Tropical Medicine and International Health doi:10.1111/j.1365-3156.2011.02754.x

VOLUME 16 NO 6 PP 756–765 JUNE 2011

Decreasing trends of bacteraemia among HIV-infected Ugandan adults: incidence, aetiology, clinical outcomes and effect of antiretroviral therapy in a semi-urban setting (2000–2008)

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Summary

OBJECTIVE To investigate the effect of antiretroviral therapy on trends of incidence, aetiology and clinical outcomes of bacteraemia among HIV-infected Ugandans in a semi-urban setting.

METHODS A cohort of HIV-1-infected Ugandans aged 15 or older was followed from 2000 to 2008. Clinical, haematological and immunological measurements were taken at 6-monthly visits. Additionally, patients reported to outpatient clinics whenever they were ill. Patients with elevated axillary temperature above 37.4 °C consistently triggered clinical assessment (with mandatory blood cultures) and empirical management protocol. Daily cotrimoxazole prophylaxis and highly active antiretroviral therapy (HAART) were introduced stepwise to eligible patients in August 2000 and February 2003, respectively. We compared the rates of bacteraemia across five calendar periods using random-effects Poisson regression for the effect of HAART at the population level.

RESULTS A total of 246 bacteraemia episodes (including multiple episodes) were documented among 188 individuals (crude incidence: 42.4 events per 1000 person-years; 95% CI: 35.0, 51.4). The most common species isolated was *Streptococcus pneumoniae*. After adjustment for current age, clinical characteristics at enrolment (CD4+ T-cell counts and WHO stage) and time since enrolment, the incidence of bacteraemia dropped significantly when HAART was widely available compared with the period when treatment was not available (adjusted hazard ratio: 0.17; 95% CI: 0.09, 0.35). No poor health outcomes (death or lack of clinical response to antibiotics) after bacteraemia occurred after complete access to HAART.

CONCLUSIONS HAART availability in a resource-poor setting substantially reduced the trends of bacteraemia among HIV-infected adults. This may further impact on future morbidity and healthcare costs of HIV-infected people.

keywords HIV, bacteraemia, highly active antiretroviral therapy, Uganda

Introduction

Bacteraemia is a frequent complication of HIV disease. A recent systematic review of studies in Africa showed that bacteraemia contributed to between 10 and 38% of all hospitalizations of patients with fever and HIV infection was a major risk factor (Reddy *et al.* 2010). Conditions predisposing HIV-infected people to bacteraemia include abnormalities in humoral- and cell-mediated immunity, severe neutrophil depletion and dysfunction and low CD4+ T-cell counts. These conditions occur more frequently at later stages of HIV disease (Brettle 1997).

Hospital-based studies in Africa prior to the advent of highly active antiretroviral therapy (HAART) showed that bacteraemia was three times more frequent among HIV-infected than among HIV-uninfected people (Gilks *et al.* 1990; Vugia *et al.* 1993; Kassa-Kelembho *et al.* 2003; Reddy *et al.* 2010) and five times more likely to cause death among HIV-infected than among HIV-uninfected patients (Arthur *et al.* 2001; Jacob *et al.* 2009). A recent

community-based study in rural Uganda showed that bacteraemia was 22 times more frequent among HIV-infected patients than among HIV-uninfected patients (Mayanja *et al.* 2010). Furthermore, an increase in bacteraemia with previously uncommon microbes such as non-typhi *Salmonella (NTS)*, *Candida albicans, Cryptococcus neoformans* and *Mycobacterium* species was observed among HIV-infected individuals (Meyer *et al.* 1994; Gordon *et al.* 2001).

Several studies from industrialized countries (mostly conducted in hospital-based settings) showed that HAART greatly altered the epidemiology of bacteraemia in HIV-infected patients (Tacconelli *et al.* 1998; Manfredi *et al.* 1999; Tumbarello *et al.* 2000; De Gaetano Donati *et al.* 2003; Meynard *et al.* 2003; Grau *et al.* 2005; Heffernan *et al.* 2005; Kapogiannis *et al.* 2008). In these studies, HAART resulted in 57–96% reduction in bacteraemia-related morbidity and mortality. Uncommon microbes in blood, such as *Pseudomonas*, mycobacteria and non-typhi *Salmonella*, were less frequently documented after HAART introduction (Tumbarello *et al.* 2000; Pedro-Botet *et al.* 2002; Hung *et al.* 2007).

Patients in poor settings, such as sub-Saharan Africa, are more likely to have a higher burden of bacteraemia and a different spectrum of aetiologies for bacteraemia (Grant *et al.* 1997; Reddy *et al.* 2010). In these settings, the effect of HAART on the epidemiology of bacteraemia and health outcomes is not sufficiently described. Such data are still sparse from outpatient settings where community-acquired bacteraemias are more common, referral bias less frequent and where the spectrum of HIV disease severity is wider than in inpatient settings.

We evaluated the effect of HAART on the trends of incidence, aetiology and clinical outcomes of bacteraemia among HIV-infected Ugandan adults attending two community-based clinics in Uganda.

Patients and methods

Study population

The study population consisted of a cohort of confirmed HIV-1-infected Ugandans aged 15 or older, living within 40 km of Entebbe, who attended one of the two outpatient clinics. At enrolment, consenting patients had to be clinically stable (WHO clinical stages 1–3) and willing to participate in research/clinical trials and to attend 6-monthly visits and interim illness visits. This cohort was established in 1995 to explore new interventions to reduce progression to AIDS/death and to collect information on the morbidity and mortality among HIV-infected persons (French *et al.* 2000). When mortality reduced following advances in patient management and the open cohort was limited to the size of about 1000 patients, we stopped the recruitment of new participants in April 2006. However, prospective observations continued until December 2008 for the analysis reported elsewhere.

Patients were seen every 6 months for clinical, haematological and immunological evaluation. At each scheduled visit, patients were examined and classified according to the WHO clinical staging systems (WHO 1993, 2005). Blood samples were collected to determine haemoglobin level, differential white cell and CD4+ T-cell counts. The FACS-count method (Becton Dickinson, San Jose, CA, USA) measured CD4+ T-cell counts, with quality control from United Kingdom External Quality Assurance Scheme (UKNEQAS).

In addition to 6-monthly visits, patients attended clinics whenever they were ill. Additional specimens for microbiological investigations supported definitive diagnosis. If at any visit a patient had a temperature above 37.4 °C, aerobic and anaerobic blood cultures were routinely obtained whenever feasible. This procedure was consistently applied throughout the study period. Prior to April 2004, brain-heart infusion and nutrient broth bottles were used for blood cultures and subcultures were performed under 5–10% carbon dioxide on chocolate agar and aerobically on blood agar. From May 2004, BD BACTEC PLUS aerobic, anaerobic and/or BACTEC Myco/F (in case of suspected mycobacteraemia) culture bottles were used and then processed with a Bactec automated fluorescent blood culture system (BD BACTEC 9120; Becton Dickinson, BD Biosciences).

For patients with suspected bacteraemia, empirical antibiotic therapy regimens (such as parenteral ceftriaxone or penicillin plus gentamycin) and other supportive treatment (such as antipyretics and hydration) were administered by three trained study doctors and two nurses. Patients also had access to free inpatient care and radiological investigations at the nearby Entebbe hospital whenever required.

From August 2000, cotrimoxazole prophylaxis was introduced in a stepwise fashion to eligible patients at 6-monthly visits. It was fully available to most participants by March 2001 (Watera *et al.* 2006). Between October 2001 and June 2002, a conjugate pneumococcal vaccine was given to a subgroup of 109 clinically stable patients to assess its safety and immunogenicity in this population (Miiro *et al.* 2005). Before 2003, HAART was only available to a very small number of patients who managed to obtain it from private sources. HAART then became partially available to some cohort participants through the randomized development of antiretroviral therapy (DART) trial in February 2003 (Mugyenyi *et al.* 2010). Three

hundred and seventy-four participants from our cohort with CD4+ T-cell counts below 200 cells/μl joined this trial and were censored at the time of joining DART. They were prioritized for recruitment into this trial as a means of providing them with HAART at a time when it was not widely available. HAART later became widely available to patients in this cohort following the national antiretroviral therapy (ART) roll-out programme in May 2005 through The AIDS Support Organization (TASO), Entebbe hospital and other public outlets.

Definitions

Outcome

Patients with axillary temperature above 37.4 °C were categorized as having experienced a febrile event. A diagnosis of bacteraemia required at least one positive bacterial blood culture result obtained during a febrile episode in the absence of parasitic or fungal organisms. Patients were categorized as having a new (recurrent) episode if they had a positive bacterial blood culture result after completing 2 weeks of a broad-spectrum antibiotic therapy. Blood isolates of coagulase-negative staphylococci were considered to be contaminated if only a single culture yielded this organism and these were excluded from this analysis.

A poor outcome after a bacteraemia event was defined as death or lack of clinical response (persistent fever) to therapy within 2 weeks after diagnosis.

HAART eligibility was defined if one of the following conditions was met: any single WHO stage 4 event; at least two recurrent WHO stage 3 events; a single WHO stage 3 event plus a concurrent CD4+ T-cell count ≤350; or CD4+ T-cell count <200 cells/μl (MoH Guidelines November 2003).

Statistical analysis

We assessed overall and species-specific incidence rates of bacteraemia. We also assessed the rate of poor outcomes among patients with bacteraemia. We observed individuals from 1st January 2000 or the date of enrolment into this cohort (if this occurred after this date), and patients were considered to be at risk of bacteraemia until death, default, date of entry into the DART trial (Mugyenyi *et al.* 2010) or the end of this study (31st December 2008), whichever occurred first. Patients were not considered to be at risk in the 2-week period following the date of diagnosis of bacteraemia. Time at risk was defined as the time since enrolment into this cohort (a proxy measure for time since HIV seroconversion) to the time of censoring. Patients were censored if they entered the DART trial because in this study, they were monitored more intensely (every 3 months) which limited comparison with patients in our cohort.

Sociodemographic, biological and clinical characteristics of patients were compared between time periods shown in Table 1 using generalized estimating equations (Wald test), multinomial regression with robust standard errors (Wald test) and mixed effects linear regression (likelihood ratio test) for binary, categorical and continuous outcomes, respectively. The incidence of bacteraemia was compared between time periods using random-effects Poisson regression to allow for repeated episodes. Likelihood ratio tests were used to evaluate the overall effect of HAART.

Analyses stratified by eligibility for HAART were also performed to assess the effect of HAART by extent of HIV disease progression. Eligibility was assessed at each visit. A patient classified by a certain WHO stage remained in that stage unless the clinical condition worsened requiring reclassification into a higher stage.

Data were double entered using Ms Access (MS Corp, Redmond, USA) and analysed using STATA 10.1 (Stata Corp, Texas, USA).

Ethical considerations

Research approval was obtained from the Science and Ethics committees of Uganda Virus Research Institute and from the Uganda National Council of Science and Technology. Informed consent and confidentiality procedures were adhered to.

Table 1 Calendar time periods during this study

Time periods	Cotrimoxazole	HAART	Start date	End date
Pre-ART1	Partially available	Not readily available	1st January 2000	28th February 2001
Pre-ART2	Readily available	Not readily available	1st March 2001	30th January 2003
Interim	Readily available	Partially available	1st February 2003	30th April 2005
ART1	Readily available	Readily available	1st May 2005	28th February 2007
ART2	Readily available	Readily available	1st March 2007	31st December 2008

HAART, highly active antiretroviral therapy.

Results

A total of 2540 patients were observed prospectively, contributed 7163 person-years observation (py). In total, 221 (9%) subjects contributed data to all 5 time periods; 131 (5%) to 4 periods; 691 (27%) to 3 periods; 825 (32%) to 2 periods and 672 (25%) to 1 period.

Sociodemographic, biological and clinical characteristics of the cohort

Table 2 shows changes in the sociodemographic, biological and clinical characteristics of patients over time. Importantly, the proportion of patients in WHO clinical stages 3 and 4 reduced by half by December 2008 and the mean

Table 2 Characteristics of patients in this study by calendar time periods

	Pre-ART1 period (*N* = 924)	Pre-ART2 period (*N* = 1056)	Interim period (*N* = 1490)	ART1 period (*N* = 1310)	ART2 period (*N* = 1243)	*P*-value
Characteristics at enrolment						
No. (%) of men	250 (27.0)	284 (26.9)	393 (26.4)	343 (26.2)	329 (26.4)	0.907
Occupation by no. (%)*						
At home	322 (34.9)	341 (32.3)	484 (32.5)	417 (31.9)	391 (31.6)	<0.001
Manual Labour	412 (44.6)	467 (44.2)	703 (47.2)	622 (47.6)	566 (45.7)	
Skilled Labour	134 (14.5)	149 (14.2)	217 (14.6)	197 (15.1)	168 (13.5)	
Unknown†	56 (6.1)	99 (9.4)	86 (5.8)	70 (5.4)	114 (9.2)	
Mean CD4 count (95% CI)‡	401 (379, 422)	395 (375, 415)	399 (384, 415)	447 (431, 464)	441 (425, 457)	<0.001
No.(%) at WHO stage 3/4§	390 (43.3)	461 (44.9)	697 (48.0)	567 (44.2)	501 (40.8)	<0.001
No.(%) eligible for HAART¶,**	297 (32.1)	341 (32.3)	464 (31.1)	301 (22.3)	274 (22.0)	<0.001
Characteristics at first visit during time period††						
Mean age in years (95% CI)‡‡	33.6 (33.1, 34.0)	34.2 (33.7, 34.7)	35.8 (35.4, 36.2)	36.9 (36.5, 37.4)	38.7 (38.3, 39.2)	<0.001
Mean time (years) since enrolment (95% CI)§§	1.5 (1.4, 1.6)	1.6 (1.5, 1.8)	1.5 (1.4, 1.6)	2.0 (1.9, 2.2)	3.3 (3.1, 3.4)	<0.001
Mean CD4 count (95% CI)¶¶	325 (305, 344)	310 (293, 328)	355 (340, 371)	398 (383, 412)	397 (384, 410)	<0.001
No.(%) at WHO stage 3/4***	257 (30.0)	304 (31.5)	354 (26.8)	268 (21.4)	176 (16.0)	<0.001
No.(%) eligible for HAART	589 (63.7)	685 (64.9)	890 (59.8)	740 (56.5)	812 (65.3)	<0.001
No.(%) Receiving HAART	1 (0.1)	2 (0.2)	47 (3.2)	231 (17.6)	586 (47.1)	<0.001
Mean duration on HAART in months (IQR)†††	–	–	–	4.1 (3.2, 4.9)	4.8 (4.0, 5.6)	0.004‡‡‡

HAART, highly active antiretroviral therapy.
*Missing occupation for four individuals each in the ART1 and ART2 periods.
†Missing data for 16, 47, 82, 45 and 39 individuals for each of the time periods, respectively.
‡Missing data for 15 20, 37, 53 and 63 individuals for each of the time periods, respectively.
§Missing data for 23, 30, 38, 27 and 15 individuals for each of the time periods, respectively.
¶Eligible for ART was categorized at the first time point during follow-up that they experienced either: (i) a single WHO stage 4 event; (ii) recurrent stage 3 events (≥2); (iii) a single WHO stage 3 event and CD4 ≤ 350; or (iv) CD4 count ≤250. Eligibility through CD4 count was only assessed at routine visits, as this outcome may be influenced by acute episodes. Once an individual became eligible for ART, they remained eligible.
**Missing data for 7, 22, 103, 99 and 86 individuals for each of the time periods, respectively.
††Either the first date of the relevant time period or the first visit of the time period, unless otherwise stated.
‡‡Missing data for 2 individuals each in the pre-ART1 and pre-ART2 periods.
§§Missing data for 1 individual in the pre-ART2 period.
¶¶Missing data for 33, 61 201, 76 and 201 individuals for each of the time periods, respectively.
***Missing data for 68, 91, 167, 59 and 140 individuals for each of the time periods, respectively.
†††Missing data for 1, 2, 47, 224 and 517 individuals, respectively.
‡‡‡*P*-value from linear regression with robust standard errors.

CD4+ T-cell count increased over time. The age of participants increased during follow-up partly because of improved survival with increased availability of HAART and partly because of the ageing of participants in the cohort.

The mean time since enrolment at the first visit up to February 2003 was 1.5 years but more than doubled by December 2008. To compensate for losses to mortality, this open cohort continued to recruit new participants up to April 2006 when recruitment stopped as the cohort was consistently above the required 1000 participants over time because of improved survival of participants.

Incidence and aetiology of bacteraemia episodes

One hundred and eighty-eight individuals experienced 246 bacteraemia episodes. Thirty subjects experienced multiple episodes (Table 3) with two subjects experiencing five episodes throughout follow-up. Accounting for repeated measurements, the crude rate over the whole follow-up was 42.4 events per 1000 py (95% CI: 35.0, 51.4). Poor clinical outcomes were experienced among 22 bacteraemia episodes (50.0 events per 1000 py, 95% CI: 24.4, 98.3). The crude rate of bacteraemia was similar among men and women (35.9 per 1000 py [95% CI: 24.9, 51.7] among men *vs.* 43.8 [95% CI: 35.1, 54.7] among women). The median age at the time for all aetiologies of bacteraemia was similar for all periods (data not shown).

The incidence and prevalence of any bacteraemia increased from the pre-ART period to the pre-ART 2 period (which coincided with peak incidence and prevalence of non-typhi *Salmonella* after which both the incidence and prevalence decreased dramatically over time (Tables 3 and 4 and Figure 1). The most common species was *Streptococcus pneumoniae*, and 103 (42%) bacteraemic episodes were a result of this pathogen over the entire period. The second most common species was non-typhi *Salmonella*. The aetiology of bacteraemia changed over time: many of the species that predominated during the pre-ART periods declined following the introduction of HAART both in absolute numbers and in relative proportions (e.g. *Streptococcus pneumonia* and non-typhi *Salmonella*). Conversely, the absolute number of less common species such as *Escherichia coli* did not decline in absolute numbers following the introduction of HAART, although this is among small numbers. However, the relative proportions of these less common bacterial species increased with time.

Effect of HAART on bacteraemia incidence

After adjustment for current age, clinical characteristics at enrolment (CD4 count and WHO stage) and time since enrolment, the rate of bacteraemia was significantly lower in the ART1 period compared with pre-ART1 [adjusted hazard ratio (AHR) 0.46, 95% CI: 0.27, 0.76]; the rate decreased further in the ART2 period (AHR 0.17, 95% CI 0.09, 0.35). All poor outcomes following bacteraemia occurred prior to complete access to HAART (Table 4).

Whilst the overall rate of bacteraemia initially increased prior to the introduction of HAART, the rate of *Streptococcus pneumoniae* bacteraemia gradually decreased over time (Figure 1), and in Table 4, it was significantly lower at all time points following the introduction of HAART

Table 3 Prevalence of bacteraemia in this nested cohort by calendar periods

	Pre-ART1 period (N = 924) n (%)	Pre-ART2 period (N = 1056) n (%)	Interim period (N = 1490) n (%)	ART1 period (N = 1310) n (%)	ART2 period (N = 1243) n (%)
Total number of events	39	100	55	39	13
No. of patients with any bacteraemia	34 (3.7)	77 (7.3)	50 (3.4)	36 (2.8)	13 (1.1)
No. of patients with >1 event	5 (0.5)	17 (1.6)	5 (0.3)	3 (0.2)	0 (0)
Individual species*					
Streptococcus pneumoniae	26 (66.7)	39 (39.0)	22 (40.0)	13 (33.3)	3 (23.1)
Non-typhi *salmonella*	5 (12.8)	36 (36.0)	13 (23.6)	11 (28.2)	1 (7.7)
Escherichia coli	3 (7.7)	3 (3.0)	4 (7.3)	4 (10.3)	4 (30.8)
Staphylococcus aureus	2 (5.1)	7 (7.0)	1 (1.8)	1 (2.6)	0 (0.0)
Mycobacterium	0 (0.0)	0 (0.0)	4 (7.3)	3 (7.7)	0 (0.0)
Other gram-negative bacteria	0 (0.0)	7 (7.0)	6 (10.9)	2 (5.1)	0 (0.0)
Other	3 (7.7)	8 (8.0)	5 (9.1)	5 (12.8)	5 (38.5)

*Some individuals experienced more than one episode per time period.

Table 4 Incidence of bacteraemia and clinical outcome over time

	No. of events (N = 2393)*	Person-years (py) observation	Rate per 1000 py (95% CI)	Crude rate ratio (95% CI)	Adjusted rate ratio† (95% CI)	P-value‡
Any bacteraemia						
Pre-ART1	38	757	52.5 (36.8, 74.7)	1.00	1.00	<0.001
Pre-ART2	93	1349	76.7 (59.6, 98.8)	1.46 (0.99, 2.16)	1.40 (0.94, 2.08)	
Interim	55	1541	45.8 (33.7, 62.1)	0.87 (0.56, 1.37)	0.89 (0.56, 1.40)	
ART1	36	1900	23.5 (16.4, 33.7)	0.45 (0.27, 0.73)	0.46 (0.27, 0.76)	
ART2	13	1616	9.9 (5.6, 17.5)	0.19 (0.10, 0.37)	0.17 (0.09, 0.35)	
Streptococcus pneumoniae						
Pre-ART1	25	757	33.3 (21.4, 51.9)	1.00	1.00	0.001
Pre-ART2	38	1349	29.8 (20.4, 43.4)	0.89 (0.53, 1.51)	0.89 (0.52, 1.51)	
Interim	22	1541	17.0 (10.7, 27.0)	0.51 (0.28, 0.95)	0.54 (0.29, 1.00)	
ART1	13	1900	7.7 (4.3, 13.6)	0.023 (0.11, 0.47)	0.25 (0.12, 0.52)	
ART2	3	1616	2.1 (0.7, 6.5)	0.06 (0.02, 0.21)	0.07 (0.02, 0.24)	
Non-typhi *salmonella*						
Pre-ART1	5	757	7.3 (2.9, 18.3)	1.00	1.00	
Pre-ART2	34	1349	29.3 (19.1, 44.9)	4.00 (1.54, 10.39)	4.15 (1.59, 10.85)	0.001
Interim	13	1541	11.0 (6.0 20.2)	1.50 (0.51, 4.38)	1.66 (0.57, 4.88)	
ART1	11	1900	7.1 (3.7, 13.6)	0.98 (0.32, 2.95)	1.14 (0.37, 3.48)	
ART2	1	1616	0.8 (0.1, 5.4)	0.10 (0.01, 0.90)	0.12 (0.01, 1.06)	
Outcome following bacteraemia§						
Pre-ART1	5	108	46.5 (19.3, 111.6)	1.00	1.00	0.001
Pre-ART2	13	175	74.2 (43.1, 127.8)	1.60 (0.57, 4.48)	1.60 (0.57, 4.48)	
Interim	4	132	30.2 (11.3, 80.5)	0.65 (0.17, 2.42)	0.65 (0.17, 2.42)	
ART1	0	119	–	–	–	
ART2	0	86	–	–	–	

*Among those with complete data on current age, CD4 at enrolment, who stage at enrolment and time since enrolment. Missing data on one of these variables for 151 subjects.
†Adjusted for current age, CD4 at enrolment, who stage at enrolment (as a binary variable, 1/2 or 3/4)and time since enrolment.
‡P value for the overall effect of time period on the rate of bacteraemia from a likelihood ratio test.
§Among those with bacteraemia only.

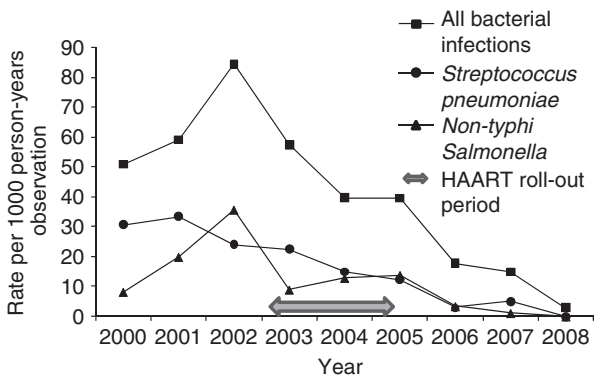

Figure 1 The incidence rate of bacteraemia over time.

(interim AHR 0.54, 95%CI: 0.29, 1.00; ART1 AHR 0.25, 95% CI: 0.12, 0.52; ART2 AHR = 0.07, 95% CI 0.02, 0.24). Similarly, the rate of non-typhi *Salmonella* decreased significantly over the time periods and was considerably lowest by December 2008 (AHR 0.12, 95% CI: 0.01, 1.06), although the number of events was very few.

As any effect of HAART on bacteraemia will only directly affect those eligible to receive it, we stratified analysis according to HAART eligibility. As expected, the rate of bacteraemia was considerably higher among eligible participants over the whole study period (adjusted rate 136.5 per 1000 py, 95% CI: 86.6, 215.1) compared with non-eligible participants (adjusted rate 29.3, 95% CI: 14.9, 57.6). Among those not eligible for HAART, very few bacteraemia events were observed prior to HAART availability and no events were observed following full access to HAART.

In Table 5, the rate of bacteraemia declined consistently following the availability of HAART and was significantly lower in the ART1 and ART2 periods compared with pre-ART1 (AHR 0.53, 95% CI: 0.31, 0.89 and AHR 0.20, 95% CI: 0.10, 0.41, respectively). A test of homogeneity provided evidence of a stronger impact among eligible participants compared with non-eligible participants (P = 0.006).

Table 5 Incidence of bacteraemia over time according to eligibility for HAART

	No. of events (N = 2393)*	Person-years observation	Rate per 1000 py (95% CI)	Crude rate ratio (95% CI)	Adjusted rate ratio† (95% CI)	P value for interaction‡
Eligible for ART						
Pre-ART1	33	517	66.5 (45.5, 97.3)	1.00	1.00	0.006
Pre-ART2	90	982	106.6 (82.0, 138.7)	1.60 (1.06, 2.43)	1.62 (1.07, 2.46)	
Interim	46	1014	58.6 (42.1, 81.6)	0.88 (0.54, 1.43)	0.95 (0.58, 1.55)	
ART1	36	1255	33.9 (23.6, 48.8)	0.51 (0.30, 0.86)	0.58 (0.34, 0.99)	
ART2	13	1172	13.1 (7.4, 23.0)	0.20 (0.10, 0.39)	0.23 (0.11, 0.47)	
Non-eligible for ART						
Pre-ART1	5	240	25.0 (10.0, 62.3)	1.00	1.00	
Pre-ART2	3	367	8.5 (2.7, 26.9)	0.34 (0.08, 1.43)	0.34 (0.08, 1.46)	
Interim	9	527	18.1 (9.1, 36.0)	0.72 (0.24, 2.23)	0.73 (0.24, 2.26)	
ART1	0	645	–	–	–	
ART2	0	444	–	–	–	

HAART, highly active antiretroviral therapy.
*Among those with complete data on current age, CD4 at enrolment, who stage at enrolment and time since enrolment. Missing data on one of these variables for 151 subjects.
†Adjusted for current age, CD4 at enrolment, who stage at enrolment (as a binary variable, 1/2 or 3/4) and time since enrolment.
‡P values for interaction between time period and ART eligibility.

Discussion

Incidence

In our semi-urban outpatient population, we show a steady decrease in the incidence of bacteraemia over time (Figure 1). The incidence of bacteraemia dropped by over 80% when HAART was fully available to patients (Table 4). The observed effect of HAART availability on bacteraemia was reproducible with analysis stratified by eligibility for HAART (Table 5). Our findings complement those from a rural Ugandan population (Mayanja *et al.* 2010), which reported a 70% reduction in the incidence of bacteraemia among HIV-infected outpatients after introducing HAART. This is expected because both studies were conducted in a similar geographical and community-based outpatient setting. HAART was also previously reported to reduce the overall incidence of malaria-associated febrile episodes from 7 to 3.1 per 100 person-years (Miiro *et al.* 2009).

The declining incidence of bacteraemia in the HAART era is consistent with observations in other settings (Tumbarello *et al.* 2000; Pedro-Botet *et al.* 2002; Mayanja *et al.* 2010). This is partly explained by the immune recovery shown by the increase in CD4+ T-cell counts following HAART (Table 2). Secondly, reduced bone marrow suppression (such as neutropaenia) following HIV viral load suppression and reduction in other opportunistic infections (such as tuberculosis that may depress the bone marrow) subsequently reduce the risk of bacteraemia.

However, we observed a peak incidence in bacteraemia (Figure 1 and Table 4) which coincided with the highest prevalence of advanced HIV disease and the lowest mean CD4+ T-cell counts in our study population (Table 2). This was largely because of an increase in the incidence of non-typhi *Salmonella*. Similar observations prior to HAART have been reported in other studies (Ssali *et al.* 1998; Arthur *et al.* 2001; Jacob *et al.* 2009; Mayanja *et al.* 2010; Reddy *et al.* 2010). During the two pre-ART periods, we did not observe any effect of cotrimoxazole prophylaxis on the incidence of bacteraemia (Tables 4 and 5) as previously reported from this cohort (Watera *et al.* 2006). This may be partly attributed to increased antimicrobial resistance to cotrimoxazole: in this cohort, 52% of pneumococcal isolates and 44% of NTS isolates were resistant to it before August 2000 (Watera *et al.* 2006). After August 2000, 89% of pneumococcal and 60% of NTS isolates were resistant to cotrimoxazole. After 2003, of the 33 pneumococcal isolates analysed in this study for sensitivity to cotrimoxazole, 32 (97%) were resistant. Likewise, 19 (95%) of the 20 NTS isolates were resistant to cotrimoxazole (data not shown). Similar observations have been reported elsewhere (Wininger & Fass 2002; Watera *et al.* 2006; Mayanja *et al.* 2010).

Aetiology

The incidence of pneumococcal bacteraemia dropped by over 90% within 4 years of providing HAART. HAART,

therefore, presents a more sustainable way of controlling the most frequent serious pathogen among HIV-positive patients than pneumococcal vaccination. Recently, a randomized trial of the 7-valent conjugate pneumococcal vaccine among HAART-naïve HIV-infected patients showed 85% protection against invasive pneumococcal disease in the first year but this effect dropped to below 25% by the second year (French *et al.* 2010). This would then require giving inaccessible multiple vaccine booster doses to increase protection against pneumococcal bacteraemia in the absence of HAART. So, HAART not only offers sustainable protection but also offers a more feasible intervention, particularly in resource-limited settings.

Consistent with what is known about bacteraemia, *Streptococcus pneumoniae* and *Escherichia coli* can be seen at any CD4+ T-cell count but NTS and mycobacterium species more commonly seen at lower CD4+ T-cell counts and they tend to disappear with improving immunological status (Tumbarello *et al.* 2000; Pedro-Botet *et al.* 2002; Hung *et al.* 2007). Prior to the advent of HAART, NTS and other species were isolated among patients with lower CD4 counts (below 70 cells/μl) at the time of infection compared with patients with higher CD4 counts who had had pneumococcal bacteraemia (data not shown). But regardless of the aetiology of bacteraemia, the median CD4+ T-cell counts after introduction of HAART in this cohort were higher than in the pre-ART periods (data not shown).

However, unlike these studies, we did not see mycobacteraemia among our HAART-naïve patients. This is most probably due to lack of diagnostic microbiology services prior to 2004. With the introduction of BACTEC Myco/F blood cultures in 2004, we saw the appearance of mycobacteraemia in the interim and early HAART era (Table 3). However, we did not observe any more cases in the later HAART period possibly because of the influence of HAART.

Mortality after bacteraemia

All poor outcomes (death or deterioration) following bacteraemia among patients in this cohort occurred prior to complete access to HAART (prior to 30th April 2005). This is consistent with HAART-naive patients, where poorer outcomes are reported among patients with more advanced HIV disease (Ssali *et al.* 1998; Arthur *et al.* 2001; Jacob *et al.* 2009). No poor outcomes were reported in the HAART era. This is consistent with other studies (Tumbarello *et al.* 2000; Pedro-Botet *et al.* 2002; Hung *et al.* 2007) that reported improved clinical outcomes during the HAART era.

Strengths and limitations

The strong points of our study are the long follow-up period with consistent procedure for obtaining blood cultures; good quality management of data and laboratory diagnosis; and having robust evidence from triangulation using stratification by eligibility for HAART.

However, our study lacked HIV-negative patient controls for comparison with HIV-infected patients. Furthermore, this population may not have been completely reflective of the true underlying population of HIV-infected patients in the community, as 374 severely immunocompromised individuals with CD4+ T-cell count ≤200 patients left this cohort to join the DART trial and we stopped following them up as per protocol. Subsequently, patients who remained in our cohort had higher CD4+ T-cell counts. It is therefore possible that the effect of HAART on bacteraemia is overestimated.

Conclusions

In conclusion, HAART considerably reduces the incidence of bacteraemia among HIV-infected patients. The aetiology and clinical outcomes of bacteraemia among HIV-infected patients may become more similar to those among the general population in the presence of HAART. This may further impact positively on future morbidity and healthcare costs of people with HIV.

Acknowledgements

The authors thank all the patients who volunteered to participate in this cohort. We also appreciate the contribution made by various staff of MRC/UVRI Uganda Research Unit on AIDS and The AIDS Support Organization (TASO) to this study. The financial support for this study was obtained from the Medical Research Council of the UK.

References

Arthur G, Nduba VN, Kariuki SM, Kimari J, Bhatt SM & Gilks CF (2001) Trends in bloodstream infections among human immunodeficiency virus-infected adults admitted to a hospital in Nairobi, Kenya, during the last decade. *Clinical Infectious Diseases* **33**, 248–256.

Brettle RP (1997) Bacterial infections in HIV: the extent and nature of the problem. *International Journal of STD and AIDS* **8**, 5–15.

De Gaetano Donati K, Tumbarello M, Tacconelli E *et al.* (2003) Impact of highly active antiretroviral therapy (HAART) on the

incidence of bacterial infections in HIV-infected subjects. *Journal of Chemotherapy* **15**, 60–65.

French N, Nakiyingi J, Carpenter LM *et al.* (2000) 23-valent pneumococcal polysaccharide vaccine in HIV-1-infected Ugandan adults: double-blind, randomised and placebo controlled trial. *Lancet* **355**, 2106–2111.

French N, Gordon SB, Mwalukomo T *et al.* (2010) A trial of a 7-valent pneumococcal conjugate vaccine in HIV-infected adults. *New England Journal of Medicine* **362**, 812–822.

Gilks CF, Brindle RJ, Otieno LS *et al.* (1990) Life-threatening bacteraemia in HIV-1 seropositive adults admitted to hospital in Nairobi, Kenya. *Lancet* **336**, 545–549.

Gordon MA, Walsh AL, Chaponda M *et al.* (2001) Bacteraemia and mortality among adult medical admissions in Malawi – predominance of non-typhi *salmonellae* and *Streptococcus pneumoniae*. *Journal of Infection* **42**, 44–49.

Grant A, Djomand G & De Cock K (1997) Natural history and spectrum of disease in adults with HIV/AIDS in Africa. *AIDS* **11**, S43–S54.

Grau I, Pallares R, Tubau F *et al.* (2005) Epidemiologic changes in bacteremic pneumococcal disease in patients with human immunodeficiency virus in the era of highly active antiretroviral therapy. *Archives of Internal Medicine* **165**, 1533–1540.

Heffernan RT, Barrett NL, Gallagher KM *et al.* (2005) Declining incidence of invasive *Streptococcus pneumoniae* infections among persons with AIDS in an era of highly active antiretroviral therapy 1995–2000. *Journal of Infectious Diseases* **191**, 2038–2045.

Hung CC, Hung MN, Hsueh PR *et al.* (2007) Risk of recurrent nontyphoid Salmonella bacteremia in HIV-infected patients in the era of highly active antiretroviral therapy and an increasing trend of fluoroquinolone resistance. *Clinical Infectious Diseases* **45**, e60–e67.

Jacob ST, Moore CC, Banura P *et al.* (2009) Severe sepsis in two Ugandan hospitals: a prospective observational study of management and outcomes in a predominantly HIV-1 infected population. *PLoS ONE* **4**, e7782.

Kapogiannis BG, Soe MM, Nesheim SR *et al.* (2008) Trends in bacteremia in the pre- and post-highly active antiretroviral therapy era among HIV-infected children in the US Perinatal AIDS Collaborative Transmission Study (1986-2004). *Pediatrics* **121**, e1229–e1239.

Kassa-Kelembho E, Mbolidi CD, Service YB, Morvan J & Minssart P (2003) Bacteremia in adults admitted to the Department of Medicine of Bangui Community Hospital (Central African Republic). *Acta Tropica* **89**, 67–72.

Manfredi R, Nanetti A, Ferri M & Chiodo F (1999) HIV-associated non-mycobacterial sepsis-bacteraemia, before and during the highly active antiretroviral therapy era. *AIDS* **13**, 1274–1276.

Mayanja BN, Todd J, Hughes P *et al.* (2010) Septicaemia in a population-based HIV clinical cohort in rural Uganda 1996–2007: incidence, aetiology, antimicrobial drug resistance and impact of antiretroviral therapy. *Tropical Medicine and International Health* **15**, 697–705.

Meyer CN, Skinhoj P & Prag J (1994) Bacteremia in HIV-positive and AIDS patients: incidence, species distribution, risk-factors, outcome, and influence of long-term prophylactic antibiotic treatment. *Scandinavian Journal of Infectious Diseases* **26**, 635–642.

Meynard JL, Guiguet M, Fonquernie L *et al.* (2003) Impact of highly active antiretroviral therapy on the occurrence of bacteraemia in HIV-infected patients and their epidemiologic characteristics. *HIV Medicine* **4**, 127–132.

Miiro G, Kayhty H, Watera C *et al.* (2005) Conjugate pneumococcal vaccine in HIV-infected Ugandans and the effect of past receipt of polysaccharide vaccine. *Journal of Infectious Diseases* **192**, 1801–1805.

Miiro G, Todd J, Mpendo J *et al.* (2009) Reduced morbidity and mortality in the first year after initiating highly active antiretroviral therapy (HAART) among Ugandan adults. *Tropical Medicine & International Health* **14**, 556–563.

Mugyenyi P, Walker AS, Hakim J *et al.* (2010) Routine versus clinically driven laboratory monitoring of HIV antiretroviral therapy in Africa (DART): a randomised non-inferiority trial. *Lancet* **375**, 123–131.

Pedro-Botet ML, Modol JM, Valles X *et al.* (2002) Changes in bloodstream infections in HIV-positive patients in a university hospital in Spain (1995-1997). *International Journal of Infectious Diseases* **6**, 17–22.

Reddy EA, Shaw AV & Crump JA (2010) Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. *Lancet Infectious Diseases* **10**, 417–432.

Ssali FN, Kamya MR, Wabwire-Mangen F *et al.* (1998) A prospective study of community-acquired bloodstream infections among febrile adults admitted to Mulago Hospital in Kampala, Uganda. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology* **19**, 484–489.

Tacconelli E, Tumbarello M, De Gaetano K, Cauda R & Ortona L (1998) Highly active antiretroviral therapy decreases the incidence of bacteremia in human immunodeficiency virus-infected individuals. *Clinical Infectious Diseases* **27**, 901–902.

Tumbarello M, Tacconelli E, Donati KG *et al.* (2000) HIV-associated bacteremia: how it has changed in the highly active antiretroviral therapy (HAART) era. *Journal of Acquired Immune Deficiency Syndromes* **23**, 145–151.

Vugia DJ, Kiehlbauch JA, Yeboue K *et al.* (1993) Pathogens and predictors of fatal septicemia associated with human immunodeficiency virus infection in Ivory Coast, west Africa. *Journal of Infectious Diseases* **168**, 564–570.

Watera C, Todd J, Muwonge R *et al.* (2006) Feasibility and effectiveness of cotrimoxazole prophylaxis for HIV-1-infected adults attending an HIV/AIDS clinic in Uganda. *Journal of Acquired Immune Deficiency Syndromes* **42**, 373–378.

WHO (1993) Proposed 'World Health Organization staging system for HIV infection and disease': preliminary testing by an international collaborative cross-sectional study. The WHO

International Collaborating Group for the Study of the WHO Staging System. *AIDS* **7**, 711–718.

WHO (2005) Interim WHO clinical staging of HIV/AIDS and HIV/AIDS case definitions for surveillance: African Region. In: WHO, G. (Ed.) who.int/hq/2005/who_HIV_2005.02.pdf.

Wininger DA & Fass RJ (2002) Impact of trimethoprim-sulfamethoxazole prophylaxis on etiology and susceptibilities of pathogens causing human immunodeficiency virus-associated bacteremia. *Antimicrobial Agents and Chemotherapy* **46**, 594–597.

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