

Effect of cotrimoxazole on causes of death, hospital admissions and antibiotic use in HIV-infected children

Veronica Mulenga^a, Deborah Ford^b, A. Sarah Walker^b, Darlington Mwenya^a, James Mwansa^a, Frederick Sinyinza^a, Kennedy Lishimpi^a, Andrew Nunn^b, Stephen Gillespie^c, Ali Zumla^d, Chifumbe Chintu^a, Diana M. Gibb^b and the CHAP Trial Team*

Background: Cotrimoxazole prophylaxis reduces morbidity and mortality in HIV-1-infected children, but mechanisms for these benefits are unclear.

Methods: CHAP was a randomized trial comparing cotrimoxazole prophylaxis with placebo in HIV-infected children in Zambia where background bacterial resistance to cotrimoxazole is high. We compared causes of mortality and hospital admissions, and antibiotic use between randomized groups.

Results: Of 534 children (median age, 4.4 years; 32% 1–2 years), 186 died and 166 had one or more hospital admissions not ending in death. Cotrimoxazole prophylaxis was associated with lower mortality, both outside hospital ($P=0.01$) and following hospital admission ($P=0.005$). The largest excess of hospital deaths in the placebo group was from respiratory infections [22/56 (39%) placebo versus 10/35 (29%) cotrimoxazole]. By 2 years, the cumulative probability of dying in hospital from a serious bacterial infection (predominantly pneumonia) was 7% on cotrimoxazole and 12% on placebo ($P=0.08$). There was a trend towards lower admission rates for serious bacterial infections in the cotrimoxazole group (19.1 per 100 child-years at risk versus 28.5 in the placebo group, $P=0.09$). Despite less total follow-up due to higher mortality, more antibiotics (particularly penicillin) were prescribed in the placebo group in year one [6083 compared to 4972 days in the cotrimoxazole group ($P=0.05$)].

Conclusions: Cotrimoxazole prophylaxis appears to mainly reduce death and hospital admissions from respiratory infections, supported further by lower rates of antibiotic prescribing. As such infections occur at high CD4 cell counts and are common in Africa, the role of continuing cotrimoxazole prophylaxis after starting antiretroviral therapy requires investigation.

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Introduction

Clinical trials and observational studies conducted in adults and children in Africa, including Cote d'Ivoire, South Africa, Malawi, Zambia and Uganda, have reported reductions of 25–46% in mortality as well as reductions in morbidity and hospital admissions

associated with cotrimoxazole prophylaxis [1–6], even in areas of high background resistance. In 2004 results of the CHAP randomized placebo-controlled trial in Zambian children, showed a 43% reduction in mortality and a 23% reduction in hospital admissions across all ages and levels of CD4 cell percentage [7]. At the time, we briefly discussed the potential mechanisms of action

From the ^aUniversity Teaching Hospital, Lusaka, Zambia, the ^bMRC Clinical Trials Unit, London, the ^cRoyal Free Hospital, London, and the ^dUniversity College London, London, UK.

Correspondence to Dr A Sarah Walker, Medical Research Council Clinical Trials Unit, 222 Euston Road, London NW1 2DA, UK. E-mail: asw@ctu.mrc.ac.uk

* See Appendix.

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of cotrimoxazole prophylaxis, which were not entirely clear in an African country such as Zambia with high and increasing background rates of bacterial resistance to the drug (J. Mwansa, personal communication; for example, 84 and 75% resistance to cotrimoxazole of non-typhoidal salmonellae [8] and *Streptococcus pneumoniae* [9]). In particular, we stressed that *Pneumocystis jiroveci* pneumonia was unlikely in this trial of children after infancy as all except one of the nasopharyngeal aspirates (NPAs) taken from hospitalized children with respiratory infections were negative for *P. jiroveci* both by immunofluorescence and polymerase chain reaction (PCR) [7].

In this paper, we report causes of death, of hospital admissions not ending in death, and antibiotic use, amongst children randomized to cotrimoxazole prophylaxis compared with placebo in the CHAP trial.

Methods

Details of the design and main results of the trial have been published elsewhere [7]. In brief, CHAP was a randomized placebo-controlled single centre trial in HIV-infected children in Lusaka, Zambia. Between March 2001 and January 2003, 534 HIV-infected children were randomized to receive cotrimoxazole prophylaxis or matching placebo. The trial was stopped early (October 2003), after a median follow-up of 19 months due to substantial and statistically significant reductions in mortality in the cotrimoxazole group.

Where a child died in hospital, causes of death were assigned by the treating paediatrician, or if the child died before seeing a paediatrician, by trial clinical officers, based on information from hospital notes and nursing staff. Subsequently all hospital deaths were reviewed and a primary cause of death assigned by two paediatricians, blind to assignment to cotrimoxazole or placebo, who had access to case report forms, death certificates, hospital notes, post-mortem findings and laboratory data. Agreement between reviewed and originally reported causes was relatively high with the notable exception of tuberculosis (reported as a cause for 17 children, primary agreed cause in only four of 17).

Hospital admission case report forms were validated against hospital notes by an independent trial manager from the MRC Clinical Trials Unit, and a trained data monitor in Lusaka. Investigations were performed on children admitted to hospital wherever possible. These included NPA samples for children with suspected respiratory infections, blood cultures, chest X-rays, thick/thin films for malaria parasites, and cerebro-spinal fluid (CSF), urine and other specimens taken for microscopy and culture where appropriate. Children with suspected tuberculosis

had NPAs, sputa and/or gastric washings examined for acid-fast bacilli wherever possible. All NPAs were examined for *P. jiroveci* by immunofluorescence, and subsequently were tested for *P. jiroveci* using PCR in the UK. Autopsies were requested from parents or carers if a child died, but only 12 were performed. Serious bacterial infections were defined as presumptive or definitive bacterial infections of an internal organ (e.g., septicæmia, meningitis, septic arthritis, pneumonia, empyema) requiring hospitalization.

Laboratory investigation of 13 blood cultures, 34 malaria films and five CSF specimens were undertaken in the month prior to death on 40 children who were admitted to hospital and subsequently died. Ninety-eight blood cultures, 166 malaria films and five CSF specimens were investigated in the month preceding or during 187 hospital admissions on 132 children admitted to hospital who survived the admission. In total 119 NPAs were undertaken in 107 children with respiratory symptoms, most during hospital admission.

Statistical analysis

Proportions were compared by Fisher's exact tests. Days in hospital or on antibiotics were compared using the Wilcoxon rank-sum test. Cause-specific hazard ratios were estimated by Cox proportional hazards regression. The cumulative incidences of death from different causes were calculated from cause-specific hazards and overall survival curves within a competing risks framework [10]. Hazard ratios based on cumulative incidence were similar to the cause-specific hazard ratios presented (data not shown). Hospital admission rates were estimated by the total number of admissions per child-year of follow-up, censoring follow-up at death outside hospital, hospital admission if the child died as an inpatient (to avoid overlap between analyses of cause of death and cause of admission), loss to follow-up (8 weeks after last clinic visit if no longer attending) or close of the trial, whichever occurred first. Statistical significance was assessed using Wald tests.

CD4 cell count results were expressed as percentage of total lymphocyte counts. Height and weight were expressed as age-adjusted z -scores with reference to UK standards for uninfected children [11]. Baseline values and values prior to death or hospital admission were those recorded nearest to randomization or the event, but before and within 6 weeks (12 weeks for CD4 cell count). All analyses were performed using Stata version 8.2 (Stata Corp., College Station, Texas, USA).

Results

At trial entry, the median age of the 534 children was 4.4 years, with 36% under 2 years (only 4% < 1 year and all

Table 1. Mortality and hospital admissions not ending in death.

	Cotrimoxazole (n = 265)	Placebo (n = 269)	CHR or IRR (95%CI)	P
Deaths				
Hospital deaths	35 (13%)	56 (21%)	0.55 (0.36–0.84)	0.005
Deaths outside hospital	39 (15%)	56 (21%)	0.60 (0.40–0.90)	0.012
All deaths	74 (28%)	112 (42%)	0.57 (0.43–0.77)	<0.001
Hospital admissions not ending in death				
≥1 admission	76 (29%)	90 (33%)	–	0.26
Admission rate per 100 child years at risk	41.0	52.5	0.78 (0.55–1.10)	0.16
Total days in hospital per child (median, IQR)				
All children	0 (0–2)	0 (0–4)	–	0.03
Children admitted	7 (4–15)	8 (4–14)	–	0.93
Total days in hospital	881	1043	–	0.03

CHR, cause-specific hazard ratio; CI, confidence interval; IQR, interquartile range; IRR, incidence rate ratio.

over 6 months), 28% 2–5 years, 21% 6–9 years and 15% over 10 years. Overall 74 (28%) children died in the cotrimoxazole group and 112 (42%) died in the placebo group [hazard ratio (HR) 0.57; 95% confidence interval (CI), 0.43–0.77; Table 1]. As reported previously, mortality was reduced across all ages (heterogeneity $P=0.82$) and across levels of baseline CD4 cell percentage ($P=0.36$) [7]. Ninety-one children died in hospital, 92 at home, two on the way to hospital, and one in a local clinic. Children receiving cotrimoxazole had similar reductions in the instantaneous risk of hospital death [cause-specific hazard ratio (CHR) 0.55; 95% CI, 0.36–0.84] and non-hospital death (CHR 0.60; 95% CI, 0.40–0.90) compared with the placebo group (Table 1).

There were 368 hospital admissions in total (described by Chintu *et al.* [7]), of which 91 were admissions prior to death in hospital. We consider here the remaining 277 admissions in 166 children; 135 admissions in 76 children on cotrimoxazole and 142 admissions in 90 children on placebo. The rate of hospital admissions not ending in death was 41 per 100 child-years at risk in the cotrimoxazole group and 53 in the placebo group [incidence rate ratio (IRR) 0.78; 95% CI, 0.55–1.10; $P=0.16$; Table 1]. The number of days spent in hospital was higher in the placebo group (1043 days) than in the cotrimoxazole group (881 days; $P=0.03$) but individual lengths of hospital stay were similar. There was no evidence of a difference in the effect of cotrimoxazole on hospital admission rates across age at trial entry (heterogeneity $P=0.90$) or baseline CD4 cell percentage ($P=0.62$) (data not shown).

Deaths by cause

A primary cause of death was assigned for all but four of the children who died in hospital (Table 2). Serious bacterial infections, predominantly pneumonia, were the leading designated cause of death (45/91), followed by diarrhoea (12/91) and malnutrition (7/91). However, of the 91 children who died in hospital, 43 (47%) died on the day of admission or the following day: the majority of causes (45) could therefore only be determined

presumptively with only 15 having laboratory confirmation of diagnosis, and a further 27 considered clinically definitive. In particular, four of the five children who died from sepsis had blood culture results: *Haemophilus influenzae* (1), *Salmonella* spp. (1) and no growth (2). Only two of eight children who died from clinical meningitis had a definitive diagnosis (*Streptococcus pneumoniae* cultured from CSF (1); both diagnoses confirmed at autopsy).

The largest imbalance in causes of hospital death between randomized groups was pneumonia or empyema (mostly diagnosed presumptively), accounting for 10 of 35 (29%) of hospital deaths in children on cotrimoxazole compared

Table 2. Primary cause of death in hospital.

Cause of death	Deaths (with cause confirmed)		
	Cotrimoxazole	Placebo	Total
Septicaemia	3 (2)	2 (0)	5 (2) ^a
Meningitis	4 (2)	4 (0)	8 (2) ^b
Pneumonia/ empyema	10 (3)	22 (6)	32 (9) ^c
Serious bacterial infections	17 (7)	28 (6)	45 (13)
Other infections ^e	1	2	3
Tuberculosis	1	3 (1)	4 (1) ^c
Diarrhoea and/or dehydration	4	8	12
Malnutrition ^f	3	4	7
Malaria	1	1 (1)	2 (1) ^d
Anaemia	2	1	3
Other ^g	5	6	11
Not known	1	3	4
All hospital deaths	35 (7)	56 (8)	91 (15)

^aBlood cultures (*Salmonella* spp., *Haemophilus influenzae*).

^bCerebro-spinal fluid culture (*Streptococcus pneumoniae*) and post-mortem (1), post-mortem (1).

^cPost-mortem results.

^dMalaria film.

^eMeasles (2), chicken pox (1).

^fMarasmus only (1), kwashiorkor only (2), marasmic kwashiorkor (4).

^gEncephalitis (3), renal failure (2), cardiac failure (3), cor pulmonale (1), Kaposi sarcoma (1), infected burns (1).

with 22 of 56 (39%) of hospital deaths on placebo ($P=0.37$). By 2 years, the cumulative probability of dying in hospital from a serious bacterial infection was 7% in the cotrimoxazole group and 12% in the placebo group ($P=0.08$, adjusted for competing risks due to other causes of death). Corresponding probabilities for hospital death due to other primary causes were 8 and 13% ($P=0.12$); and 18 and 26% for death outside hospital ($P=0.04$). There was no evidence that mortality reductions in the cotrimoxazole group varied by cause (serious bacterial infections versus other) or place of death (heterogeneity $P=0.96$).

For the 95 children who died outside of hospital, cause of death is uncertain, and was ascertained through verbal reports from relatives by a team nurse or clinical officer who visited the home. The majority were reported to have had diarrhoea and/or dehydration (30) or respiratory problems (20); for 27 children no information was available.

Hospital admissions by cause

Diagnoses at hospital admission followed a similar pattern to hospital deaths (Table 3) with pneumonia/empyema being the most common (127 admissions). Multiple diagnoses were common, with at least two distinct diagnoses reported for 122 admissions (44%). Presumed bacterial pneumonia/empyema was frequently diagnosed with other conditions, being a concurrent diagnosis in 47, 31 and 24% of admissions for tuberculosis, malaria and malnutrition. As for cause of death, definitive diagnosis with laboratory confirmation was infrequent (Table 3). *Staphylococcus aureus* and *Salmonella* species were the main isolates from blood culture; neither *S. pneumoniae* nor *H. influenzae* was isolated from blood cultures although prevalence of carriage from pernasal swabs taken from children in a planned substudy throughout the trial was 51 and 29% respectively [9]. Only four of the 55 diagnoses of malaria at admission were confirmed by the presence of parasitaemia; in the remainder, diagnosis was based on

Table 3. Hospital admissions not ending in death.

Diagnosis	Admissions (number of children) [diagnosis confirmed]			Admission rate per 100 child-years at risk			Admissions with at least one other diagnosis ^a (%)
	Cotrimoxazole	Placebo	Total	Cotrimoxazole	Placebo	IRR (95% CI) <i>P</i>	
Septicaemia	7 (7)	7 (7)	14 (14) [11] ^b	2.1	2.6	0.82 (0.29–2.32) 0.71	14 (100%)
Meningitis	3 (3)	2 (2)	5 (5)	0.9	0.7	1.23 (0.21–7.36) 0.82	1 (20%)
Pneumonia/ empyema	56 (39)	71 (47)	127 (86) [2] ^c	17.0	26.3	0.65 (0.40–1.04) 0.07	67 (53%)
Serious bacterial infections ^f	63 (42)	77 (52)	140 (94)	19.1	28.5	0.67 (0.42–1.06) 0.09	76 (54%)
Respiratory (non-serious) infections	22 (19)	20 (16)	42 (35) (34)	6.7	7.4	0.90 (0.45–1.82) 0.78	26 (62%)
Tuberculosis	20 (18)	27 (25)	47 (43) [4] ^d	6.1	10.0	0.61 (0.33–1.12) 0.11	41 (87%)
Malaria	32 (25)	23 (17)	55 (42) [4] ^e	9.7	8.5	1.14 (0.59–2.21) 0.70	41 (75%)
Diarrhoea and/or dehydration	26 (17)	17 (15)	43 (32)	7.9	6.3	1.25 (0.57–2.75) 0.57	25 (58%)
Other infections ^g	8 (7)	9 (9)	17 (16)	2.4	3.3	0.73 (0.27–1.99) 0.54	11 (65%)
Neurological ^h	2 (2)	4 (4)	6 (6)	0.6	1.5	0.41 (0.08–2.22) 0.30	3 (50%)
Malnutrition ⁱ	14 (13)	19 (15)	33 (28)	4.2	7.0	0.60 (0.27–1.35) 0.22	24 (73%)
Anaemia	4 (2)	5 (5)	9 (7)	1.2	1.8	0.66 (0.11–3.88) 0.64	9 (100%)
Other ^j	20 (13)	13 (12)	33 (25)	6.1	4.8	1.26 (0.54–2.97) 0.60	18 (55%)
Not known	1 (1)	3 (3)	4 (4)				
Total	135 (76)	142 (90)	277 (166) [21]	41.0	52.5	0.78 (0.55–1.10) 0.16	122 (44%)
Child years at risk	330	270					

CI, confidence ratio; IRR, incidence rate ratio.

^aDistinct diagnoses as listed in this table with the exception that 'Other' (^j) was split out into separate diagnoses; that is a child admitted for two non-respiratory infections would not be considered as a multiple diagnosis.

^bBlood cultures: cotrimoxazole group (6): 3 *Salmonella* spp., 2 *Staphylococcus aureus*, 1 *Klebsiella pneumoniae*, placebo group (5): 1 *Salmonella* spp., 3 *S. aureus*, 1 *Proteus mirabilis*.

^cBlood culture: cotrimoxazole group (1): *Klebsiella pneumoniae*; NPA: placebo group (1): *Pneumoniae jiroveci*.

^dGastric washings/sputum: cotrimoxazole group (1), placebo group (3).

^eMalaria film: cotrimoxazole group (1), placebo group (3).

^fSubtotal for admissions (children) with serious bacterial infections will not be the sum of admissions for septicaemia, meningitis and pneumonia/empyema because there were some admissions with > 1 diagnosis.

^gMeasles (1), chicken pox (7), skin (5), urinary (2), conjunctivitis (2).

^hEncephalitis (1), peripheral neuropathy (2), spasticity (3).

ⁱMarasmus only (15), kwashiorkor only (4), marasmic kwashiorkor (12), unspecified (2).

^jCardiac failure (4), arthritis/arthralgia(3), skin (2), rectal bleeding (1), epistaxis (1), auricular cyst (2), parotitis (2), oral thrush (10), fevers (1), Kaposi sarcoma (9), pleural effusion (1); three admissions with two diagnoses.

history and response to antimalarial drugs. Four of 47 cases of tuberculosis were confirmed by gastric washings (1) or sputum (3). Of 119 NPAs from 107 children with respiratory symptoms, only one had *P. jiroveci* identified on immunofluorescence. The child, aged 9, was on placebo and responded to high-dose cotrimoxazole therapy. Following recovery she received open label cotrimoxazole prophylaxis. Retrospective testing of 73 NPA samples for *Pneumocystis* by PCR were all negative.

The serious bacterial infections admission rate was 19.1 per 100 child-years at risk in the cotrimoxazole group and 28.5 in the placebo group (IRR 0.67; 95% CI, 0.42–1.06; $P=0.09$). A similar reduction in the admission rate for malnutrition was observed (IRR 0.60; 95% CI, 0.27–1.35; $P=0.22$), but this was based on smaller numbers and did not reach statistical significance. Although there was an apparent reduction in the admission rate for tuberculosis (IRR 0.61; 95% CI, 0.33–1.12; $P=0.11$), admission rates in the treatment groups were very similar restricting to the 32 admissions with anti-tuberculosis treatment recorded at the time (IRR 0.93; 95% CI, 0.44–1.95; $P=0.85$); in six of 15 admissions with no treatment recorded, pneumonia was a concurrent diagnosis.

By 2 years, the cumulative probability of first admission to or death in hospital with serious bacterial infections was 15% in the cotrimoxazole group and 25% in the placebo group ($P=0.05$) (Fig. 1). Comparative probabilities for other causes of first admission to or death in hospital were 24 and 28% ($P=0.27$); and 12 and 17% for death outside of hospital ($P=0.15$). The risk of first admission or hospital death with serious bacterial infections was 41% lower in the cotrimoxazole group, and without serious bacterial infections 28% lower (CHR = 0.59; 95% CI, 0.39–0.89 and CHR = 0.72; 95% CI, 0.50–1.04, respectively, heterogeneity $P=0.49$).

Age, CD4 cell percentage, weight and height at time of death or hospital admission

Underlying chronic malnutrition was prevalent in this group of children [7]; at trial entry height and weight-for-age were below the third centile in 78 and 67% children respectively; body mass index-for-age was less than the third centile in 22%. Although numbers were small there was a suggestion that children with lowest weight-for-age contributed more hospital deaths and hospital admissions in the placebo group. This was largely driven by pneumonia/empyema, for which the proportion of deaths, first and all hospital admissions in children with weight-for-age scores of -4 or less was 81, 48 and 40% in the placebo group compared with 25, 27 and 30% in the cotrimoxazole group ($P=0.02$, 0.16 and 0.31, respectively). There were no differences in the distribution of age, height-for-age or CD4 cell percentage among deaths or hospital admissions between children on cotrimoxazole and placebo.

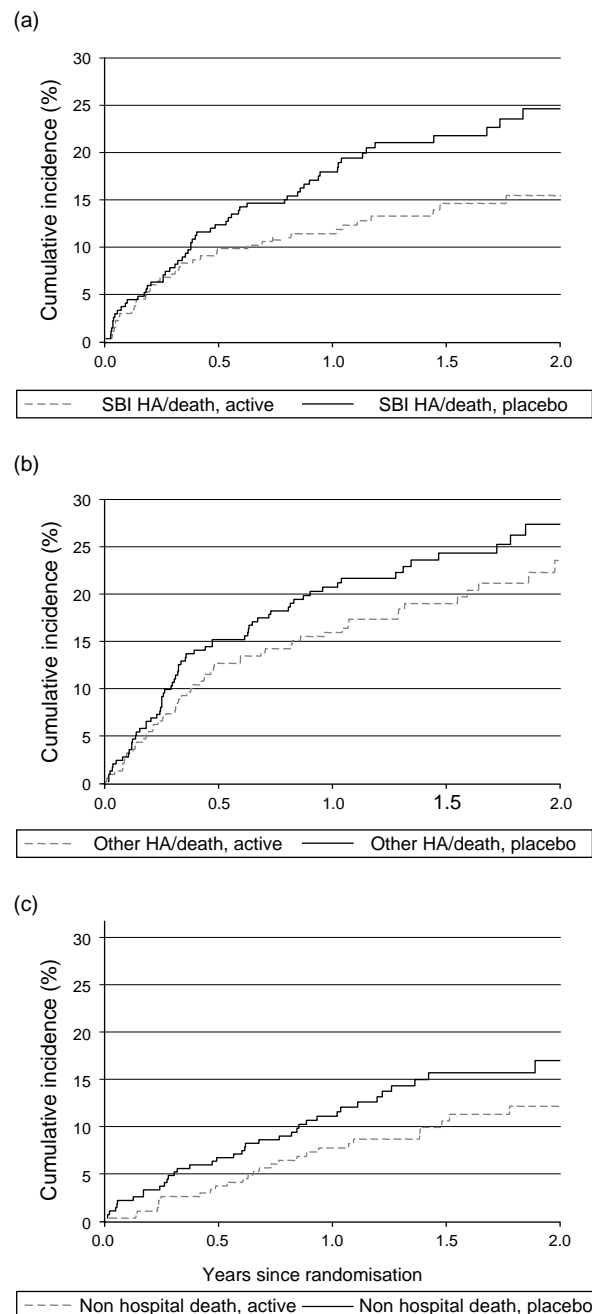


Fig. 1. Cumulative incidence of first hospital admission (HA) or death in the presence of other risks in active and placebo groups, from top: (a) hospital admission or hospital death, serious bacterial infection (SBI); (b) hospital admission or hospital death, other cause; (c) death outside hospital.

Use of antibiotics and anti-tuberculosis therapy

Most children (84%) were treated with short-term antibiotics for infections at some time during the trial. Total days on antibiotics were higher in the placebo group (7952 days) than in the cotrimoxazole group (7401 days, $P=0.68$) despite a shorter period of follow-up in the placebo group due to poorer survival. As expected, under informative censoring whereby the sickest children die

early, the difference between treatment groups was greatest in the first year of follow-up. In the first year after randomization, 91 (34%) children in the placebo group spent 10% or more of study time on antibiotics compared with 46 (17%) children in the cotrimoxazole group ($P < 0.001$). In year one, total days spent on penicillin, which was first-line antibiotic for treatment of presumed bacterial pneumonia, were significantly increased in the placebo group (1247 days, 1.7% of study time) compared with the cotrimoxazole group (843 days, 1.1%; $P = 0.001$). Treatment with penicillin in children without a respiratory-related hospital admission or death was also more common in the placebo group (565 days) compared with the cotrimoxazole group (340 days, $P = 0.004$). Use of amoxicil/ampicillin/augmentin was more similar between groups (1353 versus 1533 days) but prescribing of each of chloramphenicol, ciprofloxacin, cloxacillin, erythromycin and gentamycin was 29–51% higher in the placebo group (total 2288 days for all drugs combined for the placebo group (3.2% of time) versus 1713 days for the cotrimoxazole group (2.1%)). Finally, in children who died outside of hospital, use of antibiotics in the month prior to death was higher in the placebo group [20/56 (36%)] than in the cotrimoxazole group [6/39 (15%); $P = 0.04$]. Similar numbers of children in the placebo and cotrimoxazole groups were treated with anti-tuberculosis drugs during the trial ($P = 0.33$) and there was no evidence that days on treatment differed by treatment group ($P = 0.33$).

Discussion

In this paper, we present an analysis of likely causes of death and hospitalizations, and antibiotic use among HIV-1-infected children randomized to cotrimoxazole prophylaxis or placebo in a prospective double-blind trial in Zambia. Although many diagnoses were presumptive, this was a placebo-controlled trial and primary causes of death and hospital admission diagnoses were all assigned without knowledge of randomized group and are therefore unlikely to be biased. Overall, results from this more detailed analysis confirm our original conclusion that cotrimoxazole appears to primarily reduce death and hospitalization from lower respiratory tract infections [7]. A further factor pointing to a protective effect of cotrimoxazole prophylaxis against bacterial disease is the observation that antibiotic use in our study, particularly penicillin, was reduced in children receiving cotrimoxazole prophylaxis in spite of their longer survival.

As noted in other studies of African children [12], there were considerable difficulties in identifying definitive causes of death and hospital admissions in this study. Not only did almost half of the children die at home, but almost half of those dying in hospital died in the first 24 h,

a similar finding to other African studies [12]; many of these children did not have investigations performed in the short time they were in hospital before dying. Although a written protocol was in place for investigating children admitted to hospital in the CHAP trial, the study did not provide specific inpatient care services, and children were admitted to the same acute paediatric wards at UTH as non-trial children, where high dependency care is minimal, and overcrowding the norm in common with other paediatric wards in Africa. We ensured that investigations and treatment were free for children in the trial and they received care from members of the study team who were also following them in the trial clinic, and some of whom were also working on the routine paediatric wards. However, they received no other special care. In addition, for admissions at night and during weekends, children would not necessarily have received immediate care from study team members, but rather would have been assessed by the on-call paediatric team.

Overall 14 of 130 (11%) blood cultures grew a pathogenic organism (11 hospital admissions, two deaths and one culture not related to a death or admission). A striking feature was the lack of pneumococcal bacteraemia as *S. pneumoniae* is well recognized as a common cause of bacteraemia and pneumonia in African HIV and non-HIV infected children [12–14]. Non-typhoidal *Salmonella* is consistently one of the commonest causes of bacteraemia in children in tropical Africa [15] and *S. aureus* also occurs, particularly in older HIV-infected children with a history of chronic lung disease [13,14]; both these organisms may be easier to grow from blood culture bottles which have not been ideally handled (see below). A recent study of HIV-infected Malawian children found pneumococcus and non-typhoidal *Salmonella* to be the commonest causes of bacteraemia [5].

A possible explanation for lack of confirmed bacteraemia among children in CHAP was delayed processing of blood culture specimens. Routine laboratory services at UTH were used for the study, with the addition of further dedicated personnel to process NPAs and pernasal swabs, and equipment such as blood culture bottles to ensure an adequate supply. However, timing and handling of blood culture and CSF samples from acutely sick children may not have been ideal, particularly among those admitted at night and weekends, as transport between the paediatric wards and the laboratory could be problematic outside normal working hours. Samples were also sometimes taken after inpatient antibiotic treatment had been started, and many children were likely to have received antibiotics from local clinics before admission to hospital. In an unpublished study of 96 CSF samples from children at UTH in 2004, 40% had evidence of antibiotic activity (J. Mwansa, personal communication). Lack of sufficient blood volume could also result in low yield from blood

cultures. Of interest, the isolation rates for *S. pneumoniae* and *H. influenzae* from pernasal swabs which were transported and processed immediately after being taken at outpatient visits during the trial were not dissimilar to those reported from other African countries [9]. In contrast to acute CSF and blood culture samples, NPAs on children admitted as inpatients were undertaken by study staff on as many children with respiratory symptoms as possible, and samples examined immediately for the presence of *P. jiroveci* and stored for subsequent PCR analysis. Only one child had a positive immunofluorescence test from more than 100 tested, and even this was negative by PCR. This is consistent with the findings from an observational cohort study of Malawian children of a similar age (2–14 years) which did not identify any cases of *P. jiroveci* [16], and confirms data from industrialized countries showing that lung disease from this cause is unusual after infancy in HIV-infected children.

An alternative explanation for the lower rate of antibiotic use in the cotrimoxazole compared with the placebo group which cannot be totally excluded is that cotrimoxazole was suppressing fever due to malaria [17]. However, even though smears were undertaken routinely at outpatient visits throughout the study, as well as in any child presenting with symptoms suggestive of malaria, we observed a low rate of malaria overall (in common with some other studies in Lusaka [18]). As in the case of bacterial infections, presumptive treatment with fansidar according to Integrated Management of Childhood Illness (IMCI) guidelines would have been common at primary health facilities outside the study and may well have contributed to the low rate of observed parasitaemia.

The substantial and sustained reduction in mortality in the cotrimoxazole group in the CHAP trial may challenge the accepted relationship between efficacy and in-vitro laboratory resistance to cotrimoxazole which may be more complex than an 'all or nothing' phenomenon. There may be a different mechanism of action for cotrimoxazole as prophylaxis rather than treatment. Whereas it is well established that confirmed pneumococcal infection due to cotrimoxazole-resistant *S. pneumoniae* organisms cannot easily be treated with cotrimoxazole, its role as a prophylactic agent in preventing pneumococcal and other infections is less clear [19]. It is possible that prevention of pneumonia, which appears to be the most important effect of cotrimoxazole prophylaxis in our study, could be achieved despite the presence of resistant microflora. Of note although numbers were small, resistance levels to *Salmonella* spp. and *Staphylococcus aureus* from blood cultures, and pneumococcus and *H. influenzae* from other sources including pernasal swabs were high at around 74, 77, 76 and 57%, respectively (J. Mwansa, personal communication). Recipients of daily antibiotics replace their resident flora with resistant strains, as observed in the pernasal carriage substudy in CHAP [9], and this, in

turn, could influence the virulence and sensitivity patterns of bacteria that cause invasive disease. Several papers reporting efficacy of cotrimoxazole in adults despite high background levels of resistance have speculated about such mechanisms [20,21]. A recent paper suggesting that protection of non HIV-infected household members may occur if the HIV-infected family member takes cotrimoxazole prophylaxis might point to such a mechanism [22]. The significant reductions in total days hospitalized and death following hospitalization in spite of smaller and non-significant reductions in hospitalisations in the cotrimoxazole group observed in our study may also suggest that these children may have been less sick or had better response to treatment in hospital. We are currently undertaking detailed longitudinal serotyping studies of pernasal carriage isolates for *S. pneumoniae* and *H. influenzae* in the CHAP trial which we hope will throw further light on possible mechanisms of cotrimoxazole protection.

Bacterial infections are known to occur at relatively high CD4 cell counts [22], and even more so among children [23]. Although reductions in rates of bacterial infections have been reported among children with CD4 cell count recovery on antiretroviral therapy from well-resourced countries [24], rates are high even in HIV-negative children in resource-limited settings and therefore are likely to remain a substantial cause of morbidity for HIV-infected children even under effective antiretroviral therapy. Regardless of the mechanism of action, these data suggest that there may be a role for continuing the relatively inexpensive cotrimoxazole prophylaxis alongside antiretroviral therapy. This is in fact recommended for children under 5 years of age in recently updated WHO guidelines [25]. This question should be urgently investigated in a placebo-controlled randomized trial.

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References

1. Wiktor SZ, Sassin-Morroko M, Grant AD, Abouya L, Karon JM, Maurice C, *et al.* **Efficacy of trimethoprim-sulphamethoxazole prophylaxis to decrease morbidity and mortality in HIV-1 infected patients with tuberculosis in Abidjan, Cote d'Ivoire: a randomised controlled trial.** *Lancet* 1999; **353**:1469–1475.
2. Anglaret X, Chene G, Attia A, Toure S, Lafont S, Combe P, *et al.* **Early chemoprophylaxis with trimethoprim-sulphamethoxazole for HIV-1 infected adults in Abidjan, Cote d'Ivoire: a randomised trial.** *Lancet* 1999; **353**:1463–1468.

3. Grimwade K, Sturm AW, Nunn AJ, Mbatha D, Zungu D, Gilks CF. **Effectiveness of cotrimoxazole on mortality in adults with tuberculosis in rural South Africa.** *AIDS* 2005; **19**:163–168.
4. Badri M, Maartens G, Wood R, Ehrlich R. **Co-trimoxazole in HIV-1 infection.** *Lancet* 1999; **354**:334–335.
5. Zachariah R, Spielmann MP, Chinji C, Gomani P, Arendt V, Hargreaves NJ, *et al.* **Voluntary counselling, HIV testing and adjunctive cotrimoxazole reduces mortality in tuberculosis patients in Thyolo, Malawi.** *AIDS* 2003; **17**:1053–1061.
6. Mermin J, Lule J, Ekwaru JP, Malamba S, Downing R, Ransom R, *et al.* **Effect of cotrimoxazole prophylaxis on morbidity, mortality, CD4-cell count, and viral load in HIV infection in rural Uganda.** *Lancet* 2004; **364**:1428–1434.
7. Chintu C, Bhat GJ, Walker AS, Mulenga V, Sinyinza F, Lishimpi K, *et al.* **Cotrimoxazole as prophylaxis against opportunistic infections in HIV-infected Zambian children (CHAP): a double-blind randomized placebo-controlled trial.** *Lancet* 2004; **364**:1865–1871.
8. Mwansa J, Mutela K, Zulu I, Amadi B, Kelly P. **Antimicrobial sensitivity in Enterobacteria in AIDS patients, Zambia.** *Emerging Infect Dis J* 2002; **8**:92–93.
9. Mwenya DM, Charalambous BM, Gibb DM, Nunn A, Mwansa JCL, Gillespie SH. **Impact of co-trimoxazole on carriage and antibiotic resistance of Streptococcus pneumoniae and Haemophilus influenzae in HIV infection children in Zambia.** *Fifth International Symposium on Pneumococci and Pneumococcal Diseases.* Alice Springs, Central Australia, April 2006 [abstract PO4.12].
10. Tai BC, White IR, GebSKI V, Machin D. **On the issue of 'multiple' first failures in competing risks analysis.** *Stat Med* 2002; **21**:2243–2255.
11. Cole TJ, Freeman JV, Preece MA. **British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood.** *Stat Med* 1998; **17**:407–429.
12. Berkley JA, Maitland K, Mwangi I, Ngetsa C, Mwarumba S, Lowe BS, *et al.* **Use of clinical syndromes to target antibiotic prescribing in seriously ill children in malaria endemic area: observational study.** *BMJ* 2005; **330**:995.
13. Berkley JA, Lowe BS, Mwangi I, Williams T, Bauni E, Mwarumba S, *et al.* **Bacteremia among children admitted to a rural hospital in Kenya.** *N Engl J Med* 2005; **352**:39–47.
14. Madhi SA, Petersen K, Madhi A, Khoosal M, Klugman KP. **Increased disease burden and antibiotic resistance of bacteria causing severe community-acquired lower respiratory tract infections in human immunodeficiency type 1-infected children.** *Clin Infect Dis* 2000; **31**:170–176.
15. Graham SM, Molyneux EM, Walsh AL, Cheesbrough JS, Molyneux ME, Hart CA. **Nontyphoidal Salmonella infections of children in tropical Africa.** *Pediatr Infect Dis J* 2000; **19**:1189–1196.
16. Laufer MK, Van Oosterhout JJC, Perez MA, Kanyanganlika J, Taylor TE, Plowe CV, Graham SM. **Observational cohort study of HIV-infected African children.** *Pediatr Infect Dis J* 2006; (in press).
17. Thera MA, Sehdev PS, Coulibaly D, Traore K, Garba MN, Cissoko, *et al.* **Impact of trimethoprim-sulfamethoxazole prophylaxis on falciparum malaria infection and disease.** *J Infect Dis* 2005; **192**:1823–1829.
18. Watts TE, Wray JR, Ng'andu NH, Draper CC. **Malaria in an urban and a rural area of Zambia.** *Trans R Soc Trop Med Hyg* 1990; **84**:196–200.
19. McIntosh K. **Antimicrobial prophylaxis in children with HIV infection.** *Clin Infect Dis* 2005; **40**:146–147.
20. Anglaret X, Messou E, Ouassa T, Toure S, Dakoury-Dogbo N, Combe P, *et al.* **Pattern of bacterial diseases in a cohort of HIV-1 infected adults receiving cotrimoxazole prophylaxis in Abidjan, Cote d'Ivoire.** *AIDS* 2003; **17**:575–584.
21. van Oosterhout JJ, Laufer MK, Graham SM, Thumba F, Perez MA, Chimbiya N, *et al.* **A community based study of the incidence of trimethoprim-sulphamethoxazole-preventable infections in Malawian adults living with HIV.** *J Acquire Immune Defic Syndr* 2005; **39**:626–631.
22. Mermin J, Lule J, Ekwaru JP, Downing R, Hughes P, Bunnell R, *et al.* **Cotrimoxazole prophylaxis by HIV-infected persons in Uganda reduces morbidity and mortality among HIV-uninfected family members.** *AIDS* 2005; **19**:1035–1042.
23. Dankner WM, Lindsey JC, Levin MJ, and the Pediatric AIDS Clinical Trials Group 2001. **Correlates of opportunistic infections in children infected with the human immunodeficiency virus managed before highly active antiretroviral therapy.** *Pediatr Infect Dis J* 2001; **20**:40–48.
24. Nachman S, Gona P, Dankner W, Weinberg A, Yogev R, Gershon A, *et al.* **The rate of serious bacterial infections among HIV-infected children with immune reconstitution who have discontinued opportunistic infection prophylaxis.** *Pediatrics* 2005; **115**:e488–e494.
25. World Health Organization. **Guidelines on co-trimoxazole prophylaxis for HIV-related infections among children, adolescents and adults in resource-limited settings.** <http://www.who.int/hiv/pub/guidelines/ctx/en/index.html> Accessed 23 October 2006.

Appendix

The CHAP trial team

R. Chileshe, C. Kalengo, A. Musweu Muyawa, J. Kaluwaji, M.M. Mutengo, V. Bwalya and P. Chitambala provided counseling, clinical care and follow-up for the children and their families. M. Choongo, L. Namakube, N. Kaganson and P. Kelleher form the data entry and management team and P. Kelleher, N. Kaganson and S. Mutambo did data monitoring. L. Farelly and N. Kaganson from the Clinical Trials Unit, J. Mwansa, D. Mwenya and K. Mutela from microbiology; G Mulundu and F Kasolo from virology; M Yumbe from haematology; B Mandanda and M Mutengo from parasitology; V. Mudenda from pathology; and L. Banda, T. Chipoya and B. Chanda were support staff for the CHAP team.

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Data and Safety Monitoring Committee: T. Peto (chairman), M. Sharland, M. Quigley, and G. Biemba.