Haematological changes in adults receiving a zidovudine-containing HAART regimen in combination with cotrimoxazole in Côte d'Ivoire

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Objective: Neutropenia is the most frequent side effect of cotrimoxazole in sub-Saharan Africa. We estimated the incidence of haematological disorders during the first 6 months of a zidovudine-containing highly active antiretroviral therapy (HAART) regimen in sub-Saharan African adults receiving cotrimoxazole.

Methods: Prospective cohort study in Abidjan, with blood cell count measurement at baseline (HAART initiation), month 1, month 3 and month 6.

Results: A total of 498 adults [baseline: 80% currently on cotrimoxazole prophylaxis; median CD4 count 237/mm^3 [interquartile range (IQR) 181;316]; median neutrophil count 1647/mm^3 (IQR 1221;2256); median haemoglobin 113 g/l (IQR 102;122)] started zidovudine (AZT)/lamivudine/efavirenz. During follow-up, 118 patients had a grade 3–4 neutropenia [(56.3/100 person-years (PY)], 23 had a grade 3–4 anaemia (9.6/100 PY) and no cases of grade 3–4 thrombocytopenia. Of the 118 patients with grade 3–4 neutropenia, 86 (73%) had to stop cotrimoxazole because neutropenia persisted, and one (<1%) had to stop AZT because of persistent neutropenia after cotrimoxazole was stopped (neutropenia-related HAART modification: 0.4/100 PY). Of the 23 patients with grade 3–4 anaemia, 11 had to stop AZT (anaemia-related HAART modification: 4.4/100 PY). In patients who stopped cotrimoxazole but not AZT, the median gain in neutrophils at 1 month was +540/mm^3 (IQR +150;+896).

Conclusions: At baseline, most patients had a normal neutrophil count and 80% of them were already receiving cotrimoxazole. An unexpectedly high rate of grade 3–4 neutropenia occurred shortly after introduction of AZT. Almost all of the persistent severe neutropenia disappeared after cotrimoxazole was stopped. This suggests an accentuated drug interaction between the two drugs in these sub-Saharan African individuals. Grade 3–4 anaemia was much less frequent, but remained the first cause of AZT discontinuation.

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Introduction

In 2000, WHO/UNAIDS experts recommended that cotrimoxazole prophylaxis should be part of the minimal package of care for HIV-infected patients in sub-Saharan Africa [1]. Neutropenia is the most frequent side effect of cotrimoxazole in sub-Saharan African HIV-infected adults [2] and has been hypothesized to be more frequent in patients receiving cotrimoxazole in sub-Saharan African or American African adults than in Caucasian adults [2,3].

Zidovudine (AZT) is one of the first-line antiretroviral drugs recommended by WHO for treating HIV-infected adults in low resource countries [4]. Now that access to highly active antiretroviral therapy (HAART) is hopefully increasing, AZT will be increasingly

prescribed in settings with limited laboratory facilities compared with industrialized countries. AZT is a well-known cause of drug-induced haematotoxicity [5,6]. The incidence of haematological disorders in sub-Saharan African adults receiving cotrimoxazole in combination with AZT has never been reported.

We report here the incidence of neutropenia, anaemia and thrombopenia during the first 6 months of an AZT-containing HAART regimen in a cohort of HIV-infected adults receiving cotrimoxazole in Côte d'Ivoire.

Methods

Patients

In December 2002, a randomized trial (Trivacan ANRS 1269 trial) was launched in Abidjan, Côte d'Ivoire, with the objective of assessing two structured treatment interruption (STI) strategies of HAART compared with continuous HAART [7]. The trial was designed in two phases. Patients were included in the first phase ('pre-randomization phase') if they met the following criteria: age ≥18 years, naive of curative antiretroviral therapy, CD4+ cell count 150–350/mm^3 and written informed consent. At baseline, all patients received a continuous standardized regimen of HAART. After at least 6 months in the pre-randomization phase, patients were randomized into one of the arms of the STI trial whenever they met the following criteria: CD4 count ≥350/mm^3, undetectable viral load and absence of current opportunistic infection. The protocol of the Trivacan trial was approved by the Ethics Committee of the Ivorian Ministry of Health and the institutional review board of the ANRS.

For the present study, only data from the pre-randomization phase were considered. Patients were included in the study if: i) they were included in the pre-randomization phase of the Trivacan trial prior to 1 August 2003 and ii) they were prescribed AZT/lamivudine (3TC)/efavirenz (EFV) at baseline. There were no exclusion criteria.

Baseline and follow-up

At baseline in the pre-randomization phase, a standardized questionnaire was used to record baseline medical and socio-economic characteristics. Patients were prescribed HAART and cotrimoxazole prophylaxis [1] and were asked to return to their study centre at day 7, at 1 month and every month thereafter. During these scheduled visits, standardized questionnaires were administered to record self-reported symptoms since the last visit, signs noticed by the physicians and self-reported adherence to treatment during the previous 4 days. Between these scheduled visits, patients had free access to the study clinics whenever they had a medical problem.

At baseline and at each of the monthly visits, subjects were given numbered boxes containing the quantity of pills for 35 days, that is, 35 pills of trimethoprim 160 mg-sulfamethoxazole 800 mg (national public health drug supplier), 70 pills of Duovir® (AZT 300 mg-3TC 150; Cipla Ltd, Mumbai, India) and 105 pills of Sustiva® 200 mg (EFV 200 mg; Merck Sharp & Dohme, Haarlem, Holland) before 1 May 2003, or 35 pills of Sustiva® 600 mg after this date. Patients were asked to return the previous box at each visit with all unused tablets and to exchange it for a new one.

Blood samples were collected at baseline, month 3 and month 6 to measure blood cell count (Coulter® MAXM; Beckman Coulter, Fullerton, CA, USA), CD4 count (True Count® technique on FACScan®; Becton Dickinson, Erembodegen, Belgium), plasmatic HIV-1 RNA (real-time PCR on Taq Man technology Abi Prism 7000; Applied Biosystems, Rotkreuz, Switzerland; threshold of detection 300 copies/ml) [8], serum liver enzymes and serum creatinine. The serum HBs antigen was also looked for in the baseline sample only. An additional sample was collected at month 1 for blood cell count only. The creatinine clearance was estimated by the Cockroft–Gault equation.

At each scheduled and unscheduled visit, signs and symptoms were managed following standardized algorithms. In cases of severe anaemia with haemoglobin under 65 g/l or with a rapid haemoglobin loss, both cotrimoxazole and AZT were stopped and AZT was replaced by stavudine. In cases of a neutrophil count under 750/mm^3 without severe anaemia, the procedure was to stop cotrimoxazole and to measure the blood cell count at each further monthly visit; AZT was stopped if the neutrophil count persisted under 750/mm^3 one month after cotrimoxazole was stopped. All care was free-of-charge.

Definitions

Grades of neutropenia were successively defined as at least one neutrophil count below: 1500/mm^3 (severity grade ≥1), 1000/mm^3 (grade ≥2), 750/mm^3 (grade ≥3) or 500/mm^3 (grade 4), respectively. For each of these four definitions, the date of the neutropenia was the date of the first blood cell count showing a neutrophil count below the corresponding threshold. Neutropenia was defined either as 'prevalent' if measured at baseline or 'incident' if occurring subsequent to the baseline measurement. Similar definitions were used for anaemia and thrombocytopenia, using the following thresholds: for anaemia, at least one haemoglobin level below 105 g/l (grade ≥1), <95 g/l (grade ≥2), <80 g/l (grade ≥3) and <65 g/l (grade 4). For thrombocytopenia, at least one platelet count below 100×10^3/mm^3 (grade ≥1), $<75\times10^3$/mm^3 (grade ≥2), $<50\times10^3$/mm^3 (grade ≥3) and $<20\times10^3$/mm^3 (grade 4).

Statistical analyses

Baseline was the date of enrollment in the pre-randomization phase. The date of study termination was the date of the month 6 visit. The incidence rate of a given disorder was defined as the number of patients with at least one follow-up value above the corresponding threshold per 100 person-years (PY) of at-risk follow-up. The follow-up period during which the patient was considered to be at risk for a given disorder began on the day of enrollment and continued to the date of study termination, death, default or the date of the first blood cell count showing a value below the corresponding threshold. The disorder-free survival probability was estimated using the Kaplan–Meier method. Univariate and multivariate Cox proportional hazards regression models for first events were used to study the association between a given incident disorder, baseline characteristics and compliance to HAART treatment during follow-up. All blood cell count results were taken into account in the analyses, including scheduled and unscheduled. For each analysis involving one of the haematological disorders as defined above, patients with 'prevalent disorder' were excluded.

Results

Patients and follow-up

As of 31 July 2003, 504 patients had started AZT/3TC/EFV. Six patients (1.2%) were excluded from the analyses because no blood cell count was available during follow-up. The main baseline and follow-up characteristics of the remaining 498 patients are shown in Table 1. At baseline, 397 patients declared that they were already taking cotrimoxazole. All but three of the remaining 101 patients started cotrimoxazole at baseline (*n*=16), at month 1 (*n*=60), month 2 (*n*=18), month 3 (*n*=2) and month 4 (*n*=2).

During follow-up, five patients died and six were lost to follow-up. The percentage of patients who declared that they had missed at least one intake of antiretroviral drugs during the previous 4 days was 11% at month 1, 14% at month 2, 11% at month 3, 10% at month 4, 11% at month 5 and 11% at month 6. At month 6, 412 patients (85%) had an undetectable HIV-1 RNA plasma viral load and the median CD4 count was 401/mm^3 [interquartile range (IQR) 314–510/mm^3].

Prevalent and incident haematological abnormalities

The 498 patients in the present study had a blood cell count available at baseline. Among the 397 patients who were already taking cotrimoxazole at baseline, 58% had a normal baseline neutrophil count, 30% had grade 1 neutropenia, 9% grade 2 neutropenia and 2% grade 3 neutropenia (vs 67%, 25%, 6% and 2% in the 101 patients who had not started cotrimoxazole before baseline, respectively; *P*=0.55). During follow-up, 1786 blood cell counts were available, including 481 (97% of patients) at month 1, 491 (99%) at month 3, 487 (98%) at month 6 and 327 (in 191 patients, that is, 38% of patients) at any other time (unscheduled measurements).

Figure 1 shows the number of patients in each category for absolute neutrophil count and haemoglobin level at the baseline, month 1, month 3 and month 6 scheduled measurements. The percentage of patients with grade 3–4 neutropenia was 2% at baseline, 15% at month 1, 11% at month 3 and 10% at month 6; the percentage of patients with grade 3–4 anaemia was 2% at baseline, 2.4% at month 1, 2.8% at month 3 and 1.6% at month 6.

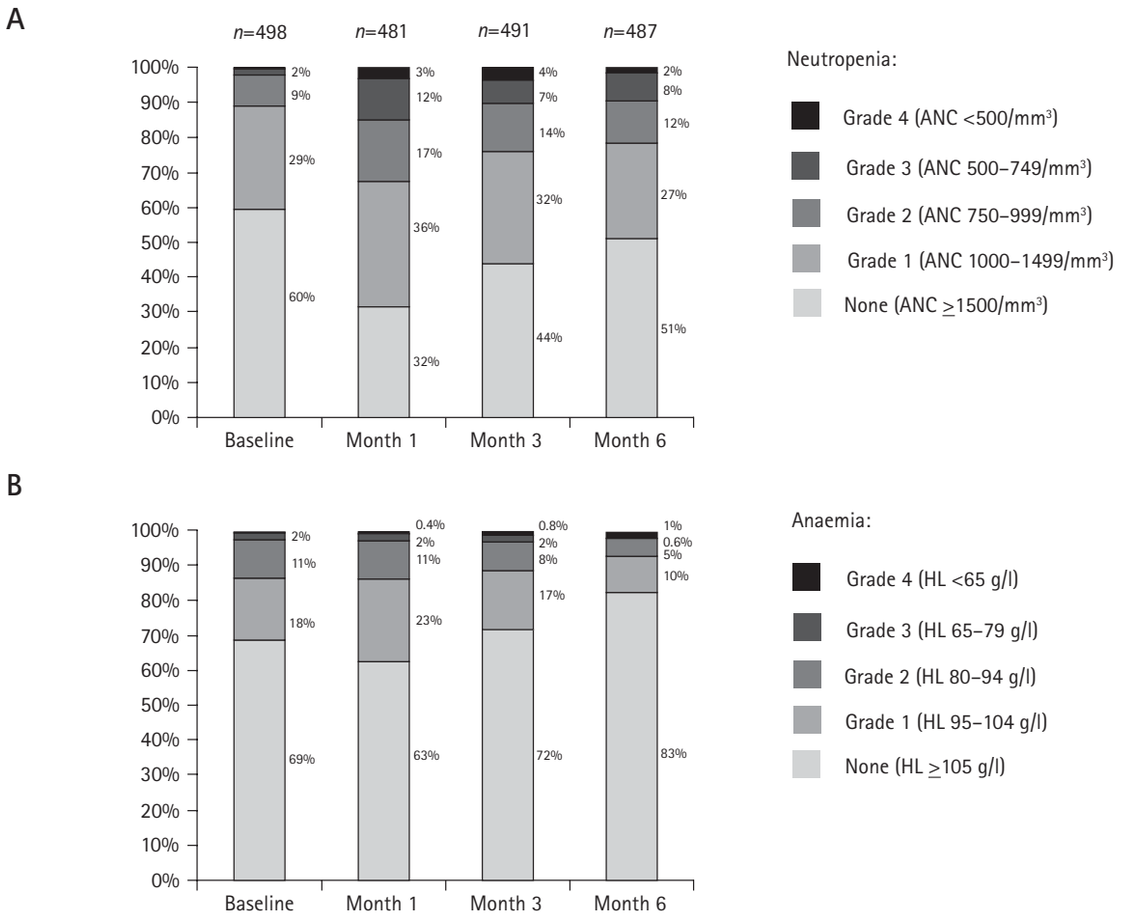
Table 2 shows the lowest values of absolute neutrophil count and haemoglobin level that were recorded during follow-up, by categories of baseline neutrophil count and baseline haemoglobin. The percentage of patients with a neutrophil count <750/mm^3 at least once during follow-up was 26% overall, 17% in patients with baseline neutrophils ≥1500/mm^3, 33% in patients with baseline neutrophils at 1000–1499/mm^3 and 46% in patients with baseline neutrophils at 750–999/mm^3. Similarly, 5% of patients had haemoglobin <80 g/l at least once during the

Table 1. Baseline and follow-up characteristics

Baseline	
Male/female, *n* (%)	137/361 (28/72)
Age, years, median (IQR)	34 (29–41)
Currently on cotrimoxazole, *n* (%)	397 (80)
Time, months, from CMX initiation, median (IQR)	12.0 (3.1–36.0)
WHO clinical stage, *n* (%)	
1	134 (27)
2	189 (38)
3	141 (28)
4	34 (7)
CD4 count, mm^3, median (IQR)	237 (181–316)
Viral load, $\log_{10}$/ml, median (IQR)	5.06 (4.49–5.54)
Neutrophil count, mm^3, median (IQR)	1647 (1221–2256)
Haemoglobin level, g/l, median (IQR)	113 (102–122)
Platelet count, ×10^3/mm^3, median (IQR)	243 (193–303)
Creatinine clearance, ml/mn, median (IQR)	104 (87–121)
Positive serum HBs antigen, *n* (%)*	71 (14)
Follow-up	
Cumulative, person-months	3046
Per patient, months, median (IQR)	6.1 (6.1–6.2)
Status on study termination	
Dead	5 (1)
Alive	487 (97.8)
Lost to follow-up	6 (1.2)

*Missing value; *n*=4; IQR, interquartile range; CMX, cotrimoxazole.

Figure 1. (A) Absolute neutrophil count and **(B)** haemoglobin level at baseline, month 1, month 3 and month 6



%, percentage of patients; ANC, absolute neutrophil count; HL, haemoglobin level.

overall follow-up. This percentage was 2% in patients with baseline haemoglobin ≥105 g/l, 2% in patients with baseline haemoglobin at 95–104 g/l and 23% in patients with baseline haemoglobin at 80–95 g/l.

Finally, Table 3 shows the incidence of neutropenia, anaemia and thrombocytopenia for each of the four thresholds defining the corresponding haematological disorder. As seen in this table, the incidence of grade 3–4 neutropenia was 56.3/100 PY (95%CI 46.2–66.5), and the incidence of grade 3–4 anaemia was 9.6/100 PY (95%CI 5.7–13.5). Of the 118 incident grade 3–4 neutropenia, only 11 were associated with a concomitant grade 3 (*n*=7) or grade 4 (*n*=4) anaemia.

Renal and liver function

The percentage of patients with creatinine clearance below 50 ml/min was 3% at baseline and 1% at month 6. The median difference in creatinine clearance between month 6 and baseline was +4.9 ml/min (IQR −10;+16.8). At baseline, 21% of patients had a transaminase value over 1.25 the upper limit of normal (ULN), including 17% with 1.25 ULN < transaminases ≤2.5 ULN, 3% with 2.5 ULN < transaminases ≤5 ULN and 1% with >5 ULN. Of the 487 patients who attended the month 6 visit, 6.6% had a transaminase value over 1.25 ULN, including 5.8% with 1.25 ULN < transaminases ≤2.5 ULN, 0.4% with 2.5 ULN < transaminases ≤5 ULN and 0.4% with transaminases >5 ULN.

Factors associated with incident anaemia and neutropenia

In the multivariate analysis, the baseline age, viral load, CD4 count, transaminases, creatinine clearance, past history of cotrimoxazole prophylaxis, WHO clinical stage, platelet count, body mass index and the adherence to treatment during follow-up were not associated with the risk of incident grade 3–4 neutropenia or grade 3–4 anaemia.

Table 2. Lowest neutrophil count and haemoglobin level during follow-up, by baseline values of neutrophil count and haemoglobin

Baseline absolute neutrophil count	Total *n* (%)	Lowest absolute neutrophil count during follow-up				
		≥1500	1000–1499	750–999	500–749	<500
≥1500/mm^3, *n* (%)	297 (100)	74 (25)	121 (41)	53 (18)	38 (13)	11 (4)
1000–1499/mm^3, *n* (%)	146 (100)	7 (5)	42 (29)	49 (34)	34 (23)	14 (10)
750–999/mm^3, *n* (%)	45 (100)	4 (9)	9 (20)	11 (24)	11 (24)	10 (22)
500–749/mm^3, *n* (%)	9 (100)	0 (0)	0 (0)	2 (22)	5 (56)	2 (22)
<500/mm^3, *n* (%)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
Overall, *n* (%)	498 (100)	85 (17)	172 (35)	115 (23)	88 (18)	38 (8)
Baseline haemoglobin	Total *n* (%)	Lowest haemoglobin during follow-up				
		≥105	95–104	80–94	65–79	<65
≥105/g/l, *n* (%)	345 (100)	255 (74)	61 (18)	20 (6)	4 (1)	5 (1)
95–104/g/l, *n* (%)	89 (100)	24 (27)	40 (45)	23 (26)	0 (0)	2 (2)
80–94/g/l, *n* (%)	54 (100)	6 (11)	18 (33)	18 (33)	10 (19)	2 (4)
65–79/g/l, *n* (%)	10 (100)	0 (0)	0 (0)	6 (60)	3 (30)	1 (10)
Overall, *n* (%)	498 (100)	285 (57)	119 (24)	67 (13%)	17 (3%)	10 (2%)

Table 3. Incidence rate of anaemia, neutropenia and thrombocytopenia

Definitions		*n*	TAR	*n**	Rate (95% CI)
Neutropenia					
Grade 1–2–3–4	(ANC <1500/mm^3)	297	69	222	319.6 (277.6–361.6)
Grade 2–3–4	(ANC <1000/mm^3)	443	157	199	126.4 (108.9–144.0)
Grade 3–4	(ANC <750/mm^3)	488	209	118	56.3 (46.2–66.5)
Grade 4	(ANC <500/mm^3)	497	239	37	15.5 (10.5–20.5)
Anaemia					
Grade 1–2–3–4	(HL <105/g/l)	345	142	90	63.4 (50.3–76.5)
Grade 2–3–4	(HL <95/g/l)	434	201	54	26.8 (19.7–34.0)
Grade 3–4	(HL <80/g/l)	488	240	23	9.6 (5.7–13.5)
Grade 4	(HL <65/g/l)	498	249	10	4.0 (1.5–6.5)
Thrombocytopenia					
Grade 1–2–3–4	(PC <100 000/mm^3)	486	243	11	4.54 (1.9–7.2)
Grade 2–3–4	(PC <75 000/mm^3)	494	249	2	0.8 (0–1.9)
Grade 3–4	(PC <50 000/mm^3)	495	250	0	–
Grade 4	(PC <20 000/mm^3)	495	250	0	–

Rate = incidence rate of the given haematological disorder per 100 PY of follow-up. *n*, number of patients included in the analyses (with no prevalent haematological disorder, that is, with baseline value of ANC, HL or PC over the corresponding threshold). *n**, number of patients with at least one value below the corresponding threshold during follow-up. ANC, absolute neutrophil count; HL, haemoglobin level; PC, platelet count; PY, person-years; TAR, time at risk.

The risk of grade 3–4 neutropenia was significantly lower in men [hazard ratio (HR) 0.61, 95% CI 0.39–0.97, *P*=0.04], higher in patients with positive serum HBs antigen at baseline (HR 1.58, 95% CI 1.00–2.52, *P*=0.05) and higher in patients with a low baseline neutrophil count. Compared with patients with a baseline neutrophil count ≥1500/mm^3, those with baseline neutrophils at 750–999/mm^3 and 1000–1499/mm^3 had an HR of grade 3–4 neutropenia of 3.24 (95% CI 1.94–5.42, *P*<0.001) and 2.31 (95% CI 1.55–3.44, *P*<0.001), respectively.

The only factor associated with the risk of grade 3–4 anaemia was low baseline haemoglobin. Compared with patients with baseline haemoglobin ≥105 g/l, those with baseline haemoglobin at 80–94 g/l and 95–104 g/l had an HR of grade 3–4 anaemia of 8.61

(95% CI 3.60–20.62, $P<0.001$) and 0.74 (95%CI 0.16–3.45, $P=0.69$), respectively.

Drug interruptions

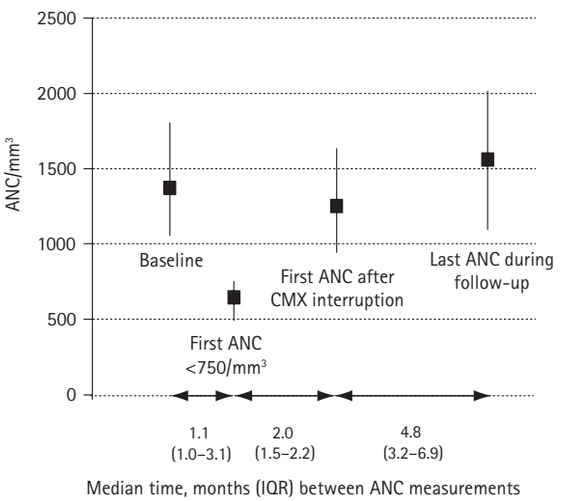
The 118 patients with incident grade 3–4 neutropenia were all receiving cotrimoxazole when the neutropenia was detected. Of these patients, 32 (27%) did not stop cotrimoxazole as the further absolute neutrophil counts were all ≥750/mm^3, but the remaining 86 (73%) stopped cotrimoxazole because of persistent severe neutropenia. Figure 2 shows, for these 86 patients, the median value and IQR of the absolute neutrophil count at the following times: baseline, lowest value before cotrimoxazole was stopped, first measurement after cotrimoxazole was stopped and last available measurement at the time of study termination. The median time between the lowest value of neutrophil count and cotrimoxazole interruption was 1.0 month (IQR 0.2–1.0). The median time between cotrimoxazole interruption and the first further neutrophil measurement was 1.0 month (IQR 0.9–1.5). The median increase in neutrophils between the lowest neutrophil count and the first measurement after cotrimoxazole was stopped was +540/mm^3 (IQR +150; +896/mm^3).

During follow-up, there were 15 modifications of the initial HAART regimen, an overall incidence of 6.0/100 PY. AZT was replaced by stavudine in one patient with persistent isolated grade 4 neutropenia after cotrimoxazole was stopped (incidence of neutropenia-related modification of HAART regimen: 0.4/100 PY, 95% CI 0.0–2.2) and in 11 patients with grade 3–4 anaemia (incidence of anaemia-related modification of HAART regimen: 4.4/100 PY, 95% CI 2.2–7.9). Of these 11 patients, five had concomitant grade 3–4 neutropenia. The other reasons for HAART regimen modification were renal insufficiency ($n=1$), neurological disorders ($n=1$) and pregnancy ($n=1$) (incidence of non-haematological motivated modification of HAART regimen: 1.2/100 PY, 95% CI 0.25–3.51).

Severe morbidity

During follow-up, there were 77 serious events, including 37 WHO stage 2, 3 or 4 classifying events (12 tuberculosis, 11 bacterial events, nine episodes of malaria, three unexplained weight loss, one oesophageal candidiasis and one chronic genital herpes) and 40 non-classifying events leading to at least 1 day in hospital (15 unexplained acute fever, 12 unexplained acute diarrhoea, seven vomiting, three cardiac insufficiency, two EFV-related neurological intolerance and one pneumothorax). The 11 bacterial events were three pneumonia, three sinusitis, two enteritis, one salpingitis, one liver abscess and one isolated bacteraemia. Four of these 11 bacterial events were with bacteraemia (two pneumonia with *Streptococcus pneumoniae*, one isolated bacteraemia with *non-typhi Salmonella* and one salpingitis with *Staphylococcus aureus*); a bacteria was also isolated from samples other than blood in three others bacterial events (two enteritis with *Shigella* sp. and one enteritis with *non-typhi Salmonella*).

Figure 2. Evolution of the absolute neutrophil count in the 86 patients who stopped cotrimoxazole because of persistent grade 3-4 neutropenia



ANC, absolute neutrophil count; CMX, cotrimoxazole; IQR, interquartile range.

Only 13 of these 77 serious events were within the 2 months before ($n=8$) or within the 2 months after ($n=5$) a grade 3 or 4 neutropenia. The eight events preceding a grade 3–4 neutropenia were three acute unexplained fever, two acute unexplained diarrhoea, one episode of malaria, one bacterial sinusitis and one pneumonia. The five events following a grade 3–4 neutropenia were two acute unexplained diarrhoea, one malaria, one sinusitis and one acute unexplained fever. None of these events led to death.

Discussion

We followed 498 HAART-naive HIV-infected adults who started AZT/3TC/EFV in combination with cotrimoxzole in Abidjan, Côte d'Ivoire. During the first 6 months of HAART, we observed an unexpectedly high incidence of 56/100 PY of grade 3–4 neutropenia. Whereas approximately three quarters of patients with grade 3–4 neutropenia had to stop cotrimoxazole, only one of them had to stop AZT. Finally, severe anaemia accounted for a rate of 4.4/100 PY of AZT discontinuation, and remained the first cause of HAART regimen modification.

To our knowledge, this is the first description of the evolution of the blood cell count in a large cohort of

adults receiving AZT in combination with cotrimoxazole in sub-Saharan Africa. Most studies which have previously reported clinical outcomes in patients receiving HAART in sub-Saharan Africa were in patients receiving non-AZT-containing HAART regimens [9,10], in patients receiving multiple HAART regimens giving no detail on tolerance by regimen [11–13] or in patients receiving HAART with no data on regimens and/or tolerance [14–18]. In one report from a large South African cohort of 285 patients receiving AZT/3TC in combination with either EFV (60%) nevirapine (38%), or indinavir (4%), the cumulative percentage of AZT discontinuation was 4.7% at 24 months and AZT was the second most frequent drug, after nevirapine, that had to be discontinued for intolerance [19]. In this study, patients were not receiving cotrimoxazole. Finally, most data on cotrimoxazole tolerance in sub-Saharan African HIV-infected patients were reported before the HAART era. In all studies that compared cotrimoxazole prophylaxis with a placebo or with the absence of treatment in Africa, the short-term incidences of grade 3–4 neutropenia and anaemia were not statistically different between patients receiving cotrimoxazole and those not receiving it [20–23]. In only one of these trials were mild events also reported, showing a significantly higher short-term rate of grade 1–2 neutropenia in patients receiving cotrimoxazole, but no difference in terms of grade 1–2 anaemia [20].

In industrialized countries, both AZT and cotrimoxazole cytotoxicity have long been well described. AZT inhibits beta-globin gene expression and has bone marrow cytotoxicity [24,25]. A non-dose-dependent sudden decrease in haemoglobin can occur shortly after initiation of therapy, while a red blood cell count and granulocyte count decrease can develop in a dose-dependent fashion [26]. Trimethoprim inhibits the dihydrofolate reductase, leading to dose-dependent neutropenia and anaemia [27,28]. In HIV practice, clinically significant severe anaemia is most frequent with AZT [29], whereas severe neutropenia is most frequent with cotrimoxazole [29]. It is a well-known fact that the combination of these drugs leads to a more frequent cytotoxicity than when they are used separately [30].

Although it is difficult to compare the rates of haematological disorders that we observed in our study with those previously reported in the literature in different settings, different populations and through studies of different designs, our figure of grade 3–4 anaemia was in the range of what has been reported in HIV-infected patients receiving AZT and cotrimoxazole in industrialized countries, while our incidence of grade 3–4 neutropenia was generally greater than what has been previously reported [3,5,29,31,32]. There may be various explanations for this. Firstly, a lower neutrophil count in individuals with African ancestry has long been described under the description 'ethnic neutropenia', as the consequence of a reduced number of bone marrow progenitor cells in comparison with the numbers present in Caucasians [33,34]. This might contribute to accentuation of the bone marrow toxicity of neutropenia-inducing drugs in HIV-infected sub-Saharan African patients. The fact is that neutropenia has been described as being more frequent in African American patients receiving HAART [31], whereas the frequency of AZT anaemia has not been found to be statistically different between Caucasian and African American patients [35]. Secondly, one can imagine that other mechanisms might accentuate the pharmacokinetic drug interaction between cotrimoxazole and AZT. At baseline, most of our patients had a normal neutrophil count or mild grade 1 neutropenia, although 80% of them were already receiving cotrimoxazole for a median of 12 months. Severe neutropenia occurred shortly after AZT was introduced, and resolved shortly after cotrimoxazole was stopped. It is known that trimethoprim decreases the renal excretion of AZT [36], but that this kinetic drug interaction has no clinical importance as long as hepatic glucuronidation is not impaired by liver disease or inhibited by other drugs [36]. In our study, the risk of neutropenia was higher in patients with positive serum HBs antigen at baseline, and chronic hepatitis may have contributed to glucuronidation impairment. Another hypothesis would be that the glucuronidation of AZT could be lower in individuals with African ancestry than in Caucasian individuals, as previously shown with other drugs [37].

During our study, the other non-antiretroviral drugs that have been the most frequently associated with myelosuppression in HIV-infected patients (for example, ganciclovir, dapsone, pyrimethamine, sulfadiazine, fluconazole or antineoplastic chemotherapy) [6,24,29,38] were rarely used. The two antiretroviral drugs that were used in combination with AZT are not likely to be a cause of most of the disorders that we observed. To our knowledge, no direct haematotoxicity of EFV has ever been described. 3TC has been implicated in rare pure red cell aplasia [39] and in neutropenia at very high concentrations [40]. Cotrimoxazole increases the area under the concentration–time curve of 3TC as a consequence of impairing the renal clearance of the drug [41]. However, this interaction is unlikely to result in a 3TC concentration-related toxicity at the usual doses [41].

Our study has several limitations. First, cotrimoxazole may induce haemolysis in G6PD-deficient HIV-infected patients [42]. The prevalence of G6PD deficiency is unknown in our population. Secondly,

myelodysplasia associated with immunosuppression renders HIV individuals more susceptible to myelosuppressive therapies [25,43]. In our study, which took place in the pre-randomization phase of a STI trial, we did not include patients with advanced immunosuppression because one of the major criteria for being randomized in the trial was a CD4 count >350/mm^3 within the 18 months following HAART initiation; patients with very low CD4 count at baseline in the pre-randomization phase were not likely to reach this criteria. This is why, in our population, two thirds of patients had a baseline CD4 count over 200/mm^3. This may explain why we did not find any association between a low CD4 count and a higher risk of drug-related haematotoxicity, as described elsewhere [6,29]. In sub-Saharan Africa, most patients actually start HAART at a lower CD4 count than the patients who participated in our trial [9–18]. Thus, our study may underestimate the true rate of haematological disorders in the population of African adults who actually start AZT in combination with cotrimoxazole. Thirdly, we did not measure the drug concentrations in the pills nor in the serum of patients.

The aim of this study was to describe the frequency of haematological disorders in patients who were prescribed both drugs. It was not to study the association between neutropenia and the frequency of infectious episodes in these patients, nor to assess the procedures for stopping cotrimoxazole and AZT at the onset of haematological toxicity. We can only make the following comments in this respect: firstly, almost one quarter of episodes of severe neutropenia resolved spontaneously as previously described in other studies [6]. In our team, we therefore continue to stop cotrimoxazole only when a grade 3–4 neutropenia is seen on at least two measurements. Secondly, the risk of infectious episodes has been shown to be lower in HIV-infected patients with drug-induced neutropenia than in neutropenic patients with haemobiological malignancies [6,38]. In the 1980s, when patients were receiving high dosages of AZT with consecutive frequent neutropenia, AZT was recommended as long as the neutrophil count remained over 500/mm^3 [44]. Our study shows that severe neutropenia will rarely motivate AZT discontinuation in HIV-infected sub-Saharan African patients, as long as cotrimoxazole is interrupted in patients with a repeated neutrophil count <750/mm^3. In our opinion, it would be well worth exploring whether cotrimoxazole could be safely continuted in sub-Saharan African adults receiving AZT with a repeated neutrophil count at 500–750/mm^3.

Finally, in our study, anaemia remained the first cause of AZT discontinuation, but our rate of anaemia-motivated discontinuation of AZT was comparable with what has been reported in industrialized countries. Furthermore, our AZT-containing HAART regimen was associated with a recovery of most baseline anaemia, as shown by the distribution of the haemoglobin level at 6 months and as previously described in the literature [45].

In conclusion, cotrimoxazole and AZT are two important first-line HIV drugs with potential haematotoxic effects. Our data suggest that in patients receiving both drugs, severe neutropenia may be more frequent in sub-Saharan Africa than in industrialized countries. Administering both drugs to individuals similar to those who participated in our study requires close monitoring of the absolute neutrophil count and the discontinuation of cotrimoxazole in cases of severe neutropenia.

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