AZT impairs immunological recovery on first-line ART: collaborative analysis of cohort studies in Southern Africa

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Objectives: Zidovudine (AZT) is recommended for first-line antiretroviral therapy (ART) in resource limited settings. AZT may, however, lead to anemia and impaired immunological response. We compared CD4 counts over 5 years between patients starting ART with and without AZT in Southern Africa.

Design: Cohort study.

Methods: Patients aged $\geq$16 years who started first-line ART in South Africa, Botswana, Zambia or Lesotho were included. We used linear mixed-effect models to compare CD4 cell count trajectories between patients on AZT-containing regimens and patients on other regimens, censoring follow-up at first treatment change. Impaired immunological recovery, defined as a CD4 count below 100 cells/μl at 1 year, was assessed in logistic regression. Analyses were adjusted for baseline CD4 count and haemoglobin level, age, gender, type of regimen, viral load monitoring and calendar year.

Results: 72 597 patients starting ART, including 19 758 (27.2%) on AZT, were analysed. Patients on AZT had higher CD4 cell counts (150 vs.128 cells/μl) and haemoglobin level (12.0 vs. 11.0 g/dl) at baseline, and were less likely to be female than those on other regimens. Adjusted differences in CD4 counts between regimens containing and not containing AZT were -16 cells/μl (95% CI $-$18 to $-$14) at 1 year and $-$56 cells/μl (95% CI $-$59 to $-$52) at 5 years. Impaired immunological recovery was more likely with AZT compared to other regimens (odds ratio 1.40, 95% CI 1.22$-$1.61).

Conclusions: In Southern Africa AZT is associated with inferior immunological recovery compared to other backbones. Replacing AZT with another NRTI could avoid unnecessary switches to second-line ART.

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Introduction

In low-income countries, the World Health Organization (WHO) recommends the use of standardized first-line antiretroviral therapy (ART) consisting of one non-nucleoside reverse transcriptase inhibitor (NNRTI) and two nucleoside reverse transcriptase inhibitors (NRTI) [1]. At present the two preferred backbones are lamivudine (3TC) or emtricitabine (FTC) + tenofovir (TDF) or 3TC + zidovudine (AZT). Although the efficacy and toxicity of these regimens have been studied extensively in clinical trials in high-income countries, data on long-term clinical outcomes in Sub-Saharan Africa are scarce.

A concern with the use of AZT is its well-known myelosuppressive effect, sometimes leading to severe hematologic toxicity [2,3]. AZT-related anaemia has been described in many studies, including in reports from sub-Saharan Africa [4,5]. As a consequence, most ART guidelines have precluded its use in patients with severe anaemia. The impaired immunological recovery related to the use of AZT has also been reported in clinical trials and observational studies, both in industrialized countries [6−11] and sub-Saharan Africa [12,13]. However, these studies were small and had limited follow-up time. In southern Africa, most of the HIV-infected patients are treated in settings where viral load monitoring is not available and are thus switched to second-line regimens according to clinical and immunological criteria. Several studies, including analyses from the International epidemiologic Databases to Evaluate AIDS (IeDEA) [14−16], have described risk factors for impaired immunological response to ART, but only few publications have examined the role of specific antiretroviral drugs in sub-Saharan Africa. To avoid unnecessary switching to expensive protease inhibitor (PI)-based second-line ART, a better understanding of the factors impairing immunological recovery is needed.

We compared 5-year CD4 cell count trajectories after ART initiation between patients on first-line regimen containing AZT and those not receiving AZT and assessed the association between AZT and severe impairment of immunological recovery in a large collaborative analysis of nine cohorts in four countries in southern Africa.

Methods

Antiretroviral treatment programmes
The International epidemiological Databases to Evaluate AIDS in Southern Africa (IeDEA-SA) is a large collaboration of ART programmes in southern Africa [17]. Data are collected at ART initiation (baseline) and each follow-up visit, using standardized instruments, and transferred to data centres at the Universities of Cape Town, Republic of South Africa (RSA) and Bern, Switzerland. All sites have ethical approval to collect data and to participate in IeDEA-SA.

We included all cohorts with at least 100 adult patients in both treatment groups (with and without AZT). Six ART programmes in RSA and one in Botswana monitored viral load and CD4 cell counts every 6 months during the first year of ART, and then yearly: Aurum Institute (community and workplace), Themba Lethu, Khayelitsha, Gugulethu, Tygerberg, in South Africa, as well as Gaborone private clinic in Botswana. Two cohorts with routine CD4 count but no viral load monitoring were also included: the Ministry of Health - Centre for Infectious Disease Research in Zambia (MoH-CIDRZ) and the SolidarMed ART program in rural Lesotho. All treatment programmes trace patients lost to follow-up.

Eligibility criteria
All patients aged 16 years and older who started a first-line ART regimen and had at least 2 CD4 cell counts performed (one at ART start and at least one during follow-up) were included. We defined first-line regimens according to the WHO treatment guidelines as a regimen including an NNRTI and 2 NRTIs. Patients with prior exposure to ART before inclusion in the treatment program were excluded.

Outcomes
We compared CD4 trajectories between patients receiving a first-line regimen including AZT with those on other ART combinations over the first 5 years of treatment. Differences in CD4 cell counts after one and 5 years of ART were compared between patients receiving AZT and those on other first-line ART regimens. Follow-up was censored at the date of the first treatment change, the last visit or the end of the study period, whichever occurred first. In a second analysis we focused on patients who started ART with severe immunodeficiency, defined as a baseline CD4 cell count below 100 cells/µl. WHO included three specific criteria in the definition of immunological treatment failure, one of which being the persistence of a CD4 cell count below 100 cells/µl. We assessed the association between AZT and impairment of immunological recovery, defined as a CD4 count remaining below 100 cells/µl 1 year after the initiation of ART.

Statistical analyses

Baseline characteristics of patients on AZT-containing regimens were compared to those of patients not on AZT using chi-squared and Mann-Whitney tests for categorical and continuous variables, respectively. We used multivariable linear mixed-effect models to analyse CD4 cell count trajectories over the first 5 years of ART, as described in detail elsewhere [16]. Differences in CD4 cell counts after 1 and 5 years of ART were compared between patients on AZT and not on AZT. CD4 cell counts were square-root transformed and trajectories modelled using fractional polynomials. Results are presented as marginal CD4 estimates and trajectories. Additionally, differences in CD4 cell counts between the two treatment groups within each cohort were described in a forest plot and overall estimates obtained using random-effect meta-analysis. We repeated all analyses in patients who remained virologically suppressed during follow-up in South Africa and Botswana and within each baseline CD4 cell count category. Predictors of impairment of immunological recovery during the first year of ART were evaluated using multivariable logistic regression. For the latter analysis, only patients who started a first-line ART regimen with a CD4 cell count below 100 cells/μl and with an available CD4 measurement between 8 and 15 months were included.

All multivariable analyses were adjusted for sex, age (16–29, 30–39 or 40 years and over), CD4 cell count at baseline (0–49, 50–99, 100–199, ≥200 cells/μl), calendar year of starting first-line ART (before 2007, 2007, 2008, 2009 and 2010), degree of anaemia at baseline, type of NNRTI (efavirenz or nevirapine) and viral load monitoring (yes or no). Anaemia was defined as severe (haemoglobin <5.0 mmol/L), moderate (5.0–<6.2 mmol/L in women and 5.0–<6.8 mmol/L in men), mild (6.2–<7.4 mmol/L in women and 6.8–<8.1 mmol/L in men) or none (≥7.4 mmol/L in women and ≥8.1 mmol/L in men). Multiple imputation was used to impute missing haemoglobin values at baseline, with analyses run on each of 20 datasets and results combined with Rubin's rules [18]. All analyses were performed using Stata software version 11 (College Station, Texas, USA).

Results

ART programmes and patients characteristics

Table 1 shows the composition of cohorts. A total of 72 597 patients on first-line ART, including 19 758 (27.2%) on an AZT-containing regimen were included in the analyses. The majority of patients were female in all cohorts except for the AURUM workplace cohort in South Africa, which was dominated by male miners. The median age ranged from 33 years in Gugulethu and

Table 1. Baseline characteristics of patients.

Sites	No. of patients	No. of patients on AZT (%)	Female (%)	Median age in years (IQR)	Median CD4 count in cells/μl (IQR)	Hemoglobin in g/dl Median (IQR)	Missing (%)	Follow-up (py)
South Africa								
Aurum_CS	7930	835 (10.5)	5016 (63.3)	36 (31–43)	130 (59–197)	11.9 (10.3–13.4)	1753 (22.1)	11 097
Aurum_WP	4205	3487 (82.9)	298 (7.1)	43 (35–49)	160 (90–228)	13.3 (11.8–14.5)	862 (20.5)	7598
Gugulethu	1784	197 (11.0)	1229 (68.9)	33 (29–39)	102 (52–159)	11.2 (9.9–12.5)	62 (3.5)	2578
Khayelitsha	3582	763 (21.3)	2443 (68.2)	33 (28–39)	90 (41–158)	10.9 (9.6–12.1)	1539 (43.0)	5869
Thembalethu	7384	208 (2.8)	4749 (64.3)	36 (31–43)	96 (39–164)	11.7 (10.2–13.1)	186 (2.5)	11 181
Tygerberg	1103	177 (16.1)	700 (63.5)	35 (29–41)	113 (49–173)	11.1 (9.7–12.5)	365 (33.1)	1762
Botswana								
Gaborone	925	713 (77.1)	572 (61.8)	36 (32–41)	127 (59–190)	11.9 (10.2–13.0)	479 (51.8)	2139
Zambia								
MoH-CIDRZ	43 284	12 249 (28.3)	27 008 (62.4)	35 (30–42)	144 (79–209)	11.1 (9.7–12.5)	3675 (8.5)	58 011
Lesotho								
SMARTLes	2400	1129 (47.0)	1639 (68.3)	39 (32–48)	151 (81–243)	11.7 (10.4–13.0)	553 (23.0)	3081
Total	72 597	19 758 (27.2)	43 654 (60.1)	36 (30–43)	134 (67–198)	11.3 (9.9–12–8)	9474 (13.1)	103 317

Aurum_CS, Aurum Community Service; Aurum_WP, aurum Workplace; AZT, zidovudine; IQR, interquartile range py: person-years; MoH-CIDRZ, Ministry of Health's Center for Infectious Diseases Research in Zambia.

Table 2. Characteristics of patients at the start of first-line ART.

	AZT (N = 19 758)	No AZT (N = 52 839)	P-value
Female gender (%)	10 320 (52.2)	33 334 (63.1)	<0.001
Median age at start (IQR)	36 (31–44)	36 (30–42)	<0.001
Median CD4 count in cells/μl (IQR)	150 (85–212)	128 (63–195)	<0.001
Median hemoglobin in g/dl (IQR)	12.0 (10.9–13.3)	11.0 (9.5–12.5)	<0.001
missing (%)	2868 (14.5)	6606 (12.5)	
Anemia			<0.001
no (%)	7319 (43.3)	13 106 (28.4)	
mild (%)	7321 (43.4)	16 074 (34.8)	
moderate (%)	2112 (12.5)	13 374 (28.9)	
severe (%)	138 (0.8)	3679 (8.0)	
Calendar year of first-line ART start (%)			<0.001
Before 2007	11 764 (59.5)	17 869 (33.8)	
2007	4295 (21.8)	9983 (18.9)	
2008	2057 (10.4)	12 079 (22.9)	
2009-10	1642 (8.3)	12 908 (24.4)	
NNRTI (%)			<0.001
NVP-based	13 466 (68.2)	25 028 (47.4)	
EFV-based	6292 (31.8)	27 811 (52.6)	
NRTI (%)			
3TC/D4T	0	33 580 (63.6)	
3TC/AZT	19 722 (99,8)	0	
XTC/TDF	0	18 440 (34.9)	
3TC/ABC	0	672 (1.2)	
other	36 (0.2)	147 (0.3)	
Virological monitoring (%)	6380 (32.3)	20 533 (38.9)	<0.001
Median follow-up time (days)	509 (224–967)	366 (197–639)	<0.001

3TC, lamivudine; ABC, abacavir; AZT, zidovudine; D4T, stavudine; EFV, efavirenz; IQR, interquartile range; NVP, nevirapine; XTC, lamivudine or emtricitabine. Anemia was categorized as follows: severe: haemoglobin <5.0 mmol/L, moderate: 5.0–<6.2 mmol/L in women and 5.0–<6.8 mmol/L in men, mild: 6.2–<7.4 mmol/L in women and 6.8–<8.1 mmol/L in men, none: ≥7.4 mmol/L in women and ≥8.1 mmol/L in men; P values from chi-squared tests or Mann-Whitney tests.

Khayelitsha to 43 years in the AURUM workplace cohort. In South Africa, the proportion of patients on AZT ranged from 2.8% in Thembalethu to 82.9% in the AURUM workplace cohort, and outside South Africa, this proportion ranged from 28.3% in the CIDRZ program in Lusaka to 77.1% in Gaborone, Botswana. Median CD4 count and haemoglobin levels at initiation of ART ranged from 90 cells/μl (interquartile range (IQR): 41–158) and 10.9 g/dl (9.6–12.1) in Khayelitsha to 160 cells/μl (90–228) and 13.3 g/dl (11.8–14.5) in the AURUM miners cohort, respectively. Data on baseline haemoglobin level was overall missing for 13.1% of patients.

Patients receiving AZT were less likely to be female, to have started ART in recent years and to be treated in the Republic of South Africa or Botswana (Table 2). Median baseline CD4 cell count (150 vs. 128 cells/μl, p < 0.001) and haemoglobin level (12.0 vs. 11.0 g/dl, p < 0.001) were higher in patients on AZT compared to those in the other treatment group (Table 2). Severe anaemia was present in 8.0% of patients initiating ART not containing AZT and in 0.8% of patients receiving AZT (p < 0.001). The most common ART combinations were 3TC/AZT/NVP (68.0%) and 3TC/AZT/EFV (31.8%) in the AZT group, and 3TC/D4T/NVP (34.3%), 3TC/D4T/EFV (29.3%), FTC/TDF/EFV (20.5%), and FTC/TDF/NVP (11.5%) in the non-AZT group.

CD4 trajectories
The total follow-up time was 34,964 and 68,353 person-years for the AZT and non-AZT groups, respectively. Figure 1 shows CD4 trajectories over the first five years of ART, by first-line treatment group. During follow-up, CD4 cell counts were similar during much of the first year on ART but substantial differences emerged from year 1 onwards. After 1 and 5 years of ART, patients on AZT reached estimated CD4 counts of 301 cells/μl (95% CI: 299 to 302) and 386 cells/μl (383 to 389), whereas those not on AZT reached CD4 cell counts of 317 cells/μl (316 to 318) and 442 cells/μl (440 to 444), respectively. Estimated differences were -16 CD4 cells/μl (95% CI: -18 to -14) at 1 year and -56 CD4 cells/μl (52 to 59) at 5 years in favour of the group of patients not receiving AZT (Web table 1, http://links.lww.com/QAD/A358). Adjusted estimates of difference in CD4 cell count between treatment groups for each cohort are shown in Figure 2. One and 5 years after the initiation of ART, most cohorts showed a higher absolute CD4 cell count in patients not receiving AZT compared to those on AZT. At each time point, only 3 out of 9 cohorts had marginally higher CD4 cells counts in patients on AZT, but these differences were not statistically significant. However, there was substantial heterogeneity between cohorts in both analyses (chi-squared test for heterogeneity: p = 0.01 at 1 year and p < 0.001 at 5 years).
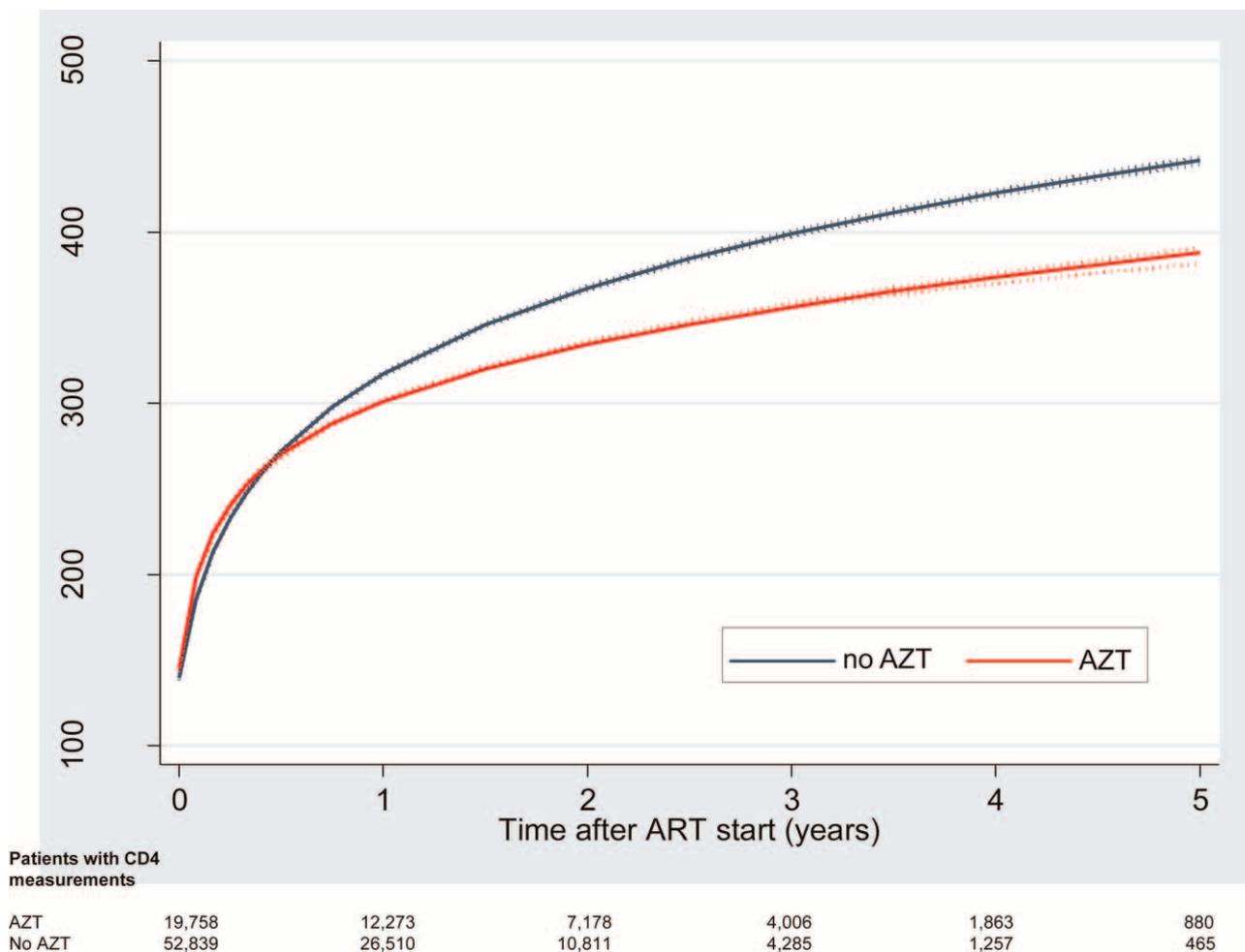
Fig. 1. Estimated CD4 trajectories in patients starting first-line ART with and without zidovudine (AZT). The dotted lines indicate 95% confidence intervals.

Several additional analyses were performed to examine immunological recovery in subgroups of patients (Web table 1, http://links.lww.com/QAD/A358). The difference between the treatment groups was most pronounced in patients who started ART with a CD4 cell count below 100 cells/μl: -66 cells/μl (95% CI: −72 to −61) at 5 years in comparing AZT with no AZT. In RSA and Botswana, where virological monitoring was available, a higher proportion of patients on AZT had at least one detectable viral load during follow-up compared to those not on AZT (39% vs. 20%, p < 0.001). However, in analyses restricted to patients who were fully suppressed 6 months after the initiation of ART and who remained so during the whole follow-up period, we found no difference in CD4 cell count at 1 year between patients on AZT and those not on AZT and a significant difference at 5 years (difference −21 cells/μl, 95% CI: −29 to −14) (Web table 1, http://links.lww.com/QAD/A358).

Predictors of impairment of immunological recovery

This analysis was based on 14,529 patients who started ART with CD4 counts below 100 cells/μl. The number of imputed haemoglobin values were 938 (15.1%) in the AZT group and 1,137 (10.4%) in the other group. 407 patients (11.4%) on AZT and 914 patients (8.3%) on other regimens remained below 100 CD4 cells/μl at 1 year (p < 0.001). In multivariate logistic regression patients on AZT were more likely to experience severe impairment of immunological recovery than those on other first-line regimens (adjusted odds ratio (aOR) 1.40, 95% CI: 1.22 to 1.61) (Table 3). When this analysis was repeated on the complete-case dataset, the result was similar (aOR: 1.38, 95% CI: 1.19–1.60, data not shown). Male patients, those older than 40 years as well as patients with very low CD4 cell counts (<50 cells/μl) at start of ART were also at increased risk of impaired immunological recovery (Table 3).
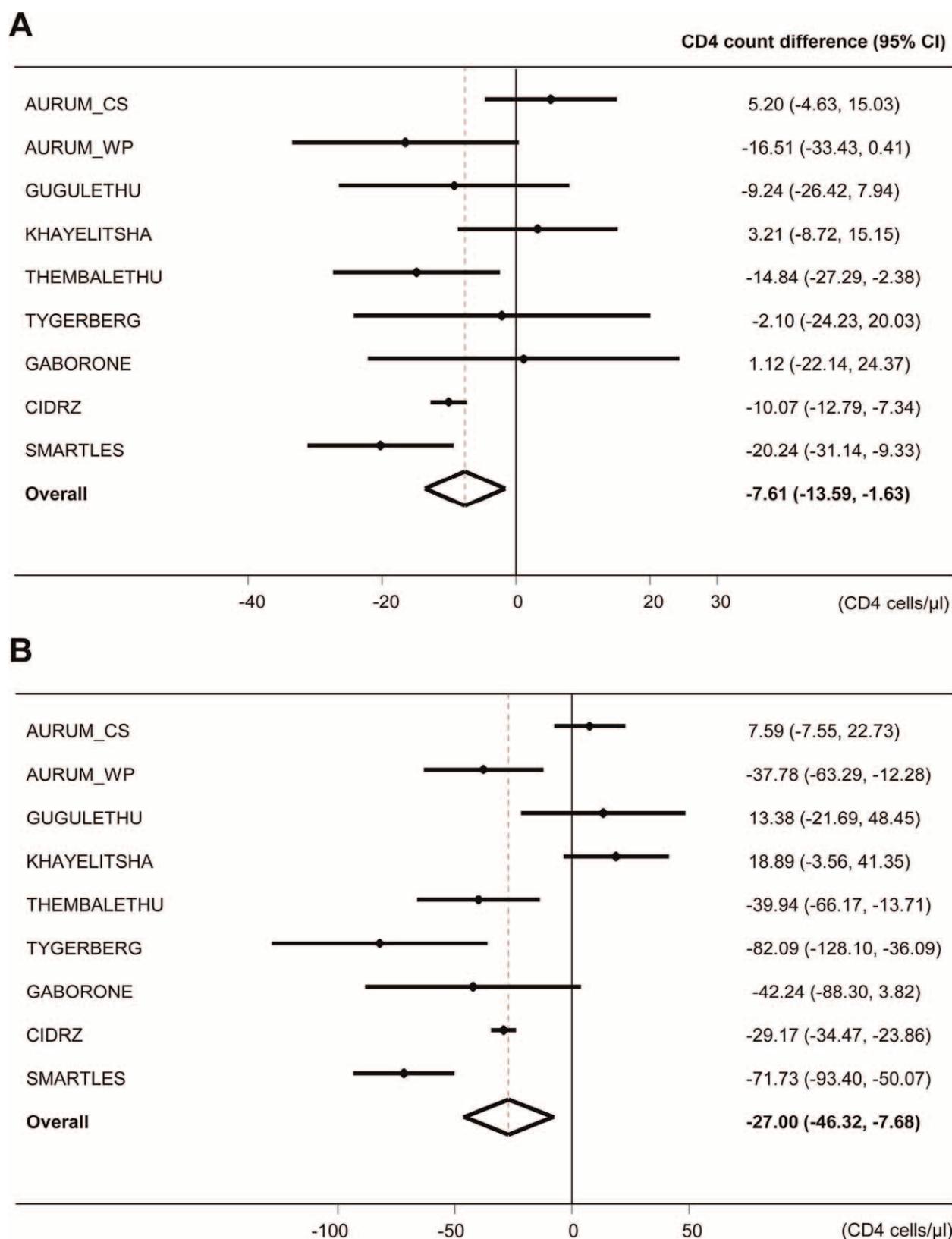
A

B

Fig. 2. Adjusted differences in CD4 counts between patients on AZT and those not on AZT after 1 (panel A) and 5 years (panel B) of ART, by cohort. I-squared (p value from test of heterogeneity): 1year (panel A): 58.7% (p = 0.013); 5 years (panel B): 87.8% (p < 0.001). Analyses were adjusted for sex, age, CD4 and anemia at baseline, calendar year, type of NNRTI and viral load monitoring.

Table 3. Predictors of severe impairment in immunological recovery (*N* = 14 529).

	Univariable analysis		Multivariable analysis	
	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
AZT				
No	1.0	<0.001	1.0	<0.001
Yes	1.42 (1.25–1.60)		1.40 (1.22–1.61)	
Sex				
Male	1.0	<0.001	1.0	<0.001
Female	0.49 (0.44–0.55)		0.56 (0.49–0.63)	
Age category (years)				
16–29	1.0	<0.001	1.0	0.01
30–39	1.28 (1.09–1.50)		1.11 (0.94–1.31)	
>39	1.64 (1.39–1.94)		1.33 (1.12–1.58)	
CD4 category (cells/µl)				
50–99	1.0	<0.001	1.0	<0.001
1–49	1.93 (1.72–2.17)		2.00 (1.77–2.25)	
Anemia				
No	1.0	<0.001	1.0	<0.001
Mild	0.83 (0.72–0.97)		0.88 (0.76–1.02)	
Moderate	0.73 (0.62–0.85)		0.77 (0.65–0.91)	
Severe	0.45 (0.33–0.61)		0.56 (0.41–0.76)	
Year of ART start				
Before 2007	1.0	0.64	1.0	0.01
2007	1.08 (0.93–1.25)		1.20 (1.03–1.39)	
2008	1.09 (0.93–1.27)		1.28 (1.08–1.52)	
2009-10	1.07 (0.85–1.35)		1.40 (1.08–1.81)	
NNRTI				
NVP	1.0	<0.001	1.0	0.72
EFV	1.31 (1.17–1.47)		1.03 (0.89–1.19)	
VL monitoring				
No	1.0	<0.001	1.0	0.01
Yes	1.22 (1.09–1.37)		1.21 (1.04–1.41)	

AZT, zidovudine; EFV, efavirenz; NNRTI, non-nucleoside reverse transcriptase inhibitor; NVP, nevirapine; VL, viral load. Anemia was categorized as follows: severe: haemoglobin <5.0 mmol/L, moderate: 5.0–<6.2 mmol/L in women and 5.0–<6.8 mmol/L in men, mild: 6.2–<7.4 mmol/L in women and 6.8–<8.1 mmol/L in men, none: ≥7.4 mmol/L in women and ≥8.1 mmol/L in men. Multivariable analysis adjusted for all variables shown in table.

Discussion

This study of nine ART programs and over 70 000 patients from four countries in southern Africa found that AZT influenced the magnitude of the CD4 count increase over the first 5 years of treatment. Patients receiving AZT had lower CD4 cell counts at 1 and 5 years and were more likely to remain severely immune suppressed during the first year of treatment. The difference in immunological recovery between patients on AZT and those on other backbones was consistent across baseline CD4 cell count categories and, to a lesser extent, was also seen in the subset of patients who had an undetectable viral load throughout the follow-up period.

Numerous studies from high income countries have assessed predictors of CD4 count increase following ART initiation [19–22]. Even though some individual characteristics, including older age, lower baseline CD4 cell counts and hepatitis C virus co-infections have consistently been shown to be associated with impaired immunological recovery, there remains controversy on the impact of specific ART components on CD4 cell recovery [11]. In accordance with the results of two small studies from Botswana and Cameroon [12,13], we

showed that patients on AZT reached significantly lower CD4 cell counts on ART compared to those on other regimens. The reasons for the association of AZT with impairment of total CD4 lymphocyte recovery are poorly understood: the most probable explanation is related to the well-known bone marrow suppression caused by AZT [2–5]. Data from the Swiss HIV Cohort Study showed reduced absolute but not relative CD4 counts in patients on AZT, suggesting a general effect on total lymphocyte count, rather than a CD4 specific effect [8].

Impaired immunological recovery in patients on ART may not have important clinical consequences when it concerns patients with high baseline CD4 counts. However, in those with very low CD4 cell counts at treatment initiation, failure to reach adequate cellular immunity not only leads to an increased mortality and HIV-associated morbidity, but also has important implications on clinical decision-making: a CD4 cell count persistently below 100 cells/µl is one of the three criteria used to diagnose immunological failure (1). Thus, in settings without access to viral load testing, many patients who remain under this threshold during ART are switched to a PI-based regimen, despite possible virological efficacy of the first-line regimen. In our

study, patients on AZT were more likely to remain severely immune suppressed after one year of ART compared to those not receiving AZT. Even though in our study the observed inferior virological efficacy of AZT-containing regimens in RSA might partially explain the impaired immunological recovery in this group, we found a difference in long-term CD4 recovery between the 2 groups when the analyses were restricted to virologically suppressed patients. This suggests that the impact of AZT on immunological recovery is, to some extent, independent of its virological efficacy.

Only few studies have compared outcomes between different ART regimens in sub-Saharan Africa [23,24]. Our study is unique regarding its sample size and length of follow-up: it involved a large number of patients from a wide range of settings in southern Africa and described immunological recovery over five years after the initiation of ART. The main limitation of observational cohort data comparing treatments in different countries lies in the lack of randomization and the heterogeneity between the treatment sites. Confounding by indication may have affected our results: patients with advanced clinical disease and low CD4 cell counts are also more likely to have severe anemia, which is the main reason for not being prescribed AZT. As a consequence, sicker patients are less likely to receive AZT in many settings. This might have led to an underestimation of the negative impact of AZT on immunological recovery. Unfortunately, due to the lack of data on pre-ART follow-up, causal modelling was not possible. Furthermore, baseline haemoglobin levels were missing in a substantial number of patients: although we addressed this issue by using multiple imputation, the missing data may have biased our results. A further limitation of our study was the wide variation in the proportion of patients on an AZT-containing regimen across countries and calendar time. This issue was described in detail in a previous multi-regional IeDEA analysis [5] and most probably reflects national treatment guidelines. Although the association between AZT and impaired immunological recovery was fairly consistent across most cohorts in our study, the results from a few treatment programs differed substantially from the overall estimates. Finally, we had no data on relative CD4 cell counts (CD4 percentage), which could have given more information on the mechanism of impaired immunological recovery under AZT-containing ART.

In conclusion, patients on first-line ART regimens including AZT are at risk of inferior CD4 cell recovery compared to those treated with other backbones. Treatment failure in patients remaining severely immune suppressed during the first year on AZT should be confirmed, where possible, with viral load testing before switching them to a second-line regimen. In patients with impaired immunological recovery but adequate virological response with an AZT-containing regimen, the replacement of AZT by another NRTI might be an adequate therapeutic approach. Further studies evaluating the role of specific ART components in immunological recovery are needed, especially from settings where virological monitoring and data on relative CD4 cell counts (or percentage) are available.

Conflicts of interest
We declare no conflict of interest.

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