positive blood culture, of whom 20 also had a positive cerebrospinal fluid (CSF) culture, and an additional five with a pathogenic organism isolated from CSF and a negative or contaminated blood culture. †Blood cultures classified as negative included 251 (6.9%) from which contaminant organisms only were cultured. ‡Three negative HIV results were based on Capillus testing only (negative predictive value 99.5%, detail not shown); all other HIV results were based on at least two concordant test results

associations of invasive bacterial disease with WHO criteria, HIV infection, fever, severe anaemia, hypoglycaemia, prostration, raised blood lactate, and recent or current malaria.

RESULTS

During the one year of the study (June 2006 to May 2007) 6470 children were admitted; 4334 (67%) were screened for inclusion; 3708 of these were eligible and enrolled, of whom 3639 had sufficient data for analysis (fig 1). The median age of children in the study was 1.6 years; 1054 (29.0%) were aged 2-11 months, 2273 (62.5%) were aged 12-59 months, and 312 (8.6%) were aged 5-13 years.

Figure 2 summarises the prevalence of and mortality associated with invasive bacterial disease, *P falciparum* by blood slide, and HIV. Invasive bacterial disease was identified in 341 (9.4%) of 3639 cases and in 58 (32%) of 184 deaths. Non-typhi *Salmonella* was the most frequently isolated bacterial pathogen, accounting for 52 (52%) of 100 organisms in slide positive children and 108 (45%) of 241 in slide negative children. Mortality among children with invasive bacterial disease (58/341, 17%) was significantly higher than that in children without invasive bacterial disease (126/3298, 3.8%) (P<0.001), and this was true regardless of the presence of *P falciparum* parasitaemia. The median duration of admission was one day for fatal admissions and three days for non-fatal admissions.

Performance of WHO criteria to target antimicrobial treatment

Overall, the sensitivity of the WHO criteria to detect invasive bacterial disease was 67.4% (95% confidence interval 65.9% to 69.0%), with a specificity of 51.5% (49.9% to 53.1%). Sensitivity was higher among slide negative children (70.5%, 68.2% to 72.9%) than among slide positive children (60.0%, 58.0% to 62.1%), and specificity was slightly higher among slide positive children (53.5%, 51.4% to 55.6%) than among slide negative children (48.1%, 45.6% to 50.7%). The sensitivity of the WHO criteria declined with increasing *P falciparum* parasite density (P<0.05, χ^2 test for trend); among children with more than 50 000 parasites/μl, only 19/36 (53%, 50% to 56%) children with invasive bacterial disease were correctly identified (table 2).

WHO criteria were significantly more sensitive in identifying invasive bacterial disease associated fatalities (82.8%, 77.3% to 88.2%) than non-fatal invasive bacterial disease (64.3%, 62.7% to 65.9%), yet 10 (17%) of 58 deaths among children with invasive bacterial disease remained unidentified by WHO criteria as needing antimicrobials.

Table 3 shows the prevalence of infecting organisms by WHO criteria of syndromic diagnosis. More than three quarters (96/120, 80%) of children with Gram

Table 3 | Number (%) of children with and without one of WHO indications for antimicrobial treatment by organism isolated from blood or cerebrospinal fluid

Guidelines indication	Non-typhi *Salmonella*	*Haemophilus influenzae* b	Other Gram negative*	Strep pneumoniae	Other Gram positive†	No organism isolated	Total
Meningo-encephalopathy	7 (3)	18 (7)	5 (2)	5 (2)	4 (2)	216 (85)	255
Malaria with shock	4 (5)	0	2 (2)	0	0	76 (93)	82
Very severe pneumonia	31 (8)	6 (2)	10 (3)	15 (4)	5 (3)	311 (82)	378
Severe pneumonia	13 (7)	5 (3)	2 (1)	9 (5)	1 (5)	171 (85)	201
Non-severe pneumonia	39 (5)	4 (1)	15 (2)	15 (2)	6 (1)	766 (91)	845
Septicaemia	1 (4)	0	1 (4)	1 (4)	0	25 (89)	28
Severe acute malnutrition	2 (5)	1 (3)	2 (5)	1 (3)	0	34 (85)	40
No WHO indication‡	63 (3.5)	5 (0.3)	24 (1.3)	10 (0.6)	9 (0.5)	1699 (93.9)	1810

One child in whom *Strep pneumoniae* was isolated from cerebrospinal fluid and non-typhi *Salmonella* was isolated from blood was classified as infected with *Strep pneumoniae* alone.
*Included 23 *E coli* and 11 *Salmonella typhi*.
†Included 17 *Staph aureus*.
‡225/1810 (12.4%) of children who did not meet an existing WHO indication for antimicrobial treatment had severe anaemia (haemoglobin <5 g/dl).

positive or *H influenzae* infections presented with clinical features that met one of the WHO criteria, whereas just over half (134/221, 61%) of children with non-*H influenzae* Gram negative infections met one of these criteria (P<0.001). Non-*H influenzae* Gram negative infections accounted for more than three quarters (151/198, 76%) of organisms isolated in children with recent or current malaria but less than half (70/143, 49%) of organisms isolated from children who were both slide negative and rapid diagnostic test negative (P<0.001) (fig 3).

Risk factors for invasive bacterial disease

We assessed clinical and laboratory features associated with invasive bacterial disease by using bivariate odds ratios and a multivariable logistic regression model (table 4). After control for the presence of any WHO criteria, HIV infection, and current or recent malaria, a significant association remained between invasive bacterial disease and axillary temperature above 38°C, severe anaemia (haemoglobin <5 g/dl), and hypoglycaemia (blood sugar <2.5 mmol/l) (table 4). Prostration was also associated with increased odds of invasive bacterial disease among children without current or recent malaria (odds ratio 2.54, 95% confidence interval 1.37 to 4.70, P=0.003).

Additions to WHO criteria to improve sensitivity

To evaluate potential candidates for addition to the WHO criteria, we identified factors from those associated with invasive bacterial disease (table 4). We added HIV first on the assumption that this would be incorporated if WHO guidelines included care of children with HIV in the "Fever" chapter. The further criteria of "severe anaemia (Hb <5 g/dl)," "prostration," and "current fever >38°C" increased the sensitivity of the WHO criteria to 86.2% (85.1% to 87.3%), with a specificity of 24.8% (23.4% to 26.2%). Table 5 shows these results by malaria slide results.

Table 4 | Logistic regression model of factors associated with invasive bacterial disease in all children in study

Factors	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)
WHO criteria	2.20 (1.74 to 2.79)**	1.67 (1.28 to 2.17)**
Meningo-encephalopathy	1.84 (1.28 to 2.64)**	
Malaria with shock	0.59 (0.34 to 1.00)	
Very severe pneumonia	2.16 (1.67 to 2.81)**	
Severe pneumonia	1.85 (1.25 to 2.74)**	
Non-severe pneumonia	0.98 (0.75 to 1.28)	
Septicaemia	5.58 (3.92 to 7.96)**	
Severe acute malnutrition	1.72 (0.72 to 4.13)	
HIV positive	2.38 (1.54 to 3.68)**	1.47 (0.91 to 2.35)
Axillary temperature >38°C	1.34 (1.07 to 1.67)*	1.44 (1.13 to 1.84)**
Prostration†	1.42 (1.03 to 1.95)*	1.01 (0.65 to 1.55)
Shock	1.14 (0.69 to 1.88)	0.90 (0.50 to 1.60)
Haemoglobin <5 g/dl	1.43 (1.08 to 1.89)*	1.56 (1.13 to 2.14)**
Glucose <2.5 mmol/l	2.33 (1.44 to 3.75)**	2.46 (1.39 to 4.35)**
Lactate >5 mmol/l	1.46 (1.07 to 1.99)*	
Slide positive	0.24 (0.19 to 0.30)**	
RDT positive, slide negative‡	2.90 (2.24 to 3.75)**	1.29 (0.95 to 1.75)
Slide positive <5000/µl	0.84 (0.58 to 1.23)	0.55 (0.36 to 0.84)**
Slide positive 5000-50 000/µl	0.27 (0.19 to 0.40)**	0.20 (0.13 to 0.31)**
Slide positive >50 000/µl	0.35 (0.24 to 0.50)**	0.24 (0.16 to 0.37)**

RDT=rapid diagnostic test.
*P<0.05.
**P<0.001.
†Inability to sit unsupported or, if age <8 months, inability to drink.
‡RDT positive and slide negative assumed to indicate recent infection with *P falciparum*.

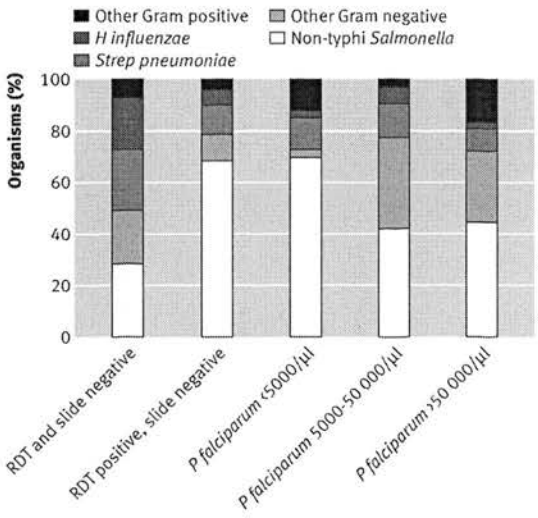


Fig 3 | Proportion of organisms isolated from children with invasive bacterial disease by *P falciparum* infection status. RDT=rapid diagnostic test

In slide positive children, the prevalence of invasive bacterial disease was 60/1035 (5.8%) if one of the WHO criteria was present compared with 26/679 (3.8%) if none was present but the child had any of severe anaemia, prostration, HIV infection, or "current fever >38°C." This contrasts with slide negative children, among whom the prevalence of invasive bacterial disease was 170/794 (21.4%) if one of the WHO criteria was present compared with 38/265 (14%) if none was present but the child had any of severe anaemia, prostration, HIV infection, or current fever >38°C. Hypoglycaemia, although strongly associated with invasive bacterial disease, applied to only 22 children with such disease; all but three of these already met WHO criteria, and all but two of them met one of the criteria classified as D in table 5.

Antimicrobial susceptibility

In children with invasive bacterial disease, the isolated organism was susceptible in vitro to the WHO recommended antibiotic in 33% to 100% of cases, with a weighted mean of 99/211 (47%). Co-trimoxazole for non-severe pneumonia and parenteral penicillin for severe pneumonia were associated with the lowest in vitro susceptibilities (32% and 33%) (table 6). Table 7 shows susceptibilities of common isolates to routinely available antibiotics.

DISCUSSION

Our findings show that, in a typical district hospital serving a population exposed to intense transmission

of *P falciparum*, WHO criteria for antimicrobial treatment failed to identify almost a third of children who had definite invasive bacterial disease. Among those who were identified by WHO criteria, the isolated organism was not susceptible to the first recommended antimicrobial drug in more than half the cases.

As expected, invasive bacterial disease was more likely in slide negative children than in slide positive children. In these co-infected children, however, invasive bacterial disease was not confined to those with low density infections that are often found incidentally in African children; a third of the invasive bacterial disease-*P falciparum* co-infections occurred in children with high density infections that are highly unlikely to be due to incidental parasitaemia.[22] This finding is consistent with those of Berkley et al, who have recently quantified the association of high density malaria infection and bacterial disease.[24] In addition, we found that invasive bacterial disease was associated with high mortality irrespective of *P falciparum* infection, highlighting the importance of considering invasive bacterial disease in children admitted to hospital irrespective of whether a malaria slide is positive. The failure of the WHO criteria to identify many of these children was to a large extent due to the high prevalence of non-typhi *Salmonella* infection and its association with severe anaemia and other non-specific clinical features that are not currently included in the WHO criteria.[8] The introduction of pneumococcal and Hib vaccines in many African countries in the next one to two years is likely to make these infections relatively more important.

Our results are consistent with increasing evidence that malaria is a risk factor for non-typhi *Salmonella* and some other Gram negative infections.[12,24,25] Reasons for this are still unclear but may relate to increased vulnerability to non-typhi *Salmonella* infection associated with haemolysis, as the association also applies in children with sickle cell disease and in animals with experimentally induced haemolysis.[26,27] The association of non-typhi *Salmonella* with "recent malaria" that we and one previous study have observed suggests that malaria may result in relatively prolonged increased vulnerability to bacterial infection, especially by enteric organisms.[3]

Validity and generalisability of findings

Although no study can automatically be generalised to other settings, our findings are similar to those of Berkley et al, who found that in an area of moderate *P falciparum* transmission the WHO criteria correctly identified 66% of invasive bacterial disease in slide positive children and 76% in slide negative children (after the exclusion of "sick young infants," a group at high risk of invasive bacterial disease who are all recommended for antimicrobial treatment by these guidelines).[9] These figures are only slightly higher than our estimates; the difference may be due to a higher proportion of non-typhi *Salmonella* infections in our study.

The diagnostic performance of the WHO criteria is likely to vary with local epidemiology, especially in relation to bacterial disease and malaria, but the consistency of our findings with several others in identifying non-typhi *Salmonella* and other Gram negative infections as leading causes of bacteraemia in African children suggest that our findings are likely to apply widely.[10,14,28-33] In addition, the low level of antimicrobial susceptibility that we found is also consistent with others.[10,32,34-36] The association between malaria and bacterial disease identified in our study is consistent with several other studies and was slightly weaker than found by Berkley et al.[18] Similarly, the association of increased mortality with bacterial disease is similar to that found in several other studies,[18,37] although a study in Malawi did not find an association between mortality and bacterial disease among children with severe malaria admitted to a paediatric intensive care unit.[11]

Table 5 | Sensitivity, specificity, and predictive values of selected additions to WHO "guidelines for care at first-referral level" criteria for presumptive treatment of invasive bacterial disease (IBD) by presence or absence of *P falciparum* parasitaemia

	Sensitivity—% (95% CI)	Specificity—% (95% CI)	PPV (%)	NPV (%)	NNT*	% fatal cases with IBD treated†
Blood slide positive for *P falciparum*						
A—guidelines criteria	60.0 (58.0 to 62.1)	53.5 (51.4 to 55.6)	5.8	96.6	17.3	69.2
B—A or HIV	62.0 (60.0 to 64.0)	52.5 (50.4 to 54.6)	5.9	96.7	17.1	69.2
C—B or severe anaemia	68.0 (66.1 to 70.0)	45.3 (43.2 to 47.4)	5.6	96.7	17.9	84.6
D—C or prostration	72.0 (70.1 to 73.9)	43.4 (41.4 to 45.5)	5.7	97.0	17.5	92.3
E—D or axillary temperature >38°C	86.0 (84.6 to 87.5)	22.3 (20.6 to 24.0)	5.0	97.1	19.9	92.3
Blood slide negative for *P falciparum*						
A—guidelines criteria	70.5 (68.2 to 72.9)	48.1 (45.6 to 50.7)	21.4	89.1	4.7	86.7
B—A or HIV	71.4 (69.0 to 73.7)	46.6 (44.1 to 49.2)	21.1	89.0	4.7	86.7
C—B or severe anaemia	75.5 (73.3 to 77.7)	42.4 (39.9 to 44.9)	20.8	89.6	4.8	88.9
D—C or prostration	75.9 (73.7 to 78.1)	42.0 (39.4 to 44.5)	20.8	89.7	4.8	91.1
E—D or axillary temperature >38°C	86.3 (84.5 to 88.1)	29.3 (26.9 to 31.6)	19.6	91.4	5.1	93.3

NPV=negative predictive value; PPV=positive predictive value.
*Number needed to treat presumptively with antimicrobials to correctly treat one child with IBD.
†Proportion of all IBD associated fatalities identified for antimicrobial treatment.

Table 6 | Proportion (%) of bacterial isolates susceptible in vitro to recommended and other commonly available antimicrobials by WHO "guidelines for care at first-referral level" criteria for antimicrobial treatment*

	Amp/chlor	Pen/chlor	Chlor	Pen/gent†	Pen	Amoxy	Co-trimox	Amp/gent†	Ceftriaxone	Pen/cipro	Azithromycin
Meningo-encephalopathy	22/34 (65)‡	19/34 (56)	16/32 (50)	22/24 (92)	5/38 (13)	13/34 (38)	6/25 (24)	28/29 (97)	31/31 (100)	12/13 (92)	26/29 (90)
Malaria with shock	2/6 (33)	2/6 (33)‡	2/6 (33)	4/5 (80)	0/6 (0)	2/6 (33)	3/5 (60)	4/5 (80)	5/6 (83)	5/5 (100)	3/8 (38)
Very severe pneumonia	32/62 (52)	31/63(49)	26/62 (42)‡	54/60 (90)	16/65 (25)	26/62 (42)	17/58 (29)	55/60 (92)	60/61 (98)	53/53 (100)	38/55 (69)
Severe pneumonia	20/29 (69)	19/30 (63)	17/29 (59)	29/29 (100)	10/30 (33)‡	18/29 (62)	9/26 (35)	29/29 (100)	30/30 (100)	23/23 (100)	27/35 (77)
Non-severe pneumonia	38/69 (55)	36/69 (52)	30/66 (46)	63/72 (88)	16/76 (21)	32/71 (45)‡	21/66 (32)	63/72 (88)	71/72 (996)	67/67 (100)	57/86 (66)
Septicaemia	1/2 (50)	1/2 (50)‡	1/2 (50)	3/3 (100)	1/3 (33)	1/2 (50)	1/2 (50)	3/3 (100)	2/2 (100)	3/3 (100)	4/5 (75)
Severe acute malnutrition	5/6 (83)	5/6 (83)	5/6 (83)	5/5 (100)	1/6 (17)	4/6 (67)	3/5 (60)	6/6 (100)‡	6/6 (100)	5/5 (100)	3/5 (60)

Amoxy=amoxicillin; amp=ampicillin; chlor=chloramphenicol; co-trimox=co-trimoxazole; cipro=ciprofloxacin; gent=gentamicin; pen=penicillin.
*Susceptibility includes "full" or "intermediate" susceptibility and follows Clinical Laboratory Standards Institute (CLSI) guidelines with exception of susceptibility of *Salmonella* isolates (noted below)[20]; not all isolates were tested for all antibiotics.
†*Salmonella* susceptibilities to gentamicin are shown as actual in vitro results, although CLSI guidelines recommend that for clinical practice all *Salmonella* should be reported as "not susceptible" owing to poor intracellular penetration of gentamicin.
‡First recommendation treatment.

A limitation of our study was our inability to enrol children at night; case fatality was higher in children admitted outside study hours, probably because "out of hours" admissions are more severely ill and possibly because initial clinical management is of lower quality at these times.[38] Mortality was also high in children who were excluded from the study because of missing data (often because they died soon after admission), and our findings are thus likely to underestimate mortality in children whose need for antimicrobials was not recognised by WHO criteria. However, children who die soon after admission probably present too late to benefit from antimicrobial treatment.

WHO guidelines

We evaluated the WHO manual "Management of the child with severe infection or severe malnutrition: guidelines for care at first-referral level,"[8] as this is the standard WHO guide for paediatric inpatient care and has been adopted as policy by the ministries of health of many resource poor countries. A WHO guideline for treatment of malaria (2006) also exists; it proposes a "low threshold" for the use of antimicrobials in children with severe malaria to treat *Salmonella* infections in particular.[17] The existence of separate clinical guidelines for malaria and general paediatric care is an unfortunate consequence of the vertical approach to malaria. Possibly as a result of this, antimicrobial treatment is not mentioned in the Tanzanian or Kenyan national guidelines for the treatment of severe malaria.

Additional indications for antimicrobials in children

Among children in this study, the addition of "severe anaemia" and "prostration" to current indications resulted in a significant improvement in the detection of invasive bacterial disease in both fatal and non-fatal cases. This is consistent with several studies that have shown an association between severe anaemia and invasive bacterial disease.[11 29 39] "Current fever"' has previously been associated with invasive bacterial disease,[40] and its inclusion as a criterion for antimicrobials resulted in a gain in sensitivity, although this was at the expense of a substantial drop in specificity. However, even in children with malaria, application of these more inclusive criteria resulted in fewer than 20 children qualifying for presumptive antimicrobial treatment to correctly identify one child with invasive bacterial disease, a figure that compares favourably with policies that recommend presumptive treatment of non-severe malaria.[17] Given the increase in mortality associated with invasive bacterial disease, this seems a reasonable proposition; however, a need exists for a more formal economic evaluation that includes such factors as the financial cost of newer

Table 7 | Proportion (%) of bacterial isolates susceptible in vitro to commonly available antimicrobials

	Penicillin/amoxicillin	Chloramphenicol	Co-trimoxazole	Gentamicin
Non-typhi *Salmonella**	49/148 (33)	53/153 (35)	49/145 (34)	147/152 (97)†
Strep pneumoniae	51/51 (100)	41/48 (85)	22/45 (49)	-
Haemophilus influenzae	12/38 (32)	16/38 (42)	2/28 (7)	22/23 (96)
E coli	2/21 (10)	5/18 (28)	1/20 (5)	9/20 (45)
Staph aureus	1/15 (7)	11/13 (85)	10/14 (71)	13/16 (81)

Susceptibility includes "full" or "intermediate" susceptibility and follows Clinical Laboratory Standards Institute (CLSI) guidelines with exception of susceptibility of *Salmonella* isolates (noted below).[20]
*Salmonella isolates were multi-resistant (resistant to amoxicillin, chloramphenicol, and co-trimoxazole) in 85/141 (60.3%) cases.
†*Salmonella* susceptibilities to gentamicin are shown as actual in vitro results, although CLSI guidelines recommend that for clinical practice all *Salmonella* should be reported as "not susceptible" owing to poor intracellular penetration of gentamicin.

WHAT IS KNOWN ON THIS TOPIC

Presumptive diagnosis of malaria is common in children admitted to hospital in Africa and is associated with failure to provide treatment for bacterial disease

Invasive bacterial disease is a major cause of mortality in African children, alone and in association with malaria

Overlapping clinical features create difficulties in distinguishing between invasive bacterial disease and malaria in African children

WHAT THIS STUDY ADDS

In a malaria endemic area, almost a third of the children with definite invasive bacterial disease were not identified by WHO criteria for the presumptive use of antimicrobials

The high prevalence of non-typhi *Salmonella* and other Gram negative infections may contribute to the poor performance of the WHO guidelines in this setting

More than half of the isolates from children in a malaria endemic area were not susceptible to the WHO recommended antimicrobial

antimicrobials and the selection of resistance that may result from their wider use.

For routine care, the high mortality associated with invasive bacterial disease, the low sensitivity of a single blood culture,[41 42] the poor quality of routine slide results, and the apparently strong bias of hospital clinicians towards the diagnosis of malaria all suggest that inclusive rather than restrictive indications for presumptive antimicrobial treatment are needed to ensure more effective treatment for children admitted to hospitals in resource poor settings.[6 43 44]

Choice of antimicrobials

Our results, which are consistent with those of other studies, show that commonly used antimicrobials often lack activity against the Gram negative organisms that are often the most common cause of bacterial infection in African children.[10 32 34-36] In our study, two thirds of the organisms identified in children who met the WHO criteria for severe pneumonia were resistant to penicillin (the first recommended antimicrobial) and more than a third were resistant to chloramphenicol or ampicillin (which might be used as an alternative). Our in vitro estimate of susceptibility to gentamicin is likely to overestimate its in vivo efficacy as, although some extracellular effect against salmonellas can be expected, salmonella is a largely intracellular organism and the limited penetration of gentamicin is likely to limit its effect.[45 46] As a consequence, gentamicin is not usually recommended for treatment of invasive salmonellosis.[20]

The choice of antimicrobials in resource poor settings is obviously constrained by cost and availability. However, as recently highlighted by Graham and English,[47] the high incidence and fatality of non-typhi *Salmonella* bacteraemia in Africa, which is often resistant to commonly used antimicrobials, constitutes a serious and neglected public health challenge. Better evidence to guide antimicrobial choices is needed, but current evidence suggests that in malaria endemic areas of Africa ciprofloxacin (not usually available parenterally) plus penicillin or ceftriaxone alone are the leading candidates for first choice antimicrobials in severely ill children.

Clinical trials of antibiotic indications in sick children

Our results raise questions that ideally should be informed by the results of a randomised clinical trial of clinical indications for the use of antibiotics. However, such a trial would need to be large. According to the data from our study and an assumption that antimicrobials in children with severe malarial anaemia and no WHO indication for antimicrobials would reduce their mortality by 50% (although a much smaller reduction would still be worthwhile), 2670 children would need to be randomised from more than 17 000 children with severe malaria. In addition, ethical concerns could exist about randomising severely anaemic children to a "no antimicrobial" arm given the strength of current evidence.[11 29 39] WHO and others need to reach a consensus on this question; if a clinical trial is feasible and ethical, it should be set up with urgency. If not, existing evidence should be used to help to modify current guidelines for the treatment of children with invasive bacterial disease in malaria endemic areas.

Conclusions

The WHO criteria for presumptive antimicrobial treatment have poor sensitivity in children living in this area endemic for *P falciparum* in Africa, and the guidelines should be re-examined. This study shows that simple and available clinical criteria may significantly increase detection of invasive bacterial disease in severe and fatal illness in African children. Currently recommended antimicrobials often lack activity against common bacterial infections in Africa, and more effective diagnosis and treatment of bacterial disease in resource poor settings are urgently needed.

We thank the staff and patients of Teule Hospital for their cooperation during the study. Aikande Shoo, Halima Mohammed, Charles Mgaya, Selina Wycliffe, Emmanuel Swai, Edward Mtili, Christina Kemi, Stella Emmanuel, Rosalia Marwa, and Simphorosa Silaye were involved in clinical and laboratory data collection. Anne B Morrissey and Susan C Morpeth provided advice and support for microbiology.

Contributors: BN was responsible for all the clinical data collection, contributed to the data analysis, and wrote the manuscript with HR. BA, DD, and JK were responsible for the laboratory analysis, drafted sections of the methods and provided critical review of the manuscript. GM was responsible for clinical data collection and contributed to the writing of the manuscript. JO was responsible for the statistical analysis and provided critical review of the manuscript. KC and FM were responsible for managing the data and provided critical review of the manuscript. HM, WM, and RM were responsible for clinical data collection and provided critical review of the manuscript. RO, JAC, and CJMW contributed to funding applications, study design, and critical review of the manuscript. HR was responsible for the study design, obtained core funding, co-wrote the manuscript, and contributed to the analysis; he is the guarantor.

Funding: Core funding for the study was provided by European Commission (Europaid) grant code SANTE/2004/078-607. BN was supported by grants from the Berkeley Fellowship, Sir Halley Stewart Trust, and Pfizer Pharmaceuticals. Pfizer Pharmaceuticals provided equipment and consumables for microbiology. Abbott Pharmaceuticals provided reagents for HIV testing. Netspear (www.netspear.org) funded the E-tests, which were performed at the KEMRI/Wellcome Trust Centre for Geographic Medicine (Coast), Kilifi, Kenya. None of the funders had a role in the design, analysis, or interpretation of results.

Competing interests: None declared.

Ethical approval: The study was approved by the ethics committees of the National Institute for Medical Research, Tanzania, and the London School of Hygiene and Tropical Medicine. Written informed consent was obtained from the caregiver of each child in the study, and pre-test counselling was provided before HIV testing.
Data sharing: Technical appendix, statistical code, and dataset are available from the corresponding author (hugh.reyburn@lshtm.ac.uk). Specific consent for data sharing was not obtained prospectively from study participants but may be granted by the Tanzanian national ethical review board.

1 Bryce J, Boschi-Pinto C, Shibuya K, Black RE. WHO estimates of the causes of death in children. *Lancet* 2005;365:1147-52.
2 Berkley JA, Mwangi I, Mellington F, Mwarumba S, Marsh K. Cerebral malaria versus bacterial meningitis in children with impaired consciousness. *QJM* 1999;92:151-7.
3 Brent AJ, Oundo JO, Mwangi I, Ochola L, Lowe B, Berkley JA. Salmonella bacteremia in Kenyan children. *Pediatr Infect Dis J* 2006;25:230-6.
4 English M, Punt J, Mwangi I, McHugh K, Marsh K. Clinical overlap between malaria and severe pneumonia in African children in hospital. *Trans R Soc Trop Med Hyg* 1996;90:658-62.
5 Evans JA, Adusei A, Timmann C, May J, Mack D, Agbenyega T, et al. High mortality of infant bacteraemia clinically indistinguishable from severe malaria. *QJM* 2004;97:591-7.
6 Reyburn H, Mbatia R, Drakeley C, Carneiro I, Mwakasungula E, Mwerinde O, et al. Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. *BMJ* 2004;329:1212.
7 Reyburn H, Mwakasungula E, Chonya S, Mtei F, Bygbjerg I, Poulsen A, et al. Clinical assessment and treatment in paediatric wards in the north-east of the United Republic of Tanzania. *Bull World Health Organ* 2008;86:132-9.
8 World Health Organization. Management of the child with a serious infection or severe malnutrition: guidelines for care at the first-referral level in developing countries. WHO, 2000.
9 Berkley JA, Maitland K, Mwangi I, Ngetsa C, Mwarumba S, Lowe BS, et al. Use of clinical syndromes to target antibiotic prescribing in seriously ill children in malaria endemic area: observational study. *BMJ* 2005;330:995.
10 Sigauque B, Roca A, Mandomando I, Morais L, Quinto L, Sacarlal J, et al. Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. *Pediatr Infect Dis J* 2009;28:108-13.
11 Bronzan RN, Taylor TE, Mwenechanya J, Tembo M, Kayira K, Bwanaisa L, et al. Bacteremia in Malawian children with severe malaria: prevalence, etiology, HIV coinfection, and outcome. *J Infect Dis* 2007;195:895-904.
12 Graham SM, Hart CA, Molyneux EM, Walsh AL, Molyneux ME. Malaria and Salmonella infections: cause or coincidence? *Trans R Soc Trop Med Hyg* 2000;94:227.
13 Graham SM, Walsh AL, Molyneux EM, Phiri AJ, Molyneux ME. Clinical presentation of non-typhoidal Salmonella bacteraemia in Malawian children. *Trans R Soc Trop Med Hyg* 2000;94:310-4.
14 Walsh AL, Phiri AJ, Graham SM, Molyneux EM, Molyneux ME. Bacteremia in febrile Malawian children: clinical and microbiologic features. *Pediatr Infect Dis J* 2000;19:312-8.
15 Maxwell CA, Chambo W, Mwaimu M, Magogo F, Carneiro IA, Curtis CF. Variation of malaria transmission and morbidity with altitude in Tanzania and with introduction of alphacypermethrin treated nets. *Malar J* 2003;2:28.
16 Edmonds S, Shangalawe L, Meadway J. Getting pregnant women on to HAART (highly active antiretroviral therapy): developing a strategy for advanced prevention of mother-to-child transmission of HIV (PMTCT+) in rural Tanzania. *HIV Med* 2009;10(suppl 1):26S.
17 World Health Organization. Guidelines for the treatment of malaria. WHO, 2006.
18 Berkley J, Mwarumba S, Bramham K, Lowe B, Marsh K. Bacteraemia complicating severe malaria in children. *Trans R Soc Trop Med Hyg* 1999;93:283-6.
19 Mayhood MK, Afwamba IA, Odhiambo CO, Ndanu E, Thielman NM, Morrissey AB, et al. Validation, performance under field conditions, and cost-effectiveness of Capillus HIV-1/HIV-2 and determine HIV-1/2 rapid human immunodeficiency virus antibody assays using sequential and parallel testing algorithms in Tanzania. *J Clin Microbiol* 2008;46:3946-51.
20 Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; nineteenth informational supplement. M100-S19, Vol 29, No 3, 2009.
21 World Health Organization. Pocket book of hospital care for children: guidelines for the management of common illnesses with limited resources. WHO, 2005.
22 Chandler CI, Drakeley CJ, Reyburn H, Carneiro I. The effect of altitude on parasite density case definitions for malaria in northeastern Tanzania. *Trop Med Int Health* 2006;11:1178-84.
23 Smith T, Schellenberg JA, Hayes R. Attributable fraction estimates and case definitions for malaria in endemic areas. *Stat Med* 1994;13:2345-58.
24 Berkley JA, Bejon P, Mwangi T, Gwer S, Maitland K, Williams TN, et al. HIV infection, malnutrition, and invasive bacterial infection among children with severe malaria. *Clin Infect Dis* 2009;49:336-43.
25 Mabey DC, Brown A, Greenwood BM. Plasmodium falciparum malaria and Salmonella infections in Gambian children. *J Infect Dis* 1987;155:1319-21.
26 Onwubalili JK. Sickle cell disease and infection. *J Infect* 1983;7:2-20.
27 Roux CM, Butler BP, Chau JY, Paixao TA, Cheung KW, Santos RL, et al. Both hemolytic anemia and malaria parasite-specific factors increase susceptibility to non-typhoidal Salmonella infection in mice. *Infect Immun* 2010; published online 25 January.
28 Archibald LK, Kazembe PN, Nwanyanwu O, Mwansambo C, Reller LB, Jarvis WR. Epidemiology of bloodstream infections in a bacille Calmette-Guerin-vaccinated pediatric population in Malawi. *J Infect Dis* 2003;188:202-8.
29 Bahwere P, Levy J, Hennart P, Donnen P, Lomoyo W, Dramaix-Wilmet M, et al. Community-acquired bacteremia among hospitalized children in rural central Africa. *Int J Infect Dis* 2001;5:180-8.
30 Shaw AV, Reddy EA, Crump JA. Etiology of community-acquired bloodstream infections in Africa. 46th annual meeting of the Infectious Diseases Society of America. Infectious Diseases Society of America, 2008.
31 Berkley JA, Lowe BS, Mwangi I, Williams T, Bauni E, Mwarumba S, et al. Bacteremia among children admitted to a rural hospital in Kenya. *N Engl J Med* 2005;352:39-47.
32 Blomberg B, Manji KP, Urassa WK, Tamim BS, Mwakagile DS, Jureen R, et al. Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: a prospective cohort study. *BMC Infect Dis* 2007;7:43.
33 Lepage P, Bogaerts J, Van Goethem C, Ntahorutaba M, Nsengumuremyi F, Hitimana DG, et al. Community-acquired bacteraemia in African children. *Lancet* 1987;1:1458-61.
34 Bejon P, Mwangi I, Ngetsa C, Mwarumba S, Berkley JA, Lowe BS, et al. Invasive Gram-negative bacilli are frequently resistant to standard antibiotics for children admitted to hospital in Kilifi, Kenya. *J Antimicrob Chemother* 2005;56:232-5.
35 Gordon MA, Graham SM, Walsh AL, Wilson L, Phiri A, Molyneux E, et al. Epidemics of invasive Salmonella enterica serovar enteritidis and S enterica serovar typhimurium infection associated with multidrug resistance among adults and children in Malawi. *Clin Infect Dis* 2008;46:963-9.
36 Scott JA, Mwarumba S, Ngetsa C, Njenga S, Lowe BS, Slack MP, et al. Progressive increase in antimicrobial resistance among invasive isolates of Haemophilus influenzae obtained from children admitted to a hospital in Kilifi, Kenya, from 1994 to 2002. *Antimicrob Agents Chemother* 2005;49:3021-4.
37 Bassat Q, Guinovart C, Sigauque B, Mandomando I, Aide P, Sacarlal J, et al. Severe malaria and concomitant bacteraemia in children admitted to a rural Mozambican hospital. *Trop Med Int Health* 2009;14:1011-9.
38 Berkley JA, Brent A, Mwangi I, English M, Maitland K, Marsh K, et al. Mortality among Kenyan children admitted to a rural district hospital on weekends as compared with weekdays. *Pediatrics* 2004;114:1737-8.
39 Calis JC, Phiri KS, Faragher EB, Brabin BJ, Bates I, Cuevas LE, et al. Severe anemia in Malawian children. *N Engl J Med* 2008;358:888-99.
40 Brent AJ, Ahmed I, Ndiritu M, Lewa P, Ngetsa C, Lowe B, et al. Incidence of clinically significant bacteraemia in children who present to hospital in Kenya: community-based observational study. *Lancet* 2006;367:482-8.
41 Cockerill FR 3rd, Wilson JW, Vetter EA, Goodman KM, Torgerson CA, Harmsen WS, et al. Optimal testing parameters for blood cultures. *Clin Infect Dis* 2004;38:1724-30.
42 Lee A, Mirrett S, Reller LB, Weinstein MP. Detection of bloodstream infections in adults: how many blood cultures are needed? *J Clin Microbiol* 2007;45:3546-8.
43 Chandler CI, Jones C, Boniface G, Juma K, Reyburn H, Whitty CJ. Guidelines and mindlines: why do clinical staff over-diagnose malaria in Tanzania? A qualitative study. *Malar J* 2008;7:53.
44 Reyburn H, Mbakilwa H, Mwangi R, Mwerinde O, Olomi R, Drakeley C, et al. Rapid diagnostic tests compared with malaria microscopy for guiding outpatient treatment of febrile illness in Tanzania: randomised trial. *BMJ* 2007;334:403.
45 Fierer J, Hatlen L, Lin JP, Estrella D, Mihalko P, Yau-Young A. Successful treatment using gentamicin liposomes of Salmonella dublin infections in mice. *Antimicrob Agents Chemother* 1990;34:343-8.
46 Kihlstrom E, Andaker L. Inability of gentamicin and fosfomycin to eliminate intracellular Enterobacteriaceae. *J Antimicrob Chemother* 1985;15:723-8.
47 Graham SM, English M. Non-typhoidal salmonellae: a management challenge for children with community-acquired invasive disease in tropical African countries. *Lancet* 2009;373:267-9.

Accepted: 25 January 2010

Tropical Medicine and International Health doi:10.1111/j.1365-3156.2008.02129.x

VOLUME 13 NO 9 PP 1213–1219 SEPTEMBER 2008

Inter-observer variation in paediatric clinical signs between different grades of staff examining children admitted to hospital in Tanzania

B. Nadjm[1,2], B. Jeffs[1], G. Mtove[2,3], W. Msuya[2,4], L. Mndeme[4], F. Mtei[2], S. Chonya[2] and H. Reyburn[1,2]

1 *London School of Hygiene and Tropical Medicine, London, UK*
2 *Joint Malaria Programme, KCMC, Moshi, Kilimanjaro, Tanzania*
3 *National Institute for Medical Research, Amani Centre, Tanga, Tanzania*
4 *Teule District Hospital, Muheza, Tanga, Tanzania*

Summary

BACKGROUND AND OBJECTIVE Children are often admitted to district hospitals in Africa without an adequate record of clinical examination, a problem that could be reduced by greater involvement of nurses in their assessment. We aimed to ascertain whether hospital nurses in a district hospital could conduct paediatric examinations as reliably as clinical staff, when provided with a short structured training session.

METHODS Hospital nurses (HN), hospital clinical officers (HCO) and research clinical officers (RCO) repeated examinations on children admitted to the paediatric ward shortly after the first examination by an RCO. Kappa scores were used to compare the agreement on the presence or absence of basic clinical signs by different categories of staff.

RESULTS Among 439 paired examinations the agreement between RCOs on clinical signs was slightly higher than for HCOs or HNs; the mean (median) Kappa scores for all signs examined were 0.54 (0.57) for RCO–RCO, 0.49 (0.49) for RCO–HCO and 0.50 (0.49) for RCO–HN. Levels of agreement were lower if children were under the age of 18 months or if they cried during the examination.

CONCLUSIONS Nurses with basic training appear to perform as well as clinically trained staff in eliciting essential signs in acutely ill children. Their role in the initial and ongoing assessment of these children should be reviewed in light of the critical shortages in clinically trained staff in African hospitals.

keywords hospital, paediatric, clinical signs, variability, staff category, training

Introduction

A relatively small number of standard clinical signs are in common use to assess children admitted to district hospitals in resource-poor settings. Many of these are specified in the integrated management of childhood illness (IMCI) and some have originated from clinical research (e.g. prostration or the Blantyre coma scale). (Molyneux *et al.* 1989; WHO 2000) Studies that have assessed the repeatability of these signs in African children suggest variable levels of agreement between two independent raters (English *et al.* 1995; Otieno *et al.* 2004), these tend to fall where signs rely on subjective judgement, require a method other than inspection or when infants are examined (Kahigwa *et al.* 2002).

WHO guidelines for care rely primarily on basic paediatric clinical signs but they are often not sought or recorded in the case notes in African district hospitals (English *et al.* 2004; Reyburn *et al.* 2008). The reasons are complex but include lack of time to assess sick children at the point of admission, often a busy and crowded outpatient clinic where clinical staff may be very stretched. In this context nursing staff may be able to contribute by checking children either before the medical consultation or after admission to the ward.

In this study we compared the repeatability of a group of standard clinical paediatric signs elicited by clinical officers who had been trained in research data collection with hospital clinical officers and hospital nurses who repeated examinations in children admitted to a busy district hospital in Tanzania. The findings may serve to increase the available options to ensure that sick children are properly assessed at or shortly after their admission to hospital.

© 2008 Blackwell Publishing Ltd

Methods

The study and site

The study was conducted in the third and fourth month of a 1-year study into the causes of illness in children aged 2 months to 13 years with a history of fever in the previous 48 h who were admitted to a district hospital in NE Tanzania. The study was initially undertaken to assess the suitability of routine staff to contribute to a standard clinical assessment that was made on admission for all children in the study.

Staff in the study

Staff in the study consisted of clinical officers (a grade of non-physician clinician with 3 years of basic clinical training) and hospital nurses (with 3–4 years of training in nursing). Clinical officers were either specifically employed as research assistants (RCOs) for the 1-year study or were employed by the hospital to provide routine care (HCOs). Hospital nurses (HNs) were all regular hospital staff. Both HNs and HCOs regularly worked on the paediatric wards.

RCOs had received didactic teaching supported by video clips that demonstrated the signs in the study; these were assembled from recordings that had been used for IMCI training or had been recorded by experienced paediatricians to demonstrate specific signs. In addition RCOs received bedside supervision on clinical examination for 3 months before the start of the study and their assessments of children in the main study were regularly checked by study physicians (BN, GM) who provided feedback and corrected any observed errors.

HCOs and HNs had a 1-h training session on clinical examination using the same training materials as had been used for RCOs followed by an interactive teaching session conducted by an experienced physician (BJ). In addition, each staff member received a 30-min individual training session where they examined children on the ward under supervision (BJ). Definitions of clinical signs were consistent throughout and are shown in Table 1.

IMCI had been part of Clinical Officer training in Tanzania since 2000 but was not in routine use at the hospital prior to the study.

Examination procedure and data analysis

On arrival at the ward all children were triaged and resuscitated as needed before the first examination. After consenting procedures, children were examined initially by a research clinical officer (RCO) followed within 1 h by a second examiner (RCO, HCO or HN according to a duty roster). Examinations were performed with no other staff present in a well-lit, quiet part of the ward, with results recorded on a standardised form listing the 17 clinical signs defined in Table 1. All examiners were blind to any clinical information (previous clinical examination, investigations or treatment) at the time of examination.

Data were double-entered in MS Access (MS Corp., Redmond, VA, USA) and analysed in Stata-8 (Statacorp, TX, USA). Correlation of findings between examiners was assessed using Cohen's Kappa score and classified by categories proposed by Landis and Koch (1977) (Table 2).

Ethics

The study was approved by the Ethics Committees of the National Institute of Medical Research, Tanzania (HQ/R.8a/Vol.IX/392) and the London School of Hygiene and Tropical Medicine (2087). Informed consent to participate was obtained from the parent or guardian in each case.

Results

A total of 439 children were examined by a RCO and then examined a second time by an HN (189), HCO (129) or another RCO (121). Table 3 shows the numbers of repeat examinations undertaken by individuals in each of the staff categories. The median time between examinations was 14.2, 13.4 and 14.2 min for RCO–HN, RCO–HCO or RCO–RCO respectively. The mean (median) age of children examined was 25 (18) months and 39% cried during the first or second examination; neither age nor crying varied by category of staff who re-examined children ($P = 0.13$ and 0.11 respectively).

Overall, the agreement between RCOs on clinical signs was slightly higher than for HCOs or HNs; the mean (median) Kappa scores for all signs examined were 0.54 (0.57) for RCO–RCO, 0.49 (0.49) for RCO–HCO and 0.50 (0.49) for RCO–HN pairs of examiners (Figure 1a).

Level of consciousness or prostration are important predictors of outcome in severely ill children and all grades of staff reached 'moderate' or 'substantial' levels of agreement for these. However, agreement on the presence of 'chest wall indrawing' between RCOs or HNs compared to the first RCO examination was 'poor' (Table 4).

Agreement between examiners, particularly RCOs and HNs, for the presence of 'delayed capillary refill' and 'temperature difference' was 'poor'. Since the ability to see capillary refill depends on a colour change in the nailbed we calculated the overall agreement (irrespective of staff grade) on 'delayed refill' (Kappa = 0.14, 95% CI 0.06–0.22) and then in the 159 children whose haemoglobin levels were <7 g/dl (Kappa = 0.083, 95% CI: 0.04–0.20)

© 2008 Blackwell Publishing Ltd

Table 1 Definition of clinical signs assessed in the study

Variable (sign)	Definition
Axillary temperature	Measured by digital thermometer in the axilla for two bleep cycles
Respiratory rate	Counted over 1 min in a non feeding child who is not crying
Lethargy	A conscious child who takes no interest in its surroundings
Ability to sit	Ability to sit unsupported if aged 8 months or more
Ability to breast feed/drink	Observed ability to breast feed or drink from a cup
Sunken eyes	A subjective assessment of sunken eyes
Very slow skin pinch	A full thickness pinch of skin taken in a longitudinal plane midway between umbilicus and the side of the body. Longer than two (2) seconds to return to normal is considered a positive response
Central cyanosis	Bluish tinge to the buccal mucosa
Blantyre score	
Eyes	Undirected or closed eyes, score 0 Directed eye movements, score 1
Motor	No movement or abnormal flexion/extension, score 0 Other movements without localisation, score 1 Movements to localise pain, score 2
Verbal	No vocal response' score 0 Weak/high pitched cry. score 1 Normal cry/speech, score 2
Jaundice	A subjective assessment based on inspection of the sclera or palms
Severe Pallor	Noted by examination of the conjunctiva and palms
Bulging fontanelle	The anterior fontanelle is assessed by palpation
Neck stiffness	Assessed by observing resistance to voluntary neck flexion and passive flexion of the neck by the researcher
Lower chest wall indrawing	Inward movement of the lower chest wall on inspiration in a non-crying child
Slow capillary refill	Assessed by depressing the fingernail or fingertip pulp for 3 s and counting the time it takes to re-perfuse. A count of greater than 3 s is considered positive
Temperature gradient	Detected by running the back of the hand down the limb
Oedema of both feet	Detected by pressing a thumb on the dorsum of the foot

compared to repeatability in the 280 children with haemoglobin ≥7 g/dl (Kappa = 0.21, 95% CI: 0.15–0.29).

We tested the repeatability of a combination of signs that would indicate the need for parenteral therapy according to IMCI criteria. These corresponded to the IMCI definitions of severe or very severe pneumonia or severe dehydration. The agreement between hospital nurses and research clinical officers for the presence or absence of one of these combinations was 'moderate' (Kappa = 0.51, 95%

Table 2 Classification of Kappa scores according to Landis and Koch (1977)

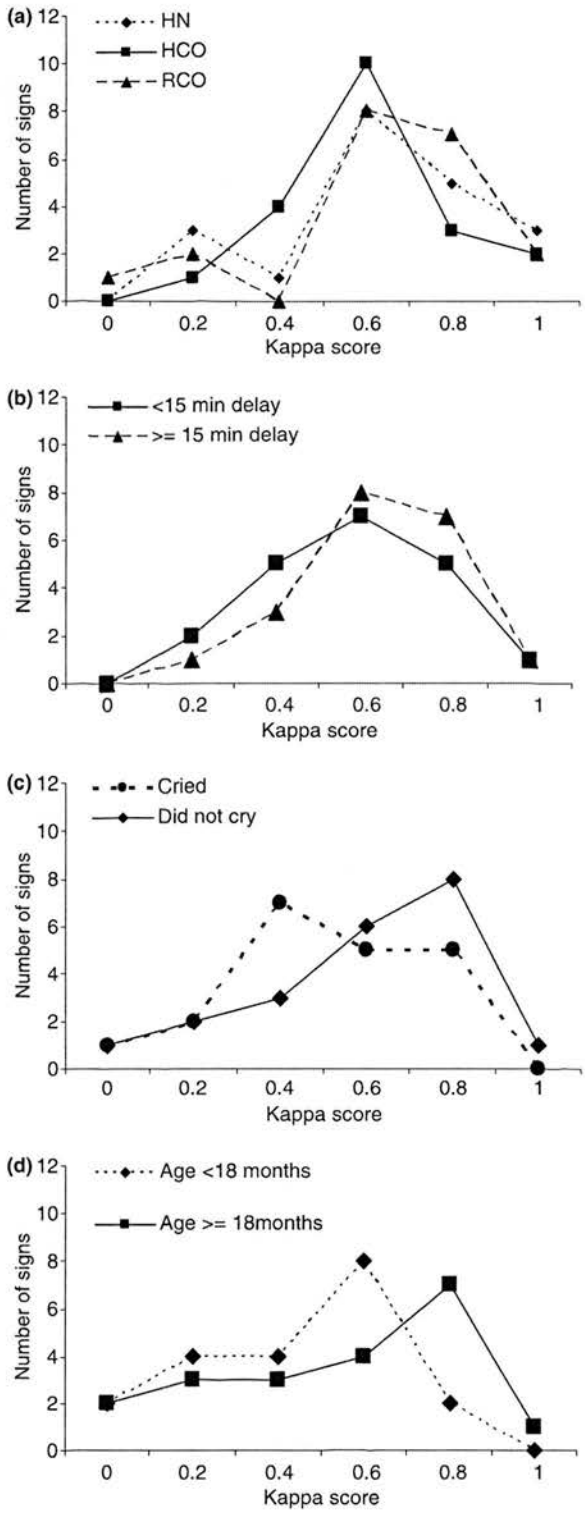
Kappa score	Level of agreement
<0	Less agreement than by chance
0–0.2	Slight
0.21–0.4	Fair
0.41–0.6	Moderate
0.61–0.8	Substantial
0.81–1.00	Almost perfect

Table 3 Numbers of repeat examinations by individual and staff category†

RCO	No. of examined	HCO	No. of examined	HN	No. of examined
1	20	1	44	1	46
2	25	2	28	2	41
3	42	3	27	3	31
4	34	4	30	4	56
				5	3
				6	9
				7	3
Total	121		129		189

†First examinations were all by one of the four RCO's; no RCO examined the same child twice.

© 2008 Blackwell Publishing Ltd



CI: 0.39–0.57) for severe or very severe pneumonia and substantial (Kappa = 0.61, 95% CI: 0.49–0.73) for severe dehydration.

A composite sign of 'severe disease' was constructed from a combination of respiratory signs, reduced consciousness, severe pallor or jaundice (Table 4). The overall Kappa value (irrespective of staff grade) was 0.61 (95% CI: 0.56–0.76) while the weighted mean Kappa of its components was 0.56.

Forty percent of signs in children over 18 months of age reached 'substantial' or better levels of agreement compared to only 10% in children under 18 months of age. Similarly, 38% of elicited signs reached substantial or better agreement in children who did not cry compared to only 25% in children who did cry. Most children (66%) were examined within 15 min of the first examination and repeatability of examination results was no better in these than the minority (34%) who were examined with a delay of more than 15 min (Figure 1b–d).

Discussion

Clinical signs elicited by different grades of staff

Up to half of paediatric deaths in African district hospitals occur within 24 h of admission (Berkley *et al.* 2003; Reyburn *et al.* 2008) and accurate assessment and prompt treatment are essential to reduce mortality. Limited laboratory support in many of these settings means that treatment decisions are generally taken on the basis of the presence or absence of basic clinical paediatric signs but these are often not sought or recorded. Clinical staff are often over-worked and the human resource crisis in African hospitals is unlikely to be resolved in the near future (Chen *et al.* 2004). In such an environment, multi-tasking among existing staff seems essential and inevitable and this could do much to address the unacceptably low levels of recording basic clinical assessments in sick children.

Our findings suggest that hospital nurses provided with basic training can reach similar levels of agreement with research-trained and hospital clinical officers in eliciting a

Figure 1 Number of signs falling into different categories of Kappa values for agreement between first (by RCO) and second examinations (by a second research RCO, HCO or HN). For different grades of staff (research clinical officers, hospital clinical officers or nurses)*. (a) Where there was more or less than 15 min delay between the end of the first examination and the beginning of the second. (b) Where children cried or did not cry during the examination. (c) Where children were more or less than 18 months of age. *(b–d) The findings from the second examination by different grades of staff have been aggregated.

© 2008 Blackwell Publishing Ltd

Table 4 Agreement on the presence of clinical signs as elicited by nurses, hospital and research clinical officers compared to first examination by research clinical officers

	Prevalence* (%)	HN		HCO		RCO		ALL	
		Kappa	(95% CI)	Kappa	(95% CI)	Kappa	(95% CI)	Kappa	(95% CI)
Respiratory signs									
Raised resp. rate for age†	294/416 (71)	0.46	(0.32–0.60)	0.54	(0.36–0.72)	0.46	(0.28–0.64)	0.47	(0.37–0.57)
Lower chest wall indrawing	60/435 (14)	0.13	(0.01–0.25)	0.63	(0.45–0.81)	0.18	(0.00.0.36)	0.31	(0.21–0.41)
Conscious level									
Prostration	64/439 (15)	0.83	(0.69–0.97)	0.83	(0.65–1.01)	0.88	(0.70–1.06)	0.84	(0.74–0.94)
Unable to sit over 8 months‡	49/436 (11)	0.63	(0.47–0.79)	0.68	(0.50–0.86)	0.85	(0.67–1.03)	0.71	(0.61–0.81)
Unable to breast feed/drink	22/439 (5)	0.69	(0.55–0.83)	0.56	(0.38–0.74)	0.71	(0.59–0.83)	0.66	(0.58–0.74)
Lethargy	68/436 (16)	0.84	(0.70–0.98)	0.57	(0.39–0.75)	0.72	(0.54–0.90)	0.63	(0.53–0.74)
Blantyre score		0.45	(0.35–0.55)	0.35	(0.23–0.47)	0.44	(0.32–0.56)	0.42	(0.34–0.50)
Eyes	39/439 (9)	0.68	(0.54–0.82)	0.64	(0.46–0.82)	0.71	(0.53–0.89)	0.68	(0.60–0.76)
Motor	38/439 (9)	0.51	(0.39–0.63)	0.09	(−0.07–0.25)	0.7	(0.54–0.86)	0.55	(0.47–0.63)
Verbal	46/439 (10)	0.57	(0.45–0.69)	0.45	(0.27–0.63)	0.62	(0.46–0.78)	0.56	(0.48–0.64)
Any reduced BCS	65/438 (15)	0.67	(0.53–0.81)	0.45	(0.27–0.63)	0.67	(0.49–0.85)	0.61	(0.53–0.69)
Any reduced consciousness	92/435 (21)	0.63	(0.49–0.77)	0.47	(0.29–0.65)	0.6	(0.42–0.78)	0.47	(0.39–0.55)
Perfusion									
Oedema of both feet	22/439 (5)	0.89	(0.75–1.03)	0.24	(0.12–0.36)	0.49	(0.31–0.67)	0.47	(0.39–0.55)
Slow Capillary refill	43/438 (10)	0.13	(0.03–0.23)	0.24	(0.12–0.36)	−0.02	(−0.20–0.16)	0.14	(0.06–0.22)
Temp difference	59/439 (13)	0.11	(−0.03 to 0.25)	0.48	(0.30–0.66)	0.19	(0.01–0.37)	0.17	(0.09–0.25)
Sunken eyes	24/439 (5)	0.27	(0.15–0.39)	0.48	(0.34–0.62)	0.47	(0.31–0.63)	0.43	(0.33–0.53)
Very slow skin pinch	15/439 (3)	0.49	(0.35–0.63)	0.32	(0.18–0.46)	0.66	(0.48–0.84)	0.48	(0.38–0.58)
Other									
Jaundice	10/439 (2)	0.49	(0.35–0.63)	0.85	(0.67–1.03)	0.49	(0.33–0.65)	0.66	(0.58–0.74)
Severe Pallor	115/439 (26)	0.48	(0.34–0.62)	0.56	(0.38–0.74)	0.5	(0.32–0.68)	0.5	(0.42–0.58)
Any sign of severe disease§	226/439 (51)	0.44	(0.30–0.58)	0.5	(0.34–0.68)	0.54	(0.36–0.72)	0.66	(0.56–0.76)

*Prevalence defined as any positive/sign examined at least once.
†Breaths per minute ≥50 if aged 2–11 months, ≥40 if aged 1–5 years, 23 children over the age of 5 years excluded.
‡Fourteen children under 8 months of age excluded.
§Any one of respiratory rate >70 b/min, chest indrawing, any reduction in Blantyre score, jaundice, severe pallor, or prostration. Three signs ('neck stiffness', 'bulging fontanelle', 'cyanosis') were dropped due to low prevalence (<3%).

range of common clinical signs. This is consistent with a previous study of primary care workers in Swaziland showing that, with training, nurses and other health workers can diagnose pneumonia and other WHO defined syndromes with acceptable sensitivity and specificity (Simoes & McGrath 1992). A study from Ethiopia similarly demonstrated that after training primary care health workers can recognise common syndromes and signs with good sensitivities compared with a paediatrician (88% for pneumonia/severe pneumonia, 76% for some/severe dehydration) (Simoes *et al.* 1997). The importance of ongoing training has been emphasised by other studies showing poorer performance where it had not been recently provided or reinforced (Brewster *et al.* 1993; Gadomski *et al.* 1993).

Clinical examination is traditionally the reserve of medical staff and this is to some extent justified by the fact that doctors take treatment decisions and have training in full clinical examination (e.g. auscultation, neurological examination). However, the essential signs tested in our study have been designed to guide care in resource-poor settings where staff may have quite basic levels of training.

In a hospital setting nurses may not be authorised to take treatment decisions (unless in an emergency) and reporting the presence of clinical signs to medical staff, especially where the finding differs from one already recently recorded in the case notes, could be seen as threatening to medical staff. However, 'medical staff' in many African countries (including Tanzania) are mostly non-physician clinicians (Mullan & Frehywot 2007) who themselves have similar entry qualifications and duration of training as nurses. Increasing the involvement of nurses in clinical assessment and decision-making is a complex task and beyond the scope of this study but our findings and those of others suggest that there is no justification for any group to claim the 'high ground' of competence in the difficult area of eliciting paediatric clinical signs.

© 2008 Blackwell Publishing Ltd

General repeatability of clinical signs

Delay between two examinations is an obvious source of error in assessing levels of agreement, but this did not impact on repeatability of signs in our study, possibly because delays were generally short. English *et al.* (1995) used videos of respiratory clinical signs in order to avoid this source of error, and these seemed to result in high levels of agreement although some medically qualified staff still did not agree with the majority on the presence of 'chest indrawing' in a significant proportion of cases.

Clinical signs of shock (delayed capillary refill and/or cool peripheries) in our study appeared to have poor repeatability and anecdotal experience from district hospitals in Tanzania suggests that the signs of normovolaemic shock (e.g. normal skin turgor with delayed capillary refill) are rarely sought or recorded, possibly due to a general lack of awareness of its importance. Our finding that the ability of staff to agree on the presence of delayed capillary refill may be reduced in anaemic children, although not reaching statistical significance, is potentially important given the large proportion of children admitted to hospitals in sub-Saharan Africa who have severe anaemia (Weber *et al.* 1997) and the positive association between shock and anaemia in sick children in malaria endemic areas (Maitland *et al.* 2005). This might at least partly explain why, even in a specialist centre, agreement between medical doctors for a 3-s delay in capillary refill (irrespective of haemoglobin level) was only 'fair' while for the presence of a lower limb temperature gradient it was 'substantial' (Otieno *et al.* 2004).

We found that signs in young children and where children had cried at some time during the examination were unreliable and this is in keeping with the results of other studies where the effect of crying or irritability on clinical examination was explored (Simoes *et al.* 1991; Kahigwa *et al.* 2002). It is clear that this group of patients requires careful and repeated assessments.

In certain areas (chest indrawing, capillary refill) the research staff agreed less often with each other than with the hospital staff. This may represent drift that had occurred over time from the standardised definitions provided in teaching sessions or training and supervision delivered over a longer period and may have led to inconsistencies.

Agreement on composite clinical signs may be higher than on their individual components. Newton *et al.* (1997) found that agreement between examiners on the Blantyre coma score was higher than for its three components although this was not confirmed in our study. However, we did find that overall agreement (irrespective of staff grade) for 'any sign of severe disease' reached 'substantial' agreement between first and second examinations while the mean of its components reached 'moderate' agreement. None of the signs included in our study were for summary subjective assessments (e.g. severe respiratory distress) but in a study conducted in a teaching hospital manned by medical graduates 'increased work of breathing' showed 'substantial' agreement between examiners despite agreement varying from 'slight' to 'moderate' for specific respiratory signs (Walsh *et al.* 2006).

Our study adds to the large body of literature on the reproducibility of examination findings. While there is considerable variation, some signs do appear to be generally reproducible: pallor has shown kappa scores ranging from 0.5 to 0.8 (Weber *et al.* 1997; Kahigwa *et al.* 2002) whilst other signs have been more variable; chest indrawing shows Kappas from 0.28 to 0.34 and sensitivities from 34 to 82% (Simoes & McGrath 1992; Brewster *et al.* 1993; Gadomski *et al.* 1993; Kahigwa *et al.* 2002). One can only assume that correctness and consistency of clinical examinations will be lower under real-life than research conditions and there is clearly a need for further studies evaluating simple and sustainable training tools to improve standards in paediatric clinical examination in resource-poor settings.

Conclusions

Nurses with basic training appear to perform as well as clinically trained staff in eliciting essential paediatric clinical signs in severely ill children admitted to hospital. This should provide a stimulus to review how children are assessed after the decision to admit to hospital; the current situation where sick children can be admitted to hospital with no more than a few lines written in the case notes is a serious problem that requires imaginative solutions and high levels of cooperation between staff grades. The discordance between staff of any grade in eliciting clinical signs suggests that sick children, particularly if crying or under the age of 18 months, need to be reassessed frequently, with effective dialogue between staff to ensure that such children receive prompt and appropriate treatment.

Acknowledgements

Thanks to the children and their caretakers who participated in the study and the staff of Teule Hospital who provided cooperation and assistance throughout. The study was funded by the European Commission. BN was supported by the Sir Halley Stewart Trust and Berkeley fellowship.

© 2008 Blackwell Publishing Ltd

References

Berkley JA, Ross A, Mwangi I *et al.* (2003) Prognostic indicators of early and late death in children admitted to district hospital in Kenya: cohort study. *British Medical Journal* **326**, 361.

Brewster DR, Pyakalyia T, Hiawalyer G & O'Connell DL (1993) Evaluation of the ARI program: a health facility survey in Simbu, Papua, New Guinea. *Papua and New Guinea Medical Journal* **36**, 285–296.

Chen L, Evans T, Anand S *et al.* (2004) Human resources for health: overcoming the crisis. *Lancet* **364**, 1984–1990.

English M, Murphy S, Mwangi I, Crawley J, Peshu N & Marsh K (1995) Interobserver variation in respiratory signs of severe malaria. *Archives of Disease in Childhood* **72**, 334–336.

English M, Esamai F, Wasunna A *et al.* (2004) Assessment of inpatient paediatric care in first referral level hospitals in 13 districts in Kenya. *Lancet* **363**, 1948–1953.

Gadomski AM, Khallaf N, el Ansary S & Black RE (1993) Assessment of respiratory rate and chest indrawing in children with ARI by primary care physicians in Egypt. *Bulletin of the World Health Organization* **71**, 523–527.

Kahigwa E, Schellenberg D, Schellenberg JA, Aponte JJ, Alonso PL & Menendez C (2002) Inter-observer variation in the assessment of clinical signs in sick Tanzanian children. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **96**, 162–166.

Maitland K, Pamba A, English M *et al.* (2005) Pre-transfusion management of children with severe malarial anaemia: a randomised controlled trial of intravascular volume expansion. *British Journal of Haematology* **128**, 393–400.

Molyneux ME, Taylor TE, Wirima JJ & Borgstein A (1989) Clinical features and prognostic indicators in paediatric cerebral malaria: a study of 131 comatose Malawian children. *Quarterly Journal of Medicine* **71**, 441–459.

Mullan F & Frehywot S (2007) Non-physician clinicians in 47 sub-Saharan African countries. *Lancet* **370**, 2158–2163.

Newton CR, Chokwe T, Schellenberg JA *et al.* (1997) Coma scales for children with severe falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 161–165.

Otieno H, Were E, Ahmed I, Charo E, Brent A & Maitland K (2004) Are bedside features of shock reproducible between different observers? *Archives of Disease in Childhood* **89**, 977–979.

Reyburn H, Mwakasungula E, Chonya S *et al.* (2008) Clinical assessment and treatment in paediatric wards in the north-east of the United Republic of Tanzania. *Bulletin of the World Health Organisation* **86**, 132–139.

Simoes EA & McGrath EJ (1992) Recognition of pneumonia by primary health care workers in Swaziland with a simple clinical algorithm. *Lancet* **340**, 1502–1503.

Simoes EAF, Roark R, Berman S, Esler LL & Murphy J (1991) Respiratory rate:measurement of variability and accuracy at different counting periods. *Archives of Diseases in Childhood* **66**, 1199–1203.

Simoes EA, Desta T, Tessema T, Gerbresellassie T, Dagnew M & Gove S (1997) Performance of health workers after training in integrated management of childhood illness in Gondar, Ethiopia. *Bulletin of World Health Organization* **75**(Suppl 1), 43–53.

Walsh P, Gonzales A, Satar A & Rothenberg SJ (2006) The interrater reliability of a validated bronchiolitis severity assessment tool. *Pediatric Emergency Care* **22**, 316–320.

Weber MW, Kellingray SD, Palmer A, Jaffar S, Mulholland EK & Greenwood BM (1997) Pallor as a clinical sign of severe anaemia in children: an investigation in the Gambia. *Bulletin of World Health Organanization* **75**(Suppl 1), 113–118.

WHO (2000) *Management of the Child with a Serious Infection or Severe Malnutrition*. WHO, Geneva.

Corresponding Author **Hugh Reyburn**, London School of Hygiene and Tropical Medicine, Keppel St, London WCIE7HT, UK. Tel.: +255 754 377374; Fax: +255 272 752990; E-mail: hugh.reyburn@lshtm.ac.uk

© 2008 Blackwell Publishing Ltd