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Background

- Neutrophil counts are known to be lower in uninfected Africans than in other populations. Neutropenia is also a known feature of advanced HIV infection and a side-effect of zidovudine (AZT), and it increases the risk of severe bacterial infections (SBI).

Methods

- 3316 previously untreated HIV-infected adults in Zimbabwe and Uganda, with a CD4 cell count <200/ μ L, initiated zidovudine-containing ART in the DART trial. We estimated the prevalence of grade 4 ($\leq 0.50 \times 10^3$ cells/ μ L) neutropenia at twelve-weekly scheduled Absolute Neutrophil Count (ANC) as part of the full blood counts to 216 weeks, and outcomes in terms of resolution or zidovudine substitution. Associations between baseline factors, grade 4 neutropenia and concurrent SBIs were identified using multiple logistic regression.
- Results of 11 participants are excluded from this analysis because of pre-existing Grade 4 Neutropenia at study enrolment.

Results

Of 57740 scheduled tests by Week 216, 1144 (2.0%) were grade 4 neutropenia, occurring in 769 (23.2%) of 3316, including recurrent episodes in 239 (7.2%) of 3316 of the study participants. The baseline characteristics of the participants are summarised in Table 1.

Table 1: Baseline characteristics of the study participants

		Experienced grade 4 Neutropenia during follow-up		P value
		No (n= 2,547)	Yes (n=769)	
Sex, n(%)	Male	874 (34.3%)	286 (37.2%)	0.143
	Female	1673 (65.7%)	483 (62.8%)	
Age	Mean (sd)	37.6 (7.6)	37.4 (8.1)	0.560
Baseline WHO stage, n(%)	2	551 (21.6%)	122 (15.9%)	0.001
	3	1423 (55.9%)	441 (57.4%)	
	4	573 (22.5%)	206 (26.8%)	
Initial antiretroviral regimen, n(%)	AZT 3TC ABC	235 (9.23%)	65 (8.45%)	0.022
	AZT 3TC NVP	415 (16.29%)	132 (17.17%)	
	AZT 3TC TDF	1897 (74.48%)	572 (74.38%)	
Had prior ART for PMTCT, n(%)	No	2503 (98.3%)	752 (97.8%)	0.382
	Yes	44 (1.7%)	17 (2.2%)	
On Cotrimoxazole, n(%)	No	1010 (39.7%)	309 (40.2%)	0.793
	Yes	1537 (60.4%)	460 (59.8%)	
On Isoniazid, n(%)	No	2515 (98.7%)	762 (99.1%)	0.435
	Yes	32 (1.3%)	7 (0.9%)	
Viral Load (copies/ml)	Median [IQR]	292739 [100747, 630450]	299593 [101624, 674629]	0.386
CD4 (cells/ml)	Median [IQR]	93 [39, 143]	56 [16, 121]	<0.0001
Haemoglobin (g/dL)	Median [IQR]	11.5 [10.3, 12.7]	11.2 [10.0, 12.5]	0.0003
Total WBC Count ($\times 10^3$ /uL)	Median [IQR]	3.8 [3, 4.7]	3.3 [2.5, 4.3]	<0.0001
Lymphocyte Count ($\times 10^3$ /uL)	Median [IQR]	1.33 [1.0, 1.8]	1.18 [0.8, 1.7]	<0.0001
Neutrophil Count ($\times 10^3$ /uL)	Median [IQR]	1.62 [1.2, 2.2]	1.23 [0.9, 1.7]	<0.0001
Platelet Count ($\times 10^3$ /uL)	Median [IQR]	205 [159, 260]	200 [151, 262]	0.086
Body Mass Index (kg/m ²)	Median [IQR]	21.3 [19.3, 23.8]	20.6 [18.5, 22.8]	<0.0001
Body Surface Area (m ²)	Median [IQR]	1.6 [1.5, 1.7]	1.6 [1.5, 1.7]	0.002

NB: Not all the participants had baseline Viral loads available

Timing and duration of the Grade 4 neutropenia events

- The median time [IQR] to onset of the first episode of neutropenia was 12 weeks [4,60], Table 2.
- Overall, the 1144 severe neutropenia episodes lasted for a median of 84 [56, 84] days.
 - 1084 (94.7%) episodes resolved with a median duration of 84 [57, 84] days and 15 (1.4%) episodes were unresolved by study week 216, after a median follow up of 60 [56, 84] days.
 - Nearly all the resolution of the grade 4 neutropenia occurred without discontinuation of AZT, which was done in only 33 (2.9%) of the 1144 episodes; in 5 of these 33 events, there was concurrent grade 3 or grade 4 anaemia.
- There were 45 deaths with unresolved grade 4 neutropenia (4% of the grade 4 neutropenia events), with a median duration of 6 [2, 42] days.

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Overall, during the follow up period, death occurred in 112 of 769 individuals that ever experienced any grade 4 neutropenia, compared to 240 of 2547 that experienced no grade 4 neutropenia (OR 1.64, 95% CI 1.28 - 2.10, P-value < 0.0001).

Table 2: Time to onset of grade 4 Neutropenia

N th Neutropenia episode	Number of Episodes	Mean week of Onset	Median week of onset
1	769	39	12
2	239	62	36
3	80	80	60
4	25	85	72
5	15	117	132
6	7	123	144
7	3	112	84
8	2	90	90
9	2	125	125
10	1	144	144
11	1	168	168
All Episodes	1144	50	24

On multiple logistic regression, individuals with low baseline ANC, CD4, Haemoglobin, BMI and those on Cotrimoxazole were more likely to develop grade 4 neutropenia, Table 3.

Table 3: Baseline predictors for Grade 4 Neutropenia

Predictor	O.R.	P-value	95% Conf. interval	
Country	Uganda	1	Ref	
	Zimbabwe	0.48	<0.001	0.38 ; 0.59
Sex	Male	1	Ref	
	Female	0.79	0.018	0.65 ; 0.96
ART regimen	TDF	1	Ref	
	NVP	1.31	0.025	1.03 ; 1.67
	ABC	0.87	0.39	0.64 ; 1.19
Neutrophils	Per 0.25 increase up to 1.375	0.61	<0.001	0.55 ; 0.67
	Per 0.25 increase past 1.375	0.97	0.037	0.93 ; 0.998
Log _e (CD4)	Per unit increase	0.75	<0.001	0.70 ; 0.81
Haemoglobin	Per 2.5 increase	0.77	<0.001	0.67 ; 0.88
BMI	Per 2.5 kg/m ² increase	0.92	0.017	0.87 ; 0.99
On Cotrimoxazole	No	1	Ref	
	Yes	1.35	0.001	1.12 ; 1.62

Severe Bacterial Events

- Severe bacterial infections occurred in 204 (26.5%) of 769 individuals that ever experienced a grade 4 neutropenia, compared with 559 (21.9%) of 2547 that never experienced any grade 4 neutropenia (OR 1.28, 95% CI 1.06 - 1.55, P-value = 0.008). Only 31 episodes of SBI were however documented during ongoing grade 4 neutropenia, in 26 individuals.
- Although cotrimoxazole was associated with development of grade 4 neutropenia in this population, no association was seen with SBIs. Among the 769 individuals experiencing the first episode of neutropenia, severe bacterial infection occurred in 130 of 460 individuals on cotrimoxazole and among 74 of 309 that were not on cotrimoxazole (OR 1.25, 95% CI 0.89 - 1.77, P-value 0.213).

Conclusions

- Grade 4 Neutropenia more commonly occurs during the initial 12 weeks of AZT-based ART in Africa. The risk of severe neutropenia is increased with advanced disease and use of Cotrimoxazole. In resource limited settings, the targeted use of Blood counts at 12 weeks will identify the majority of severe neutropenia episodes in this population.**
- In a large majority of patients the grade 4 neutropenia resolves without necessitating ART substitution.**