DECLARATION

I, *DAVID MARTIN LE ROUX* hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Date:10 September 2010

Revised and re-submitted: 22 November 2010

Incidence of bacteraemia in HIV-infected children in Africa, and the impact of highly active antiretroviral therapy

by

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SUBMITTED TO THE UNIVERSITY OF CAPE TOWN
In fulfilment of the requirements for the degree

Master of Medicine (MMed) Paediatrics

Faculty of Health Sciences
UNIVERSITY OF CAPE TOWN

Date of submission: 10 September 2010
Revised and re-submitted 22 November 2010

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Contents

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Study Title: Impact of Isoniazid Preventative Therapy on Incidence of Bacteraemia in a Cohort of HIV-Infected Children in South Africa.

Research Question:

Is there a difference in the incidence of bacteraemias between children using Isoniazid Preventive Therapy (IPT) versus placebo?

Rationale:

Background: From November 2002 till December 2006, a placebo-controlled, randomized trial investigated the incidence of tuberculosis and the overall mortality in a cohort of HIV-infected children in Cape Town, South Africa. They were randomized to receive either Isoniazid Preventive Therapy (IPT) or placebo. In addition, they were randomized to receive trimethoprim/sulfamethoxaxole prophylaxis on either a daily or a three-times-per-week schedule. This will be a secondary analysis of the cohort looking specifically at the incidence of invasive bacterial infections.

Aim: To describe the incidence of bacteraemia, and the spectrum of organisms cultured. To determine if there was a difference in the incidence of bacteraemia between children using Isoniazid Preventive Therapy (IPT) versus placebo; and to determine if there was a difference in the incidence of bacteraemias between the groups using daily versus thrice-weekly trimethoprim/sulphamethoxazole prophylaxis.

Methods:

Study Design: Prospective, placebo-controlled, randomized trial, designed to detect a difference in the incidence of Tuberculosis. The primary outcome has been published, and an limited data set has been made available for analysis of the blood culture results.

Population:

Sample Size: 324

Inclusion and Exclusion Criteria: All children enrolled in the original trial are included in this secondary analysis.

Gender, Age and Locale: Males and females, age range from 4 weeks to 12 years old, living in Cape Town, South Africa

Disclosure/Consent Process:

Description of the Consent Process: Signed informed consent was obtained from the parent or legal guardian of the child before enrolment

Safety Monitoring:

Trial is already terminated so no further safety monitoring is required

Confidentiality Assurances:

Data Security: I have received abstracted data sets with no personal identifiers

Collaborative Agreements:

The trial was run at 2 sites in Cape Town, South Africa. The Principle Investigators are based at University of Stellenbosch and the University of Cape Town.

Letter(s) of Collaboration: I have letters of authorization from both of the Principle Investigators to allow me to access the abstracted data set.

Prof Heather Zar, University of Cape Town and Prof Mark Cotton, University of Stellenbosch

Bacteraemia in HIV-infected children in Africa: a literature review

David M le Roux

Background

Bacterial infections are a major cause of morbidity and mortality among HIV-infected adults[1] and children.[2] Opportunistic infections in children are a marker of immune suppression, as well as a driver of further immunological decline.[3] HIV-infected children are at increased risk for both superficial (skin, otitis) and invasive bacterial infections.[4] Children with acute disease complicated by bacteraemia have high morbidity and mortality.[5]

Similarly to HIV-infected adults, children have impaired clonal maturation of T helper 1 lymphocytes, with defective interleukin-2 and interferon gamma production, resulting in increased susceptibility to intracellular organisms, viruses and malignancy. [4, 6] All children have poor immune responses to bacterial polysaccharide antigens in the first 2 years of life. In HIV-infected children, B-lymphocyte dysfunction produces non-specific polyclonal immunoglobulin instead of functional antigen-specific antibodies.[5, 7, 8] In addition, decreased natural killer cell activity and impaired neutrophil bactericidal function result in particular predisposition to infection with encapsulated bacteria such as *Streptococcus pneumoniae.[4]* This increased susceptibility to pneumococcal septicaemia was described early in the HIV epidemic, with risk of bacteraemia increased 10 to 40 times in HIV-infected children compared to age-matched, HIV-uninfected children.[9]

Children with severe malnutrition also have impaired immunity and are at increased risk of bacteraemia.[10, 11] Many HIV-infected children suffer from severe malnutrition [12], which further increases susceptibility to invasive bacterial infections.[2]

Invasive disease due to *Streptococcus pneumoniae* and *Haemophilus influenzae* type B (HIB) may be prevented by vaccination. HIV-infected children have less robust immune response to vaccination.[13, 14] The risk of HIB vaccine failure in HIV-infected children not receiving anti retroviral therapy is estimated to be 35 times greater than HIV-uninfected children.[15] Coverage of these vaccines remains low: the World Health Organisation estimated in 2008 that in African countries, only 43%

of the children under 1 year received HIB vaccination, and only 1 African country was routinely providing pneumococcal conjugate vaccine.[16]

A recent systematic review and meta-analysis of community-acquired bloodstream infections in Africa showed that bacteraemia was more common in Africa than in many industrialized countries.[17] The authors demonstrated that in tropical countries, bacteraemia and malaria parasitaemia often coexist. There is a wide variation in incidence rates and causative organisms across Africa. That review analysed results from studies that included both HIV-infected and uninfected African adults and children. Due to stringent selection criteria, only 6 studies that included HIV-infected children were presented; none of the children were receiving highly active antiretroviral therapy (HAART).

The impact of (HAART) on the incidence and outcomes of bacteraemia in HIV-infected children in Africa has not yet been described.

Objectives

This literature review focuses on bacteraemia among HIV-infected children in Africa. The objectives are: to describe the prevalent organisms causing bacteraemia; to identify potential predictors of bacteraemia; and to evaluate the effect of HIV infection on risk of bacteraemia; and effect HIV infection on case fatality ratios.

The incidence of bacteraemia in African children with HIV is compared to that described in Europe and North America during the pre-HAART era. The paucity of data regarding HAART and bacteraemia in Africa is discussed, and the impact of HAART on incidence and type of bacteraemia is examined, with a discussion on how the evidence from North America and Europe might apply to Africa.

Search strategy

The literature search was limited to English-language articles in MEDLINE (www.ncbi.nlm.nih.gov), using two search strings:
“Africa and (bacteraemia OR bloodstream infection)”
“(bacteraemia OR bloodstream infection) AND (child OR pediatric) AND (HIV OR AIDS)”

Results were limited to humans. "Children" were defined as less than 18 years old. Article abstracts were obtained and reviewed. Abstracts that included only neonates, only adults, or children with only haematological/oncological disease were excluded. Sickle cell disease is prevalent in West and Central Africa, and is a known risk factor for invasive bacterial disease. However for the purpose of this review, papers that dealt exclusively with sickle cell disease and not HIV infection were not considered for inclusion. Papers reporting only malaria without bacteraemia were also rejected. Laboratory-based articles that dealt only with microbiology but did not have any clinical information were excluded. Letters, comments, guidelines and treatment protocols were excluded. Articles that reported a patient cohort that was already included in a larger or more comprehensive study were excluded. The remaining articles were retrieved and reviewed in full text, and the same criteria applied, figure 1. Reference lists of all full text articles reviewed were screened for other possible articles. Conference abstracts, grey literature and unpublished reports were not included.

Diagnosis of tuberculosis is difficult in children; as most children have paucibacillary pulmonary disease or primary lymph node disease, mycobacterial blood culture has traditionally been considered to have low yield.[18] Furthermore, there is a rapidly expanding field of research focusing on novel methods of tuberculosis diagnosis.[19] Thus articles that focused solely on mycobacterial culture were not included.

Quality criteria for study entry were less stringent than in the systematic review by Reddy et al.[17] Studies were included even if incidence of bacteraemia was an incidental finding and not the *a priori* outcome of the research. The major criterion for inclusion was that all included articles had to present raw numbers of HIV-infected children who had bacteraemia, separately from adults or HIV-uninfected children. Studies were rejected if they presented only the background prevalence of HIV-infection in the study population or in the region. Many studies were retrospective reviews. Articles had widely varying approaches to distinguishing pathogens from contaminants, but no article was excluded solely on the basis of the interpretation of contamination.

As study methodology and reporting varied significantly between reports, a meta-analysis of the data was not feasible.

Results

The online database search was done on 23 August 2010. The two search strings yielded 722 articles (Fig 1). Forty articles were reviewed in full text and 21 were included in the analysis. The 21 studies were done in 8 countries in Southern and East African (Kenya, Malawi, Mozambique, South Africa, Tanzania, Uganda, Zambia and Zimbabwe). No studies from Central, West or North Africa were found that met the inclusion criteria. Two studies were conducted in rural hospitals; the other 19 were conducted in urban centres and academic hospitals. The data was collected between 1993 and 2007, table 1. In 5 prospective studies the main outcome under investigation was incidence of bacteraemia.[2, 18, 20-22] In 10 prospective studies the incidence of bacteraemia was a secondary outcome; primary outcomes for these studies included malaria, pneumonia, anaemia, opportunistic infections and efficacy of cotrimoxazole (CTX) preventive therapy.[23-32]. There were 4 record reviews of infections by specific organisms, namely meningococcal disease [33] *Shigella* [34], *Streptococcus pneumoniae* [35], and *Pseudomonas.[36]* There were 2 retrospective case-note reviews: one on diarrhoea admissions and one on bacterial infections in hospitalised HIV-infected children.[37, 38]

There were 922 bacteraemias detected in 3619 HIV-infected children. The most common Gram-positive isolates were *S. pneumoniae* followed by *Staphylococcus aureus*; the most common Gram negative isolates were non-typhoidal *Salmonellae* (NTS). In 5 studies[18, 20, 21, 27, 30] NTS was reported to be the most common organism.

Of the 16 studies that included both HIV-infected and uninfected children, 14 studies calculated some measure of excess risk of bacteraemia among HIV-infected children. Two retrospective studies showed decreased risk of bacteraemia among HIV-infected children. Children with HIV were 0.66 times as likely to have *Shigella* bacteraemia[34], and 0.55 times as likely to have *Pseudomonas* bacteraemia[36]. All of the prospective studies showed increased risk of bacteraemia in HIV-infected children, from 1.4 [18] to 15 [37] times greater than HIV-uninfected children.

Four studies used the size of the population served by the hospital to calculate a population-level incidence rate of bacteraemia. In rural Kenya, an incidence rate of 505 bacteraemias per 100 000 persons per year for children under 5 years was reported.[2] In this context, HIV-infected children had a 3.3 times increased odds of

any bacteraemia when compared to HIV-uninfected children. In urban Johannesburg, South Africa, HIV-infected children under 2 years had an incidence rate of 1844 pneumococcal bacteraemias per 100 000 persons per year, while HIV-uninfected children had an incidence rate of 5 per 100 000 persons per year, giving a 37-fold increase in incidence.[23] In a subsequent study in the same area, the increase in relative risk of bacteraemia due to HIV infection ranged from 13.4 for *Salmonella* bacteraemia to 97.9 for *E. coli* bacteraemia.[9] In a retrospective review of meningococcal reporting in Gauteng, South Africa, HIV infection conferred a 61-times increased risk of meningococcal bacteraemia.[33]

Two longitudinal cohort studies of HIV-infected children reported incidence rates of bacteraemia: 2.3 per 100 person-years in Zambia[27] and 7.0 per 100 person-years in Cape Town, South Africa.[32] In both cases, bacteraemia was not an *a priori* objective of the study, but was detected incidentally during clinical management of acute illness. These estimates may therefore under-estimate the actual incidence rate of bacteraemia in those children.

Three studies reported increased case fatality associated with bacteraemia, but no significant association with HIV infection.[2, 25, 37] Three studies reported significantly increased case fatality associated with both bacteraemia and HIV infection,[21, 22, 31] and one reported increased case fatality only among children who were both HIV-infected and had bacteraemia.[20] Estimates of association with excess case fatality for children with bacteraemia ranged from 2.03 increase odds (95% CI 1.05 to 5.39) [22] to a risk ratio of 4.97 (95%CI 4.41 to 5.60)[2]. Two studies from Malawi reported a non-significant trend towards increased deaths associated with either bacteraemia or HIV infection.[18, 30] Two studies, both investigating hypoxic pneumonia in South African infants and children, reported increased case fatality amongst HIV-infected children, but no significant association with bacteraemia.[25, 29] In the study of meningococcal disease, HIV-infected children were more likely to have bacteraemia than meningitis; compared to children with meningitis, children with bacteraemia were more likely to die (OR 2.1, 95% CI 1.1 – 3.9). [33] Eight studies did not discuss deaths, or did not describe factors associated with increased risk of death.

There were no studies from Africa that specifically compared the incidence of bacteraemia in HIV-infected children before and after starting HAART. Of the 4 studies that reported such incidence rate ratios, 3 were from North America and one

from Europe. Despite quite different patient characteristics, these studies had similar findings, with a 70 to 89% decrease in bacteraemia observed post-HAART (incidence rate ratio 0.11 to 0.3), table 2.

Discussion:

Invasive bacterial infections are common in HIV-infected children in Africa, and are associated with high morbidity and mortality. The articles assessed in this review demonstrate the heterogeneity of paediatric HIV care in Africa, and the wide range of infectious risks to which African children are exposed. The excess risk of dying due to bacteraemia among HIV-infected children is variable, depending on the causative organism, the clinical setting and the geographical context of the study population. The studies that did not show association with mortality were in highly selected subgroups of children who already had significant background disease, such as severe malnutrition[37] or severe malarial anaemia[30], as opposed to studies assessing general paediatric patients.[2]

There were major differences in the prevalence of different organisms, especially non-typhi *Salmonella* and *S. pneumoniae*. Non-typhi *Salmonella* (NTS) are endemic in tropical Africa, with seasonal peaks in the rainy season; contamination of food and water is common, and it has also been suggested that person-to-person spread may occur in some contexts.[39] Furthermore, malaria appears to increase the risk of invasive NTS infection. This association might be explained by malarial trophozoite-induced haemolysis, resulting in the release of iron, thus creating a favourable environment for siderophilic organisms such as Salmonella. [30, 31]

The low incidence of *S pneumonia* and *H influenzae* in some studies from tropical countries is remarkable. One author described in detail the difficulties in processing blood culture specimens in their hospital, and suggested that fastidious organisms would be less likely to be detected in that context.[27] As some of the study sites in tropical African countries were rural and used an off-site microbiology lab, this issue may have confounded the relative frequency of detection of NTS and *S. pneumoniae* in these studies. All of the studies were completed before introduction of pneumococcal conjugate vaccines, and many tropical countries were not using *Haemophilus influenzae* type B vaccine at the time of the study. The infrequency of positive cultures of these organisms in tropical Africa probably represents under-detection rather than absence of disease.

Another concern with limited microbiological support is incomplete identification of bacteria. In particular, the incidence of *S. aureus* can be over-estimated if coagulase-negative staphylococci are not correctly identified.[40]

There are a number of other reasons why the reported incidences might represent under-estimates of actual disease burden. Blood cultures are known to be insensitive in detecting bacterial infection, with bacteria identified in blood culture in only 20-30% of patients with invasive bacterial disease.[9, 41] Volume of blood taken for culture has been shown to influence the detection of bacteraemia.[2] As it is difficult to draw large volumes of blood from small children, inadequate blood specimens may also have contributed to under-detection of bacteraemia. Furthermore, many children in Africa receive antibiotics before presenting to the health facility[2, 27] and this may also decrease the rate of positive blood cultures. In addition, even in carefully controlled clinical trials, with highly selected and motivated families with good access to health services, many deaths occur at home. [2, 27, 32] Despite verbal autopsies, in some cases an adequate cause of death is never established. Many of these out-of-hospital deaths may be due to bacterial infections: in one study of post mortem blood cultures done within 3 hours of death on 212 Zimbabwean children who died at home, 92 (43%) had positive bacterial growth, with the most common organism being Klebsiella. [42]

There is strong and consistent association of bacteraemia and increased risk of death among HIV-infected children in Africa. Some authors suggested that the observed increased mortality was largely due to increased incidence of bacteraemia.[20, 33] However, three large studies were able to demonstrate that HIV infection was associated with an increase in mortality independent of the incidence of bacteraemia.[21, 22, 31] These three studies had enrolled many infants and young children (median ages 8.5, [21] 5,[22] and 11[31] months), who were at high risk of rapid disease progression and death without anti-retroviral therapy.[43] These young children should be prioritised for provision of HAART.

The decreased incidence rates of bacteraemia observed in children in the post HAART era in America and Europe reflect the immune restoration and increased survival observed in children on HAART. However, three of the trials evaluated in this review are subject to the “ageing cohort” effect,[44-46] where the same children are followed over time. The incidence rates of bacteraemia post-HAART are

compared to incidence rates that occurred in the same children before HAART was available. As individual risk of bacteraemia decreases as a child gets older,[2] the effect of HAART may be confounded by the natural decreased incidence that would be expected over time. This effect was partly resolved in one pooled analysis of 4 birth cohorts, where follow-up time was censored at 72 months, the age when risk of bacteraemia starts to decrease.[5] Thus the incidence rates of bacteraemia with and without HAART were compared among children of similar age; this estimated incidence rate ratio of 0.3 is the most conservative estimate of all of the studies, but is probably the most reliable quantification of the independent effect of HAART on bacteraemia risk.

Conclusion

Bacteraemia is common in HIV-infected African children, and is associated with a significantly increased risk of death. There is noticeable geographical variation in the incidence and outcome of bacteraemia, related to both local prevalence of disease (differences in types of bacteria, and prevalence of malaria) and capacity for the detection of bacteraemia. There have been no trials investigating the effect of HAART on incidence of bacteraemia in Africa. However, data from North America and Europe suggest that HAART confers a 70-89% decreased risk of bacteraemia in HIV-infected children. This is an issue that should be prospectively evaluated, and should strengthen the call to expand anti-retroviral program coverage to children in Africa.

Tables and figures

Fig 1: Selection of eligible articles

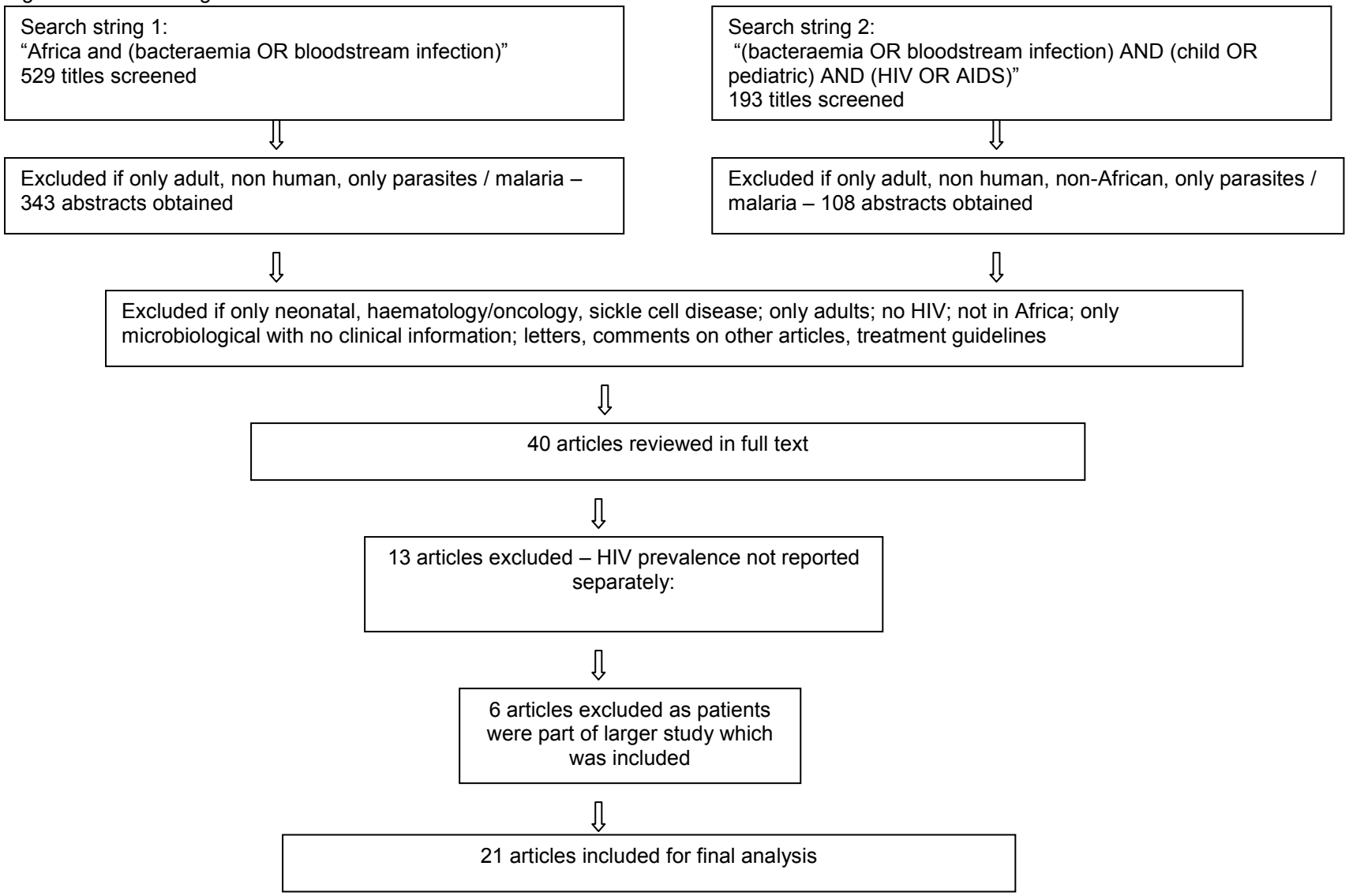

Table 1. Bacteraemia among HIV-infected children in Africa.

First Author	Location, study dates	Study design	Main outcome of study	Number of children	Age range	Number HIV-infected children	Number of bacteraemia: HIV-infected vs HIV- uninfected	Organisms	Comments on case fatality ratio (CFR)
Archibald [18]	Lilongwe Malawi; 1998	Prospective, blood culture drawn for all acute admissions	Predictors of bacteraemia	229	Median 2.7 years	HIV infected: 63/229 (28%); HIV uninfected 160/229 (70%) Not tested: 6/229(3%)	12/63 (19%) vs 22/160 (14%)	NTS, *E. coli*	Neither bacteraemia nor HIV affected CFR (data not given)
Bachou [20]	Kampala, Uganda; 2003-2004	Blood culture drawn for all children <60 months with severe malnutrition	Incidence of bacteraemia in children admitted with malnutrition	450	Median 17 months (range 4-60)	HIV infected: 149/450 (33%); HIV uninfected: 259/450 (58%) Unknown: 42/450 (9%)	30/149 (20%) vs 39/259 (15%)	NTS, *S. aureus*	With bacteraemia: 22/76 (29%) died; without bacteraemia 85/369 (23%) died. Among HIV-infected children, 13/30 (43%) with bacteraemia died;

First Author	Location, study dates	Study design	Main outcome of study	Number of children	Age range	Number HIV-infected children	Number of bacteraemia: HIV-infected vs HIV- uninfected	Organisms	Comments on case fatality ratio (CFR)
Berkley [47]	Rural Kenya;1998-2002	Blood culture drawn for all children <13 years	Incidence of bacteraemia	16570	0-13 (88% <5yr)	HIV infected: 243; HIV uninfected: 1838; unknown 14483	HIV infected: 186/243 (77%) vs 908/16327 (6%) HIV uninfected or unknown	*S pneumoniae*, NTS	With bacteraemia: 308/1094 (28%) died; without bacteraemia 876/15 476 (6%) died. Mortality of HIV-infected children not given
Blomberg [21]	Dar es Salaam, Tanzania; 2001-2002	Blood culture drawn from all children with severe disease**	Incidence of bacteraemia in children	1787 children (1828 episodes)	Median 8.5 months	HIV infected: 152 (9%) HIV uninfected: 753 (42%) Unknown: 882 (49%)	28/160 (18%) admissions vs 119/766 (16%) admissions	NTS	With bacteraemia: 80/229 (35%) died; without bacteraemia 197/1403 (14%) died; risk ratio 2.49 (2.00 – 3.01). Mortality of HIV-infected children not given
Bronzan [30]	Blantyre, Malawi; 1996-2005	Prospective: blood culture drawn from all children with severe malaria	Incidence of bacteraemia in severe malaria	1388	Median 40 months	HIV infected: 178 (13%); HIV uninfected: 941 (68%); Unknown: 269 (19%)	HIV infected: 13/178 (7%) HIV uninfected: 38/941 (4%) Unknown: 13/269 (5%)	NTS	With bacteraemia: 9/64 (14%) died; without bacteraemia 109/1323 (8%) died; Mortality of HIV-infected children not given

First Author	Location, study dates	Study design	Main outcome of study	Number of children	Age range	Number HIV-infected children	Number of bacteraemia: HIV-infected vs HIV- uninfected	Organisms	Comments on case fatality ratio (CFR)
Chhagan [37]	Durban, South Africa; 2001	Retrospective review of diarrhoea admissions	Co-morbidity and CFR of diarrhoeal disease	319	Median 8.5 months (IQR 4 to 19 months)	HIV infected: 214/319 (67%) HIV uninfected: 103/319 (32%) Unknown: 2/319	34/194 vs 1/94	*Klebsiella* sp / *E. coli*	Children with bacteraemia: 14/35 (40%) died; without bacteraemia 32/284 (11%) died; HIV infected: 42/212 (20%) died; HIV uninfected 4/103 (4%) died
Cohen [33]	Gauteng, South Africa; 2003-2007	Review of meningococcal case reporting	Incidence of meningitis/ bacteraemia, HIV infected vs uninfected, all ages	181 children (<14); 127 adults (>15)	Median 6 yr (IQR 3-25 years)	54/181 (30%)	54/181 (30%)	*Neisseria meningitidis*	All patients: HIV infected: 27/138 died (20%); HIV uninfected 18/170 (11%) died; not present children CFR separately
Davies [34]	Johannes-burg, South Africa; 1996-1998	Review of all cases of *Shigella* bacteraemia (all ages)	Describe risk factors and outcomes of *Shigella* bacteraemia	20 children (<18) ; 14 adults (>18)	Children: median 7 months	HIV infected: 6/20 (30%) HIV uninfected: 9/20 (45%) Unknown:5/20 (25%)	6/15 vs 9/15	*Shigella flexneri*	No child deaths

First Author	Location, study dates	Study design	Main outcome of study	Number of children	Age range	Number HIV-infected children	Number of bacteraemia: HIV-infected vs HIV- uninfected	Organisms	Comments on case fatality ratio (CFR)
Jaspan [38]	Cape Town, South Africa; 2002-2006	Record review of hospitalised HIV-infected children	Describe bacterial disease and antibiotic sensitivity in HIV-infected children	141	Median 1.2 years (IQR 0.5-2.3 years)	141 (100%)	128	*S. pneumoniae*	13/141 (9%) died; did not describe difference in case fatality if had bacteraemia
Jones [23]	Johannes-burg, South Africa; 1996	Prospective study of *S. pneumoniae* infection, all ages	Burden of disease of *S. pneumoniae* in HIV infected and uninfected	49 children (<13); 127 adults (>13)	2 weeks to 12 years	25/49 (51%)	25 vs 24	*S. pneumoniae*	HIV infected: 2/25 (8%) died; HIV uninfected 1/24 (4%); risk ratio 1.9 (0.19-19.8)
Karstaedt [35]	Johannes-burg, South Africa; 1996-1997	Retrospective review of *S. pneumoniae* bacteraemia	Determine incidence of *S pneumoniae* bacteraemia	194		HIV infected: 67; HIV uninfected: 44; Unknown: 83	HIV infected: 67; HIV uninfected: 44; Unknown: 83	*S pneumoniae*	42/194 (22%) died, did not distinguish HIV infected from HIV uninfected
Lala [24]	Johannes-burg, South Africa; 1997-1998	Blood culture drawn on all children with pneumonia	Value of CRP in pneumonia	570	6 weeks to 5 years	244/570 (43%)	38/244 vs 25/326	*S pneumoniae*	(Did not describe CFR)

First Author	Location, study dates	Study design	Main outcome of study	Number of children	Age range	Number HIV-infected children	Number of bacteraemia: HIV-infected vs HIV- uninfected	Organisms	Comments on case fatality ratio (CFR)
Madhi CID 2002 [25]	Johannes-burg, South Africa; 2000-2001	Blood culture drawn on all children with pneumonia	Effectiveness of CPT for PJP prophylaxis	185	Median 4.5 months	185 (100%)	18 bacteraemias in 231 episodes of pneumonia	*S pneumoniae*	Among children with proven PJP, higher CFR if also had bacteraemia: 2/20 with bacteraemia died vs 3/78 without bacteraemia; risk ratio 3.9 (0.85 – 17.9)
Madhi CID 2000 [9]	Johannes-burg, South Africa; 1997-1998	Blood culture drawn on all children with severe pneumonia	Aetiology and outcomes of pneumonia	1215	2-59 months	HIV infected: 548 / 1215 (45%); HIV uninfected 617/1215 (51%); unknown 50/1215 (4%)	HIV infected 82/548 (15%) vs 40/617 (6%) in HIV uninfected	*S pneumoniae*	HIV infected children with bacteraemia: 14/82 (17%) died: HIV infected children without bacteraemia 58/466 (12%) died; risk ratio 1.4 (0.80 – 2.34)
Mulenga [27]	Lusaka, Zambia; 2001-2003	RCT of CTX vs placebo in HIV-infected children	Survival and bacterial infections comparing CTX vs placebo	534	Median 4.4 years at baseline (36% <2yr)	534 (100%)	14/534 (2.3/100PY)	NTS	186 (35%) died; did not describe difference in mortality if children had bacteraemia or not

First Author	Location, study dates	Study design	Main outcome of study	Number of children	Age range	Number HIV-infected children	Number of bacteraemia: HIV-infected vs HIV- uninfected	Organisms	Comments on case fatality ratio (CFR)
Nathoo [22]	Harare, Zimbabwe; 1993-1994	Blood culture drawn on all pyrexial children <8 years	Incidence of community acquired bacteraemia	309	Median 5 months	168/309 (54%)	67/168 (40%) vs 28/141 (20%)	*S. aureus*, *S pneumoniae*	Among HIV infected children: 20/67 (30%) with bacteraemia died, vs 18/101 (18%) without bacteraemia; risk ratio 1.67 (0.96 – 2.92)
Perovic [36]	Johannes-burg, South Africa; 1998-1999	Review of all cases of *Pseudomonas aeruginosa*	Describe risk factors and outcomes	38 children 44 adults	(Not stated)	12/38 (32%)	12/38 (32%) vs 22/38 (68%)	*Pseudomonas aeruginosa*	Described increased case fatality among HIV-infected adults with bacteraemia; did not describe case fatality in HIV-infected children
Sigauque [31]	Rural Mozam-bique; 2004-2006	Blood cultures drawn on all children <2 years	Characteristics of children with severe pneumonia	757	0-23 months	HIV infected: 53/757 (7%); HIV uninfected: 149/202 (20%); unknown 555/757 (73%)	10/39 (26%) vs 14/111 (13%)	*S. pneumoniae*	Children with bacteraemia 15/58 (26%) died, children without bacteraemia 62/497 (12%) died;

First Author	Location, study dates	Study design	Main outcome of study	Number of children	Age range	Number HIV-infected children	Number of bacteraemia: HIV-infected vs HIV- uninfected	Organisms	Comments on case fatality ratio (CFR)
Westwood [28]	Cape Town, South Africa; 1996	Cohort of HIV-infected children followed for 1 year	Incidence of bacterial infections	108	Median 16 months	108 (100%)	31	*S. pneumoniae*	No deaths reported
Zar Acta Paed 2001 [29]	Cape Town, South Africa; 1998	Prospective study of children admitted with severe pneumonia	Aetiology and outcome of HIV-infected children with pneumonia	250	Median 6 months	151 (60.4)	HIV infected 20/151 (13%) vs 15/99 (15%) HIV uninfected	*S. pneumoniae*	HIV infected 31/151 (21%) died vs 8/99 (8%) HIV uninfected; did not describe CFR for children with bacteraemia
Zar AIDS 2010 [32]	Cape Town, South Africa; 2003-2007	Prospective study of CPT HIV-infected children; blood cultures drawn when clinically indicated	Mortality and morbidity on intermittent vs daily CTX prophylaxis	324	Median 22 months at baseline	324 (100%)	30 children had 47 episodes of bacteraemia	*S. pneumoniae*	Children with bacteraemia: 13/30 (43%) died vs 40/294 (14%) without bacteraemia; Risk ratio 3.18 (1.93 to 5.25).

vs = versus

*NTS = Non-typhoid *Salmonella*

**PJP = *Pneumocystis jirovecii* pneumonia

CTX = co-trimoxazole

CPT – co-trimoxazole preventive therapy

Table 2: Impact of HAART on incidence of bacteraemia in HIV-infected children

1st Author	Location; study date	Study design	Number of patients	Age range	Main outcome measure	Incidence of bacteraemia pre- vs post-HAART; incidence rate ratio
Gona [44]	PACTG 219C sites in USA and Puerto Rico; 2000-2004	Prospective	2767	Median 9.9 years; disenrolled when age 24 years	Incidence of opportunistic infections in children on HAART compared to a pre-HAART study (Dankner 2001)	Pre: 3.3 / 100PY Post: 0.35 / 100PY IRR 0.11
Guellin [45]	Madrid, Spain; 1990-2006	Retrospective review of clinical cohort	366	Median 2.6 years at enrollment	Incidence of opportunistic infections in three calendar periods	Pre: 2.85 /100PY Post: 0.36 /100PY IRR 0.13
Kapogiannis [5]	4 cities in USA; Enrolled 1986 to 1990; ended follow up 2004	Longitudinal data from birth 4 cohorts	364	Birth to 72 months	Incidence rate of bacteraemia pre vs post HAART	Pre: 9.8 / 100PY Post: 3 / 100PY IRR 0.3
Steenhoff [46]	Philadelphia, USA; 1989-2006	Retrospective record review	256	Med 1.1years (range birth to 18 years)	Incidence of invasive pneumococcal disease pre-HAART and pre PCV	Pre: 1.86 / 100 PY Post: 0.29 / 100PY IRR 0.16

*IRR = incidence rate ratio
**PCV = Pneumococcal conjugate vaccine

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Abstract

Aims

Bacteraemia is an important cause of morbidity and mortality in HIV-infected children, especially in the absence of highly active anti-retroviral therapy (HAART). The incidence of bacteraemia in a cohort of HIV-infected children from South Africa is described.

Methods

HIV-infected children enrolled in a randomised controlled trial evaluating 2 regimens of co-trimoxazole preventative therapy (CPT) were followed for 5 years from January 2003. Blood cultures were taken during acute admissions, when clinically indicated.

Results

324 children were followed for 672 person-years; 364 blood cultures were taken from 125 children. 47 bacteraemias occurred in 30 children; 22 (47%) were Gram positive and 25 (53%) Gram negative bacteria. *S. pneumoniae* was the most common isolate, comprising 32% of bacteraemias.

53 (16%) children died during the study. Children with documented bacteraemia had higher risk of death than those without bacteraemia (risk ratio 3.18, 95% CI 1.93 to 5.05). Children receiving intermittent CPT had higher incidence of bacteraemia than those receiving daily CPT (9.63 vs. 4.08 per 100 person-years, incidence rate ratio (IRR) 2.36, (95% CI 1.21 to 4.86). Children established on HAART had lower incidence than those not on HAART (2.2 vs. 8.5 per 100 person years, IRR 0.26, 95% CI 0.09 to 0.61); however, the incidence rate of bacteraemia was significantly higher in the first 3 months of commencing HAART compared to those not on HAART(IRR 3.82, 95% CI 1.84 – 7.58).

Conclusion

Bacteraemia in HIV-infected children is common and associated with high mortality; lower rates of bacteraemia occurred in children receiving daily CPT and in those well established on HAART.

Keywords:

Africa, children, HIV, bacteraemia, anti-retroviral therapy, co-trimoxazole, preventive therapy

Introduction

Invasive bacterial infections are a major cause of mortality and morbidity among HIV-infected children in Africa.[1] As children with HIV are particularly susceptible to infection with encapsulated bacteria,[2] the incidence of bacteraemia in HIV-infected children is 40 to 100-fold that of similar-aged HIV-uninfected children.[3] Treatment with highly active antiretroviral therapy (HAART) has been shown to decrease mortality in many clinical trial and programmatic settings in sub-Saharan Africa[4, 5] with similar early treatment outcomes and immune recovery to those seen in Europe and North America.[4] HAART is also associated with decreased incidence of bacteraemia.[2, 6, 7] Co-trimoxazole (CTX) preventive therapy (CPT), initially used for prophylaxis against *Pneumocystis jirovecii*, decreases mortality of HIV-infected African children, [8] with significant reductions in bacterial infections.[9]

In a cohort of HIV-infected children in Cape Town, South Africa, it was previously reported that, compared to daily CPT, intermittent CPT was associated with longer hospitalisation and increased incidence of bacteraemia. [10] The aim of this study was to further investigate risk factors for bacteraemia.

Methods

A prospective trial to compare different dosing schedules of CPT in HIV-infected children commenced in January 2003. The study had a factorial design, with two levels of randomisation and hence four comparison groups: children were randomised to receive either daily or three times a week CPT, and then further randomised to receive either isoniazid (INH) or placebo on the same dosing schedule, figure 1. After interim analysis showed significantly reduced mortality in the INH groups, the placebo arm was terminated on 17 May 2004, on the recommendation of the Data Safety Monitoring Board.[11] Thereafter, all children receiving placebo were changed to INH, continuing the same dosing schedule (daily or thrice weekly) as before. After May 2004, a further group of children were recruited into the study, on open-label INH and CPT, randomised to either daily or three times a week. The trial was run in two tertiary hospitals in Cape Town, South Africa (Red Cross War Memorial Children's Hospital, University of Cape Town and Tygerberg Children's Hospital, Stellenbosch University). Blood culture results from January 2003 until December 2007 were analysed.

Participants

Participants were HIV-infected children ≥ 8 weeks old, attending either hospital. Additional inclusion criteria and exclusion criteria have been described.[11] Written informed consent was obtained from a parent or legal guardian. HIV status was confirmed at enrolment by 2 enzyme linked immunosorbent assays (Abbott AxSYM HIV antibody/antigen ELISA) in children older than 15 months; in younger children, the polymerase chain reaction (Amplicor HIV-1, Roche Diagnostic Systems) was used.

Medication

Dosage of CTX was calculated at 5mg/kg/dose of the trimethoprim component. Dose was adjusted according to weight at each visit and rounded up to the nearest 0.5 millilitre. INH or placebo was given on the same dosing schedule as the CTX. INH was given in tablet form (100 mg tablets, Be-Tabs Pharmaceuticals, Johannesburg, South Africa) as quarter or half tablets, for a dose of approximately 10mg/kg (range 8-12mg/kg). Placebo tablets were manufactured to look identical to INH.

In 2003, HAART was not yet widely available in South Africa but was obtained for some children through pharmaceutical trials or charitable donations. From 2004, due to the HAART roll-out programme, study participants qualifying on local criteria could access HAART.

Allocation

Randomisation was stratified by site, to account for potential differences in study site population and for the benefit of precision. The trial statistician generated random variable block lists; study pharmacists labelled study drugs sequentially. Study pharmacists issued study medication at scheduled study visits. All participants and investigators were blinded to the placebo/INH allocation, but not to the CPT dosing schedule.

Bacteriology

Blood cultures were taken by emergency room or hospital ward staff at the treating clinician's discretion. Details of a hospital admission were recorded by a study team member and all blood results were followed up in person or telephonically. Children were admitted to the tertiary academic study hospitals or their affiliated secondary-level (community) hospitals. Indications for blood cultures are similar in these

hospitals and include clinical sepsis, suspected bacterial meningitis, pyrexia <3 months old, severe malnutrition requiring admission, spiking fever or severe pneumonia. Cultures were drawn in a sterile manner according to written hospital protocols. Cultures were taken in Bactec Peds Plus/F bottles (Becton-Dickenson) and incubated in the Bactec 9240 blood culture system. Isolates from positive blood cultures were identified and had susceptibility testing performed according to standard laboratory practices in the two laboratories serving the respective hospitals. As most children were recruited during or soon after hospital admission, and due to the frequent hospitalisations of children in the study, differences between community-acquired and hospital-acquired bacteraemias were not explored. When isolates of coagulase-negative staphylococcus, micrococcus or viridans streptococcus were detected, the records were reviewed to determine if this was a likely contaminant or pathogen. In no children were these bacteria cultured repeatedly, or in a context suggestive of endocarditis or central line infection. They were therefore regarded as contaminants. Cultures that grew other organisms were considered indicative of bacteraemia. Blood cultures with no reported growth after 5 days were considered negative. Children were managed according to hospital antibiotic guidelines; antibiotic choices were amended once susceptibility results were known. Bacterial resistance is reported for the most commonly used antibiotics, or those that would be considered in the first line of therapy. Specimens for TB were processed separately; these results have been reported.[11]

Statistical analysis and Sample size

For summary statistics, continuous variables are expressed as medians (interquartile range, IQR). Categorical variables are expressed as number (percentage).

Risk ratios were used to compare mortality proportion of children who had ever had a bacteraemia to those who did not have bacteraemia. Crude rates of bacteraemia between the two dosing schedules and across time-varying age and HAART exposure categories were compared by dividing the number of bacteraemias observed by the person-time accrued in each category. Age was dichotomised at 12 months; HAART was categorised as (1) not on HAART, (2) on HAART for less than 3 months (at high risk for immune reconstitution inflammatory syndrome) or (3) more than 3 months on HAART. Follow-up time was calculated from enrolment to last known time alive.

The main outcome measure for multivariate analysis was number of episodes of bacteraemia. The main exposure variable was dosing schedule (comparing intermittent to daily CPT, using intention to treat analysis). Counts of bacteraemia over 5 years were modelled using a negative binomial model, after comparing different count models (Poisson, zero inflated Poisson) with the Vuong statistic, and with Akaikes Information Criterion (AIC).[13] As some patients had repeated episodes, we adjusted for potential within-individual correlation by using generalised estimating equations (GEE), clustering on each child.[14]

Baseline characteristics that were explored included age, sex, dosing schedule, INH/placebo allocation, nutrition status (weight-for-age, weight-for-height and height-for-age Z-scores), study site, CD4 count, CD4 percentage at baseline, and WHO clinical stage at baseline. Age and exposure to HAART were included as categorical time-varying covariates. Variables associated with the outcome at the α=0.1 level were included for multivariate analysis. Manual and automated backwards and forwards step-wise model building procedures were used; potential models were compared with the AIC. The final model chosen presented the best AIC value and included study site, dosing schedule, weight-for-age Z-score, WHO clinical category, age and HAART exposure category.

Z scores were calculated according to the World Health Organization Standard Growth Charts[12] using Epi-Info statistical program, and then saved in the data base. Subsequent analyses were done in STATA statistical software, version 10 (StataCorp LP, College Station, Texas, USA). All p-values are two-tailed.

Sample size

The *a priori* defined study sample size was 400; this sample size was primarily determined by the expected mortality effect of INH compared to placebo[11]. Due to the early and significant mortality difference in the INH/placebo comparison, study enrolment was terminated before this sample size was achieved. Therefore all our findings are presented with 95% confidence intervals which reflect the precision achieved by the realised sample size of 324 individuals (47 events).

Results

Baseline characteristics

Three hundred and thirty nine children were enrolled; fifteen children were excluded (ten tested HIV-negative, five were lost to follow-up within a month after randomisation), figure 1. Therefore, 324 children (181 males (56%)) were followed for 672.1 child years (interquartile range (IQR) 1.03 to 4.04 years). 28 (9%) children were lost to follow-up and 38 (11.7%) withdrew on personal request or relocated. The median age at enrolment was 23 months (IQR 9.5 to 48.6 months); 98 children (30.3%) were less than 1 year old. One hundred and sixty five (50.9%) children were randomised to three times per week CPT, while 159 (49.1%) were randomised to daily, table 1.

One hundred and twenty three (38%) children were underweight for age at enrolment (Z score of less than -2); the median weight-for-age Z score was -1.5. Most had moderate to severe immune suppression: 271 (84%) were either WHO clinical category 3 or 4. Median CD4 percentage was 20% (IQR 13.6 to 26.9%). Although only 28 (12%) were on HAART at enrolment, a further 179 (55%) started HAART during follow-up, table 1. Adherence to study medication was excellent.[15]

Blood stream infections

Three hundred and sixty four blood cultures were taken from 125 children; 77 children had more than 1 blood culture taken. There were 27 contaminants (8%); 2 cultures grew Candida species and 288 cultures (80%) had no growth after 5 days. Forty-seven bacteraemias occurred in 30 children; 8 children had more than one bacteraemia. Twenty-five (53%) pathogens were Gram negative while 22 (47%) were Gram positive bacteria. Of the 47 bacteraemias, 14 (30%) occurred more than 72 hours after admission to hospital (7 *Enterobacteriaceae*, 2 *Haemophilus sp*, 2 *Pseudomonas sp*, 2 *S aureus* and 1 *S. pneumoniae*). *Haemophilus influenzae* type B was not detected. *Streptococcus pneumoniae* was the most common single isolate, occurring in one-third of all positive cultures, table 2.

Antimicrobial resistance patterns were available for 45 bacteria; 7 (58%) of the 12 *Enterobacteriaceae* produced extended spectrum beta-lactamase (ESBL). Five of these isolates were also resistant to gentamicin; one *K.pneumoniae* was also resistant to amikacin. Of the *S. pneumoniae*, 7 of the 14 (50%) isolates showed reduced susceptibility to penicillin (MIC >0,06 ug/ml); one was also resistant to cefotaxime (MIC 4.0 ug/ml). All 5 *Staphylococcus aureus* isolates were resistant to

CTX; 4 (80%) were methicillin resistant (MRSA). Of 30 bacteria tested against CTX, 25 (83%) were resistant, table 2. There was no significant difference in CTX resistance in bacteria cultured from patients randomised to intermittent versus daily CPT. Ten (77%) of 13 bacteria isolated from children receiving daily prophylaxis were resistant to CTX, compared to 32 (94%) of 34 bacteria from children receiving intermittent prophylaxis (risk ratio 0.74, 95% CI 0.45 – 1.19).

Mortality

Fifty three of 324 children (16%) died. Children with an episode of bacteraemia had higher mortality than those without bacteraemia: 13/30 (43%) with bacteraemia died versus 40/294 (14%) without bacteraemia (risk ratio for mortality 3.18, 95% CI 1.93 to 5.25). Of the 13 children who died, 4 (31%) had Gram positive and 9 (69%) had Gram negative bacteraemia. Five (38%) of the 13 deaths occurred within 14 days of a bacteraemia; all of these deaths were due to clinical sepsis. Two (40%) of these 5 deaths were related to a resistant pathogen (both due to ESBL-producing *Klebsiella pneumoniae*). As reported, mortality was similar among children receiving intermittent CPT (24 of 165, 14%) compared to daily CPT (29 of 159, 18%; hazard ratio 0.75 95% CI 0.44-1.29).[10]

Characteristics of children with bacteraemia

Children randomised to intermittent CPT had more episodes of bacteraemia than those randomised to daily CPT. Nineteen children from the intermittent group had 34 bacteraemias (6 had recurrent infections, crude incidence rate (IR) 9.6 per 100 person-years), compared to 11 children from the daily group, who had 13 bacteraemias (2 had recurrent infections, crude IR 4.08 per 100 person years; incidence rate ratio (IRR) for bacteraemia 2.36, 95% CI 1.21 – 4.86, table 3). There were significantly more episodes of Gram negative bacteraemia in children receiving intermittent compared to daily prophylaxis [20 (80%) vs. 5 (20%), IRR 3.61 (95% CI 1.31 – 12.3)]. There was also a trend towards more Gram positive bacteraemias in the intermittent [14, (64%)] compared to the daily group [8, (36%)], (IRR1.58, 95% CI 0.62-4.34).

Prior to the termination of the placebo arm of the trial in May 2004, there was no difference in the number of children who had bacteraemia while receiving INH (9 of 131) compared to placebo (9 of 132), risk ratio 1.03 (95% CI 0.43 – 2.53).

Twenty-six (55%) of the 47 bacteraemias occurred in children not on HAART; 14 (30%) occurred within 3 months after starting HAART (crude IRR compared to children not on HAART 3.82, 95% CI 1.84 – 7.58) while only 7 (15%) occurred once established on HAART for more than 3 months (crude IRR compared to children not on HAART 0.26, 95% CI 0.09 – 0.61), table 3.

Bacteraemia was more common in infants and young children than in older children (median age at bacteraemia 18 months, IQR 10.5 to 37.5 months). There were 15 (32%) bacteraemias in children aged less than 12 months; 14 (93%) were due to Gram negative bacteria. There were 21 (45%) bacteraemias in the second year of life (8 (38%) due to *S. pneumoniae)*; and 11 (23%) bacteraemias in children older than 2 years, 7 (64%) due to *S. pneumoniae.*

Risk factors for bacteraemia

In the multiple regression model (negative binomial) controlling for the effects of age, study site, HAART status and degree of immune suppression, children receiving intermittent CPT had a higher incidence rate of bacteraemia than daily CPT (IRR 2.33, 95% CI 0.87 – 6.24), table 4. Children recently started on HAART had a two-fold increased incidence rate of bacteraemia compared to those not on HAART (IRR 2.25, 95% CI 0.87 – 5.87), whereas children established on HAART had a significantly decreased incidence rate compared to children not on HAART (IRR 0.18, 95% CI 0.04 – 0.83). Weight-for-age at enrolment remained a strong predictor for bacteraemia, with a 30% decreased incidence rate of bacteraemia with every unit increase in Z score, table 4.

Discussion

In this study of HIV-infected children, malnutrition, young age and lack of HAART were risk factors for bacteraemia. Novel findings were increased incidence of bacteraemia when CPT was given thrice-weekly compared to daily, and a higher incidence of bacteraemia in children in the first months after commencing HAART, with the incidence substantially reduced once HAART was established.

The observed pre-HAART incidence rate of 8.5 bacteraemias per 100 person-years is similar to rates reported from other pre-HAART studies of bacteraemia in HIV-infected children of similar age. As bacteraemia is more common in younger children, the studies that included older children and teenagers[7, 16] had lower incidence rates (2.8 and 3.3 per 100 person-years) than when follow-up was

censored at 72 months of age (9.8 per 100 person-years)[2]. The highest incidence rate (23 per 100 person years) was observed in a small cohort of young children followed from birth to 36 months, less than half of whom were receiving CPT.[17]

Four studies have reported 70 to 89% decreases in incidence rates of bacteraemia in children after starting HAART.[2, 6, 7, 18] The magnitude of decreased incidence rate ratio was similar irrespective of whether infants, children or teenagers were included in the analysis. This is consistent with the highly significant 83% reduction in adjusted incidence rate ratio observed in this cohort.

Increased incidence of bacteraemia during the first 3 months after starting HAART was observed. As the allocation to HAART was not randomised but based on clinical need, a higher incidence of bacteraemia in the first 3 months may reflect that starting HAART in this context was a marker of disease severity, and children with the most advanced disease had the highest risk of bacteraemia. The effect of HAART in restoring immunity may take months to years in children with severe immune suppression; these children would still be highly susceptible to infection in the first 3 months after initiating HAART. The significant reduction in bacteraemia after 3 months of HAART reflects the expected overall beneficial effect on immune function and decreased susceptibility to infection [5] and is consistent with previous studies.

Increased incidence of bacterial infections has previously been described soon after starting HAART in severely immune suppressed children. In a study of the incidence of pneumococcal disease in HIV-infected children in Philadelphia, USA, a 14% increase in cases of invasive pneumococcal disease was observed in the “transition year” as children were started on HAART.[18] In a cohort in Thailand, 35% of children needed hospital admission after starting HAART; 62% of the admissions were for “pneumonia and other bacterial infections”.[19] Immune reconstitution inflammatory syndrome (IRIS) has been well described in children;[20] common manifestations are muco-cutaneous eruptions and exacerbation or unmasking of tuberculosis.[21] Consensus criteria for definition of an IRIS event state that “symptoms cannot be explained by an alternate infection.”[22] However, in a cohort of Ugandan children, 6.5% of IRIS events were attributed to bacterial pneumonia,[23] and *S. pneumoniae* sepsis with empyema and bacteraemia was described in a case from a paediatric study in South Africa.[24]

Daily CPT compared to placebo has been shown to decrease mortality of HIV-infected African children,[8] with significant reductions in bacterial infections.[9] It has previously been reported that daily CPT provides better protection against bacteraemia than intermittent CPT and is associated with decreased duration of hospitalisation.[10] A multicentre study among HIV-infected adults in the USA showed that daily CPT was associated with an 18% decreased incidence of bacterial pneumonia compared to thrice weekly CPT, but did not report the incidence of bacteraemia or a difference in incidence of Gram positive and Gram negative infections.[25]

The higher incidence of bacteraemia observed among children receiving intermittent CPT was predominantly due to Gram negative organisms. It is possible that there is a different threshold for protection from Gram negative and Gram positive bacteraemia, although the numbers are small. We postulate that intermittent CPT provided some protection against Gram positive infections, and there was additional benefit when CPT was given daily. However intermittent CPT provided inadequate protection against invasive Gram negative infections, but daily CPT was effective, resulting in a significant 3-fold decreased incidence of bacteraemia.

S pneumoniae, the most common pathogen, is a frequent cause of invasive disease in HIV-infected children. [3, 26] In South Africa, the highest incidence of invasive pneumococcal disease (IPD) occurs in children under 1 year,[27] but *S. pneumoniae* bacteraemia was only detected in children older than 1 year. It is possible that CPT is more effective at preventing IPD in infants than in older children. This trial was completed before the launch of the pneumococcal conjugate vaccine in South Africa. It is anticipated that with inclusion in the national immunisation schedule, substantial morbidity and mortality may be prevented in HIV-infected children.[28]

The vaccination schedule remained constant throughout the duration of the trial, and included 3 doses of *Haemophilus influenzae* type B conjugate vaccine. Although this vaccine has been shown to have up to 35 times increased risk of vaccine failure in HIV-infected children in South Africa compared to HIV-uninfected children,[29] there was still some degree of protection, as no isolates of *Haemophilus influenzae* type B were isolated.

No *Salmonella sp* were detected in this cohort. Non-typhi Salmonella were occasional bloodstream pathogens in previous studies among children in Cape

Town; prevalence of CPT was not reported.[30-32] Many studies from East or central Africa report a high incidence of both *S. typhi* and non-typhi strains, even among children receiving CPT, [1, 9] as well as high incidence of CTX-resistant Salmonellae.[1, 9, 33, 34] In South Africa, 19% of non-Typhi Salmonella are resistant to CTX.[27] It is possible that CPT provides *in vivo* protection from invasive disease despite *in vitro* CTX resistance.[35]

High prevalence of antibiotic resistance was observed. There are several possible contributing factors. Firstly, HIV-infected children are known to be colonised with resistant organisms, as was shown by a high prevalence of colonisation with MRSA and ESBL-producing-*Enterobacteriaceae* in nasopharyngeal cultures at baseline in this cohort.[36] It has been suggested that antibiotic resistance is common among the families and communities of the study population, and that many young infants may be colonised with resistant organisms even before they are admitted to hospital.[36] Secondly, some children were enrolled soon after a hospital admission, where they may have acquired resistant organisms; the frequency and length of hospitalizations in which colonisation with hospital flora may have occurred has been described[10]. Thirdly, high levels of antibiotic resistance have been described among children with severe malnutrition admitted with pneumonia[37] and many of the children in this cohort were also malnourished.

The negative binomial regression is a more robust method for modelling count data than Poisson regression, but gives more conservative confidence intervals.[13] When comparing intermittent to daily CPT, the univariate (unadjusted) incidence rate ratio from the regression model had a similar point estimate (2.36) to the crude incidence rate ratio (2.44) which was calculated by dividing the number of bacteraemias by the person time in each prophylaxis group. However the IRR from the regression model is not statistically significant due to the conservative nature of the model. It is interesting that in the multivariate model, after adjusting for age, WHO clinical stage, degree of malnutrition and HAART status, the point estimate and confidence intervals did not change. This implies that the protective effect of daily CPT is independent of these other factors, and clinical benefit can be anticipated at all ages and degrees of immune suppression and malnutrition

There are a number of limitations to this study. Blood cultures are known to be insensitive in detecting bacterial disease.[38] Furthermore, the blood cultures obtained for our study were taken for clinical indications and not routinely, possibly

under-representing the true incidence of bacteraemia in this population. Only those children brought to hospital could have had blood cultures taken, so those who died at home were excluded. However, only 5 children died outside a hospital facility.[11] Treatment with antibiotics by a primary-care provider before referral may have further decreased the detection rate; it was not possible to objectively measure antibiotic use prior to hospitalisation. As cultures were taken by different clinical care providers and not by members of the study team, there may have been varying techniques, and different volumes of blood taken. The high number of contaminants indicates that despite availability of standard guidelines, sterile technique is frequently not possible in the emergency room. Some of the contaminants may have masked pathogenic organism growth, which would also lead to an under-estimate of incidence. For these reasons, our estimate of 7.0 bacteraemias per 100 person-years is almost certainly an underestimate.

Conclusion

Bacteraemia in HIV infected children is common, is predominantly due to common organisms such as *S pneumoniae,* and is associated with high mortality. Effective preventative measures such as pneumococcal vaccines, daily CPT and HAART should urgently be scaled up for HIV-infected children in Africa.

Collaborators and author's contribution

David le Roux did the analysis and wrote the manuscript.
Heather Zar and Mark Cotton conceived the study, wrote the protocol and grant for funding and supervised the study and manuscript. Stanzi le Roux was a trial physician and assisted with data analysis Andrew Whitelaw contributed to the laboratory methods and interpretation. Carl Lombard contributed to study design, was responsible for the randomization list and did initial statistical analysis.

Acknowledgements:

Funding for the study was provided by the Rockefeller Foundation, USA; the MRC South Africa; the National Research Foundation, South Africa; The South African Thoracic Society, and the Department of Health, South Africa.
We thank the children and their caregivers for participating, and the members of the INH study team; and also Dr Marie Diener-West, Dr Rosa Crum and Dr Bill Moss for statistical advice and encouragement.

Data and Safety Monitoring Committee: Dr J. Kaplan (chair), Dr W. El Sadr, Professor P. Donald and Professor N. Beyers; local DSMB: Professor P. Donald (chair),
Professor N. Beyers and Professor M. Klein.

Table 1: Comparison of children assigned to intermittent or daily trimethoprim-sulphamethoxazole prophylaxis regimens at randomisation

	Intermittent (n=165)	Daily (n=159)	Total (N=324)
Age (months):			
Median	21.8(9.5 – 52.3)	24.6(9.7 – 45.6)	23.0(9.5 – 48.6)
<12 months	29.7% (49)	30.8% (49)	30.3% (98)
>12 months	70.3% (116)	69.2% (110)	69.8% (226)
Male	56.9% (94)	54.7% (87)	55.8% (181)
Weight for age (z-score)	-1.3(-1.8 to -1.0)	-1.6(-1.9 to -1.2)	-1.5(-1.8 to -1.2)
Weight for height (z-score)	-0.23(-0.41 to -0.10)	-0.24(-0.47 to -0.02)	-0.24(-0.38 to -0.02)
Height for age (z-score)	-1.90 (-2.65 to -0.76)	-1.91 (-2.79 to -1.02)	-1.95 (-2.76 to -0.94)
World Health Organisation Clinical Staging			
1 or 2	28	25	53
3	99	95	194
4	38	39	77
CD4 (% lymphocytes)	20.4(14.3 – 28)	19.0(12.1 – 25.2)	19.96(13.6 – 26.9)
On HAART:			
At enrolment	8.5% (14)	8.8% (14)	8.6% (28)
Total received HAART during study period	63.0% (104)	64.8% (103)	63.9% (207)

Figures are medians (interquartile range) for continuous variables, or percentage (number) for categorical variables

HAART, highly active antiretroviral therapy

No P-values for pairwise comparisons of baseline characteristics at randomisation were significant

Table 2: Pathogenic bacteria and antimicrobial resistance.

Pathogens	Number (%) (N=47)	Number resistant to selected antibiotics / Total number tested (%)
Gram Positive		
S. pneumoniae	15 (32 %)	CTX - 2/2 (100%) Penicillin / Ampicillin - 7/14 (50%) Erythromycin - 2/9 (22%) Cefotaxime - 1/10 (10%)
S. aureus	5 (11 %)	CTX - 5/5 (100%) Cloxacillin - 4/5 (80%) Vancomycin - 0/5 Ciprofloxacin - 0/5 Clindamicin - 0/5 Erythromycin - 4/5 (80%)
Group G streptococcus	1 (2 %)	Penicillin - 0/1
Streptococcus sanguinis	1 (2 %)	Penicillin - 0/1
Total Gram positive	22 (47%)	
Gram negative		
***Enterobacteriaceae* ***		CTX – 11/13 (85%)
Klebsiella sp	7 (15 %)	Gentamicin 7/11 (64%)
Citrobacter freundii	2 (4 %)	Amikacin 1/12 (8%)
E. coli	3 (6 %)	Ceftriaxone** – 7/12 (58%)
Enterobacter cloacae	1 (2 %)	Ciprofloxacin – 2/10 (20%)
		Imipenem / Meropenem - 0/9
Shigella flexneri	1 (2 %)	* Antibiotic resistance for *Enterobacteriaceae* shown for group, not individual organisms **Resistance to ceftriaxone denotes ESBL production

Pathogens	Number (%) (N=47)	Number resistant to selected antibiotics / Total number tested (%)
Other Gram Negative		
Acinetobacter sp	4 (9 %)	CTX 2/4 (50%) Gentamicin 0/4
Campylobacter species	1 (2 %)	Ceftriaxone 0/1 Ciprofloxacin 1/1 (100%)**
Gram negative bacillus	1 (2 %)	CTX 0/1 Ceftriaxone 0/1
Haemophilus influenzae	3 (6 %)	CTX– 3/3 (100%) Ampicillin - 0/3
Pseudomonas aeruginosa	2 (4%)	CTX - 2/2 (100%) Amikacin 1/2 Ceftazidime - 1/2 (50%) Piptazobactam 0/2 Meropenem – 0/2
Total Gram negative	25 (53%)	
Total	**47 (100%)**	

* Antibiotic resistance for *Enterobacteriaceae* shown for group, not individual organisms

** Sensitivity test for Campylobacter based on laboratory-specified criteria

Table 3: Incidence rates* of bacteraemia by prophylaxis regimen, age category and HAART exposure time.

	Person time (person years)	Number of bacteraemias	Incidence Rate (per 100 person-years)	Incidence rate ratio (95% CI)	p-value
HAART exposure:					
Not on HAART	306.3	26	8.5	1	-
≤ 3 months on HAART	43.2	14	32.4	**3.82 (1.84 – 7.58)**	**0.0002**
> 3 months on HAART	321.9	7	2.2	**0.26 (0.09 – 0.61)**	**0.0005**
Age:					
< 12 months	30.47	15	49.23	1	
≥ 12 months	640.86	32	4.99	**0.10 (0.05 – 0.20)**	**<0.0001**
Prophylaxis regimen:					
Daily	318.31	13	4.08	1	
Thrice weekly	353.02	34	9.63	**2.36 (1.21 – 4.86)**	**0.006**
Total study	**671.33**	**47**	**7.00**	-	-

* Rates are unadjusted crude rates calculated by number of bacteraemias over exposure time

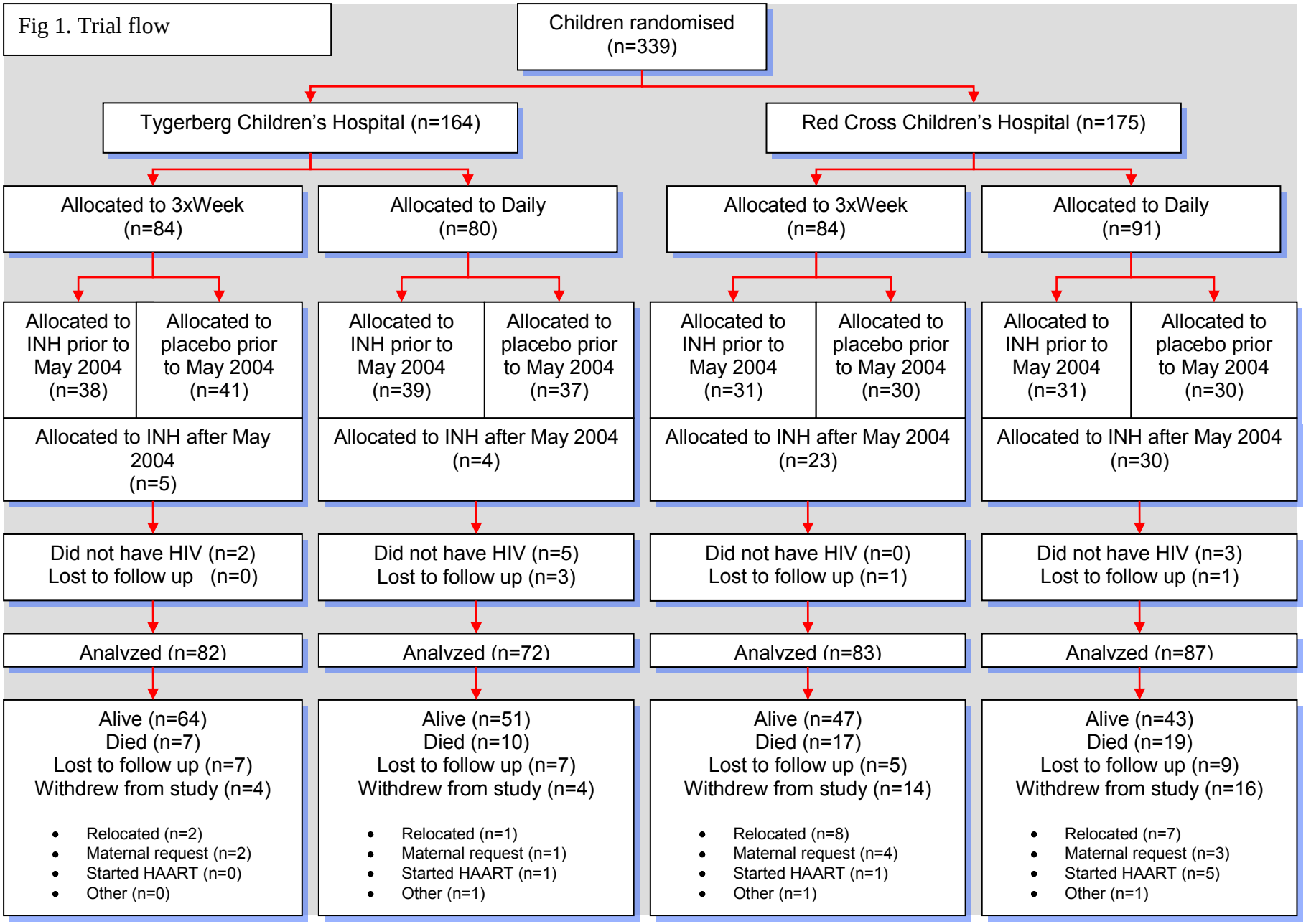
Table 4:

Incidence rate ratio of bacteraemias: unadjusted and adjusted, from negative binomial regression

	Unadjusted Incidence Rate Ratio	95% CI	p-value	Adjusted Incidence Rate Ratio*	95% CI	p-value
Prophylaxis regimen:						
Daily	1	-		1	-	
Intermittent	2.44	0.92 – 6.46	0.07	2.33	0.87 – 6.24	0.09
Age:						
Age <12 months	1			1		
Age ≥12 months	**0.23**	**0.08 – 0.63**	**0.005**	**0.16**	**0.05 – 0.48**	**0.001**
HAART exposure:						
Not on HAART	1			1		
HAART <3 months	1.30	0.47 – 3.66	0.61	2.25	0.87 – 5.87	0.10
HAART >3 months	**0.10**	**0.02 – 0.43**	**0.002**	**0.18**	**0.04 – 0.83**	**0.03**
Weight-for-age Z score	**0.51**	**0.36 – 0.74**	**<0.0001**	**0.70**	**0.49 – 1.02**	**0.06**

* Adjusted for study site and WHO clinical category at enrolment

Fig 1. Trial flow



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Appendices

1) Letter to Prof Zar
2) Ethics approval letter
3) Ethics renewal
4) Examiners reports
5) Response to examiners
6) Instructions for authors: Pediatric Infectious Disease Journal

UNIVERSITY OF CAPE TOWN

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01 June 2009

REC REF: 250/2009

Prof H Zar
Paediatrics
Red Cross Children's Hospital

Dear Prof Zar

PROJECT TITLE: IMPACT OF ISONIAZID PREVENTATIVE THERAPY ON INCIDENCE OF BACTERAEMIA IN A COHORT HIV-INFECTED CHILDREN IN SOUTH AFRICA: A SECONDARY ANALYSIS OF EXISTING DATA.

Thank you for submitting your study to the Research Ethics Committee for review.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study.

Approval is granted for one year till the 05th June 2010.

Please submit an annual progress report if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close our file.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

Signed by candidate

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

sariefdien

From: Dianne Pryce <Dianne.Pryce@uct.ac.za> Tuesday - August 10, 2010 2:38 PM

To: David Le Roux <David.LeRoux@uct.ac.za>

Subject: Approval of Study Proposal

Dear Mr Le Roux

Candidature approval (LRXDAV004)

Degree	MMed in Paediatrics
Title	Impact of isoniazid preventative therapy on incidence of bacteraemia in a cohort of HIV-infected children in South Africa: a secondary analysis of existing data
Department	Child & Adolescent Health
Supervisor	Prof H Zar
Ethics Approval	250/2009

I am pleased to advise that the Chair of the Dissertations Committee has approved your candidature for the above degree on behalf of the Committee. Formal approval was obtained by publication in the Dean's Circular, Med05/2010.

Sincerely

Dianne Pryce (Mrs)
Postgraduate Administrative Officer
Room N2.19, Wernher & Beit Building North
Health Sciences Campus
Anzio Rd, Observatory, 7925
Tel: 021 406 6809
Personal Fax: 086 505 3830

Comments:

Title: Incidence of bacteraemia in HIV-infected children in Africa, and the impact of highly active antiretroviral therapy.

I do not think negative findings of the study to asses the influence of INH should be seen in a negative light, in fact this adds to the understanding of the mortality reduction seen in the BMJ paper. As such I therefore do not think the literature review necessarily add to the value of the work presented.

The implications of the findings for practice are not fully explored.

Title: Bacteraemia in HIV-infected children in Africa: a literature review.

1) Background

- Bacteraemia is not the most extreme form of invasive infection
- References in the basic immunology section references are not to papers where these findings are made but rather to papers where the findings are referenced.
- Discussing the role of vaccination and vaccine responses in HIV-infected children is appropriate and access to vaccines in Africa can also be addressed.

2) Objectives

- Given the lack data the objectives regarding HIV-infected children is unrealistic.

3) Search strategy

- Define age cut of for child
- Why exclude low HIV prevalence studies?
- There are studies not on this description that describe the risk of bacteremia in children <3 months on cART.

4) Results

- Discuss each study

5) Discussion

- The term "bacterial sepsis " should be defined as sepsis is a distinct entity from bacteraaemia. Death in bacteraemic patients is not necessarily due to sepsis.

6) References

- There are errors in the references formatting.

Title: Bacteraemia in a cohort of HIV-infected children from Cape Town, South Africa

1) Introduction

Minor

- Add an additional comment for the decrease in mortality with use of cART. In the NEJM paper Violari *et al* access to cART was timely and controlled and most importantly in young children. I therefore think an additional reference for older children and those under program circumstances in Africa is appropriate.
- I would also mention that cART equally effective in African children with a mention as to the expected differences in response with the appropriate references.
- Comment on the effectiveness of blood culture to detect bacteraemia in children.

Major

- Comment on the vaccination schedule at the time as well as the response of HIV infected children to routine vaccinations. Add the appropriate references.

2) Methods

General

Minor

- There is inconsistent use of Tygerberg Hospital and Tygerberg Children's Hospital. May I suggest Tygerberg Children's Hospital (TCH).

Medication

Minor

- Describe the dosing accuracy of the trimethoprim-sulfamethoxazole better, ie 5mg/kg - was this given as a single daily dose, was the volume rounded up and was the total dose per week the same for daily and 3 times per week? State clearly that weight and or age band categories where not used.

Bacteriology

Minor

- Uncomplicate by stating that cultures where performed at treating clinician's discretion
- Remove the word "secondary " from the sentence on admitting hospitals unless you are sure that children where never in a primary level hospital.
- You are reporting some data on resistance in the results section, please mention how/why those antibiotics where chosen

Major

- Since you made no distinction between hospital and community infections and did not investigate the indications for admission ie pneumonia vs endocarditis you cannot exclude isolates as contaminants with out further justification or altering the definition. An example of erroneous exclusion could be coagulase-negative staphylococcus in a child with a central catheter or a thrombophlebitis. Correcting is essential.

Statistical analysis and sample size

Minor

- Reference the clinical and immunological staging and nutritional status assessment documents.

Major

- State how Z-scores where calculated and which standards where used. State what is considered under weight and moderate and severe malnutrition.
- The CDC method for immune classification was used. I do realize that this was also the method used in the main paper but since this an independent analysis WHO staging is better and should be used. This is particularly important in children < 12 months where the CDC staging system may be under appreciating the level of suppression the most. I will make further comments in the results section. Correcting is essential.

3) Results

Baseline characteristics

Minor

- States the that "most children where under weight at enrolment" please clarify that this is **under weight for age** . Also this is in contradiction with the statement that 38% of children had WAZ -2. Please see comment in the methods section as well.

Major

- Please look at my comment on the CD4% / count use. 30.3% of children enrolled where < 12 months of age and 26.7% of children where immune stage 1 with a median CD4% of 19.9%. Clearly there may be discrepancy in the data brought on by age related classification – in contrast only 11% where clinical N or A. Though clinical staging can probably not be adjusted in retrospect I think it is imperative that the immune classification be corrected and or that the author address this issue satisfactory prior to the approval of the thesis.

Table 1

Minor

- Add the word "trimethoprim-sulfametoxazole" after daily to improve clarity of the title.

Major

- See comment on CD4 Staging in the results section
- Add a comparator statistic to illustrate lack of difference in the groups

Blood stream infections

Minor

- See previous comment of the definition of contamination.
- In the paragraph at the bottom of page 5 - at the moment you are addressing enterobactereaceae and pseudomonas resistance in the same sentence when you are addressing the aminoglycoside sensitivity profile, this may be confusing.
- Where the H. Flu type B?

Major

- See comment on CD4 Staging in the results section
- Add a comparator statistic to illustrate lack of difference in the groups

Table 2

Minor

- Enterobactereaceae table has formatting error
- Indicate where ESBL findings would be appropriate
- Indicate aminoglycoside and piptazobactam resistance with pseudomonas

Mortality

Minor

- Do you have information on the indication cause of death in children? Given the resistance patterns where there errors in antibiotic choice that may have contributed to death?
- Is it appropriate to compare children bacteraemia to all children? Given the limitations of detecting bacteraemia it may be inappropriate to state that children with bacteraemia had higher mortality than those with out.

Predictors of bacteraemia

Major

- To assess the possibility of cART being associated with bacteraemia - ie a form of IRIS comparing children 3 months prior to initiation with 3 months after initiation. This remains a question despite your findings.

Table 3

Minor

- Add the prophylaxis regimen in the title and subtitle

Table 4

Minor

- Add data in CD4

3) Discussion

Major

- The difference in gram-negative and gram-positive organisms with age is very interesting especially the lower s. pneumo in younger "theoretically" more susceptible age group. It also seems to be in contrast with some prior findings from South Africa. Can you compare with existing data on pneumococcal disease in younger children?
- Also given the resistance patterns of the gram negative organisms why is there a difference between the 2 regimens?
- Discuss the vaccination responses -Where the H. Flu Type B?
- Can you add comment on the implications of resistance for antibiotic choices and practice?
- The discussion on possible IRIS should include data on hospitalization in the 1st 3 months after initiation and the previously reported increased bacteraemia in those patients.

4) References

- There are errors in the references formatting.

Report on Dissertation of Dr David Le Roux for MMed in Pediatrics, University of Cape Town

Introduction

This dissertation, which is concerned with bacteremia in HIV-infected children, consists of two sections: 1. a review of the literature of bacteremia in African children with HIV infection; 2. a study of bacteremia in HIV- infected children in Cape Town.

Literature review:

The method by which the literature reviewed was chosen is well-described, as are the limitations posed by the diversity of studies. This is a difficult type of review to perform because the present author was seeking information which might not have been the main emphasis of the study reviewed, and, therefore, might not have been presented in a manner easy to extract.

Presentation of the data: this is done in a table and commented upon in the text.

Abbreviations should be explained, and one term should be used throughout, e.g sometimes "median" is used, sometimes "med".

The table should be arranged such that one row is not split over two different pages.

Data:

The number of episodes of bacteremia are derived from different types of studies: those in which all bacteremias in all children were recorded, with HIV-positive and negative children considered separately; those in which all bacteremias in only HIV-infected were studied, those in which bacteremia due to a specific organism (*Streptococcus pneumoniae)* in only HIV-positive children was studied, and those in which bacteremia due to specific organisms in HIV-infected and uninfected children was studied (*Shigella* and *Neisseria meningitidis*).

This variety makes it impossible to analyze the data together.

However the author has drawn reasonable conclusions.

Comments:

1.I count a total of 919, not 989 cases of bacteremia in HIV-infected children.

2.The use of epidemiologic terms:

a) In the study by Berkley et al (reference 2, or 11, which are the same), the subjects were not followed over a period of time. The incidence is estimated based on an estimate of the total population, and is expressed as cases per **100,000 persons per year,** not as cases **per 100,000 person-years.**

b) Mortality is generally is an expression of number of deaths per population, e.g per 1000. What is described in the table is presumably proportion of children with bacteremia who died (since the population size was not known). The term for this is **case fatality rate**.

3. Actual data (Table 1): it is not clear to me why, in reference 15, there were 69 HIV-infected patients, and the denominator for bacteremia in HIV-infected children was 149; similarly, in the data from reference 16, there were 152 HIV-infected children, and yet the denominator was 160.

Reference 16: what does IMCI mean?

4. the case fatality rates (and the numbers from which these were derived), should be provided in the last column of the table.

5. There is no comment on sickle cell disease as a risk factor for bacteremia in children in Africa. Although this is rare in South Africa, it is prevalent in central and west Africa.

Bacteraemia in a cohort of HIV-infected children from Cape Town, South Africa
This study entails an analysis of data of children who had been enrolled in a study to evaluate the benefit if isoniazid (INH) prophylaxis for HIV-infected children. The present study does not address the INH prophylaxis. It addresses rates of bacteremia in two groups of children, namely those receiving cotrimoxazole prophylaxis daily, and those receiving the same drug three times per week. Risk factors (here referred to as predictors, a term with which I do not agree) for developing bacteremia are examined, namely age, nutritional status, category of severity if HIV infection, and receipt of highly active antiretroviral therapy (HAART).

Methods: these are well-explained; the statistical methods are appropriate.

Results: these are presented in tables, which are clear.

Findings: these are similar to those of other studies of bacteraemia in HIV-infected children, namely increased rates in infants, and in children with malnutrition. In addition, children who had not received highly active retroviral therapy (HAART) or had received it for less than three months, were at an increased risk of bacteraemia. Intermittent (three times per week) cotrimoxazole prophylaxis was associated with a higher rate of bacteraemia than daily prophylaxis. Although the children receiving HAART for fewer than 3 months had a higher risk of bacteraemia than those not receiving HAART at all in an unadjusted comparison, when adjusted for immune status at enrollment, this difference did not reach statistical significance. This is appropriately addressed in the discussion.
The high rate of antibiotic resistance among Gram-negative rods and possible reasons for this are discussed, namely hospitalization (i.e. that these infections might have been hospital-acquired), malnutrition, and HIV infection. It would be of interest to know what the rates of resistance are among such organisms in patients in the same community, but who did not have these risk factors.
The limitations have been made clear and the conclusions are justified and important. I think that a major conclusion, namely that HIV-infected infants receive HAART as early as possible, should be emphasized.

Questions and comments:
1. Blood stream infections (page 5): the numbers do not add up:
361 blood cultures taken
- 27 contaminants
- 2 fungi
-288 negative
= 44
However there were 47 episodes of bacteremia, and none of the isolates described in Table 2 were fungi.

2. Page 5: Regarding antibiotic resistance: it is stated: "40% of the *S. pneumoniae* isolates were of intermediate resistance to penicillin and ampicillin;" However Table 2 indicates that 50% of isolates were resistant to penicillin or ampicillin. It would be useful to have the details of the degree of resistance for this organism reported, because penicillin or ampicillin can be used to treat patients with infection caused by intermediately resistant isolates, unless there is meningitis.

3. Page 6: Characteristic of children with bacteraemias:
It is stated that there were similar numbers of Gram positive bacteraemias in both groups. I disagree with this statement. The percentages given do not reflect the proportion of patients with Gram-positive bacteraemia, but the proportions of Gram-positive bacteraemias in the different groups, which is not useful information. When calculated, 14/165 (8.5%) of children receiving intermittent therapy and 8/159 (5%) of children receiving daily therapy had Gram-positive bacteraemia. Although these proportions are not statistically different (chi-square 1.5, p value 0.2), they are not similar.
Table 1: this describes the children at the time of entry into the study. I think that a column with p values for each variable should be added, to demonstrate that the groups were comparable (or otherwise) at the time of entry.
Although the text reports different distributions of different organisms in children less than and older than 1 year, the details are not shown in the table. Furthermore, although table 2 indicates that there were a total of 15 isolates of *Streptococcus pneumoniae*, and 2 of other streptococci, the text (page7) describes 9 Streptococcus species in the second year of life, and 7 cases of specifically *Streptococcus pneumoniae* after the second year of life. This is confusing. I conclude that most of the "Streptococcus species" isolates were *Streptococcus pneumoniae.*

4. Table 2: Although an asterisk indicated that the susceptibilities for the Enterobacteriaceae are for the group, it is located on the next page. Therefore I think that these susceptibilities should be shown below this group of organisms, not alongside it.

5. Although INH treatment was not the subject of this study, before May 2004 some of the children received INH and some did not. Did INH have any influence on the risk of bacteraemia?

6. The author refers to "we", which implies that there were collaborators. I believe that collaborators should be named, and that their contribution to the work should be stated.

Summary Comment:
This is a very nice study of bacteremia in HIV-infected children, of which the data has been thoroughly analyzed. It should be submitted for publication in the medical literature.

MMed Thesis: Bacteraemia in HIV-infected children in Africa, and the impact of highly active antiretroviral therapy

Dr David le Roux LRXDAV004

Response to examiner's comments:

A) Literature review:

1) Reviewer 1:

Background

Comment that bacteraemia is the most extreme form of invasive form of infection has been removed

References to basic immunology have been revised

Comment on vaccine effectiveness in HIV infected children and vaccine coverage in Africa added.

Objectives

Objectives have been revised to be more realistic

Search strategy

Child defined as less than 18 years

Reference to low-HIV preference studies omitted

The incidence of bacteraemia in children recently started on cART was not a major objective of the literature review, as this would have involved an extensive review of immune reconstitution inflammatory syndrome in children and adults. Thus the main focus of the literature review was on the incidence of bacteraemia in HIV-infected children in Africa, with a short description of the decreased incidence of bacteraemia post starting cART.

Results

Each study is listed and described in the table, and salient point elaborated on in the discussion.

Discussion

Ambiguous references to "sepsis" have been removed

References

References have been re-formatted

2) Reviewer 2:

Abbreviations have been explained, inconsistencies revised

Table re-formatted so that one row is not split over 2 pages

Comments

1) Total of bacteraemias revised to 919 (not 949)

2) a) Description of incidence rates in Berkley's study amended to rates per 100 000 persons per year, not person-years

b) Incorrect use of "mortality" replaced by "case fatality ratio" throughout

3) Discrepancy between number of HIV-infected children in a study and number of bacteraemias observed is due to recurrent bacteraemias: some children had more than one

episode of bacteraemia. Table 1 Column heading changed to "Number of bacteraemias" and explanation to this effect added to results
4) Case fatality ratios (and the numbers they were derived from) have been added to the last column.
5) Comment on sickle cell disease and predisposition to bacterial infection added to "Search strategy"

2) Research article:

Reviewer 1:
1) Introduction:

- Impact of HAART on mortality now includes comment on clinical trial as well as programmatic settings, and effectiveness of HAART among African children; added extra references.
- Effectiveness of blood culture to detect bacteraemia are addressed in the discussion
- Vaccination schedule and response among HIV infected children addressed in discussion

2) Methods

- General: Inconsistent use of Tygerberg Children's Hospital corrected
- Medication: Exact dose of CTX on each regimen clarified.
- Bacteriology:
    - Added statement that cultures were taken on treating clinician's discretion
    - Concept of "secondary level hospital" clarified in the text;
    - Explanation given for choice of antibiotics
    - Description about decision to classify bacteria as contaminants vs pathogens added.
- Statistical analysis
    - Description of Z scores and references added.
    - CDC immune staging replaced with WHO clinical staging

3) Results:

- Baseline characteristics:
    - Statement about children being under weight (for age) has been corrected and qualified.
    - CDC immune staging replaced with WHO clinical staging
- Table 1:
    - Changed title to "Comparison of children assigned to intermittent or daily trimethoprim-sulphamethoxazole prophylaxis regimens at randomization"
    - CDC clinical staging replaced with WHO clinical staging
    - CDC immune staging removed from Table 1
    - Comment regarding a comparator statistic (p value) to illustrate lack of difference in two groups at randomization has been added to table 1.
- Bloodstream infections:
    - Results of resistance testing re-phrased so that Pseudomonas is not confused with the Enterobactereacea.

- Comment added regarding absence of *Haemophilus influenzae* type B

Table 2:

- ESBL-producing *Enterobacteriacea* identified in the table

- Mortality
  - Comment added regarding cause of death in children who died soon after bacteraemia
  - Sensitivity of blood culture to detect invasive bacterial disease is addressed in the discussion
- Predictors of bacteraemia (Paragraph title changed to "Risk factors for bacteraemia")
  - Possibility that bacteraemia may be a form of IRIS is discussed in the discussion
- Table 4
  - Replaced CDC immune category with WHO clinical category; recalculated the adjusted incidence rate ratios.

4) Discussion

- Comment added regarding unexpected low incidence of *S. pneumoniae* bacteraemia in children aged <1 year
- Comment added on difference in incidence of Gram negative and Gram positive bacteraemia.
- Comment added regarding absence of *H influenzae type B* and response to vaccination with Hib conjugate vaccine
- Comment added regarding antibiotics and prescribing practice.
- The incidence of hospitalisation in 1st three months after starting HAART has been addressed in other publications; a detailed description of immune reconstitution inflammatory syndrome and the incidence of hospitalization is not within the scope of this article.

5) References

- Formatting revised.

2) Reviewer 2

1) Number of blood cultures, no growth, contaminant and bacteraemia corrected
2) Number of penicillin-resistant pneumococcus corrected; definition of resistance (penicillin MIC) added
3) Characteristics of children with bacteraemia

- Sentence regarding Gram positive bacteraemias in the different prophylaxis groups rephrased: there is non-significant trend towards increased bacteraemias in children receiving intermittent therapy
- Paragraph regarding age-related incidence of bacteraemia clarified, and incidence of *S pneumoniae* explained.

4) Table 2: comment regarding sensitivities of *Enterobacteriaceae* moved to be on same page as the antibiotic descriptions.

5) Influence of INH on bacteraemia: top of page 9 – there was no difference between the placebo and the INH groups.
6) Collaborators named and their contributions identified

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Journal article

1. Trujillo M, Correa N, Olsen K, et al. Cefprozil concentrations in middle ear fluid. *Pediatr Infect Dis J*. 2000;19:268 –270.

Book chapter

2. Grose C. Bacterial myositis and pyomyositis. In: Feigin RD, Cherry JD, eds. *Textbook of Pediatric Infectious Diseases*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 1998:704 – 708.

Entire book

3. Nelson JD, Bradley JS. *Nelson's Pocket Book of Pediatric Antimicrobial Therapy*. 14th ed. Philadelphia: Lippincott Williams & Wilkins; 2000.

Proceedings

4. Harrigan PR, Dong W, Weber AE, et al. Highly mutated RT and protease [Abstract I-115]. In: 38th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, September 24 to 27, 1998. Washington, DC: American Society for Microbiology; 1998.

Online journals

5. Friedman SA. Preeclampsia. Obstet Gynecol. [serial online]. January 1988;71:22–37. Available from: BRS Information Technologies, McLean, VA. Accessed December 15, 1990.

World Wide Web

6. Gostin LO. Drug use and HIV/AIDS [JAMA HIV/AIDS web site]. June 1, 1996. Available at: http://www.ama-assn.org/ special/hiv/ ethics. Accessed June 26, 1997.

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