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Detectable plasma HIV RNA is associated with sensory neuropathy in HIV patients treated without stavudine

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Introduction

Anti-retroviral therapy (ART) reduces morbidity and increases life expectancy among people living with HIV, but some patients still experience symptoms, including sensory neuropathy (HIV-SN). Neuropathies resulting from HIV infection itself and toxic side-effects of ART have similar presentations, and include neuropathic pain, tingling and numbness.[1-3] Neuropathy was the dose limiting toxicity in early trials of some of the nucleoside analogue reverse transcriptase inhibitors (NRTIs) used to treat HIV: zalcitabine (ddC), didanosine (ddI) and stavudine (d4T). Hence rates of HIV-SN rose when these drugs entered clinical use, and exposure to ART emerged as a neuropathy risk factor. Stavudine was used in first line ART until well into the 21st century, especially in resource limited countries. The prevalence of HIV-SN in patients receiving stavudine at Cipto Mangunkusumo Hospital, Jakarta, Indonesia in 2006 was 34%. In this cohort, the observed associations with HIV-SN among stavudine treated patients were increasing age and height.[4,5] In black South African HIV patients exposed to stavudine, identical assessment methods revealed an HIV-SN prevalence of 57%.[6] Again, age and height were the observed associations with HIV-SN. The association between increasing age and HIV-SN was evident in a large US-based cohort study, where the prevalence of HIV-SN increased with age and time on ART despite a decline in the use of neurotoxic ART.[7]

Use of stavudine declined as alternative treatments became available. However, some HIV patients still complain of burning and tingling sensations in their legs and arms - likely symptoms of HIV-SN. Here we describe the prevalence of HIV-SN in an Indonesian population receiving ART without stavudine. We also examined associations with HIV-SN in this setting. The populations served by our clinic are similar to 2006, and the HIV-SN assessment methods were identical to the previous study[4]. Since 2014, healthcare for all Indonesians has been covered by government insurance. With more people aware of health issues and seeking treatment, numbers of patients in the clinics have increased.

Methods

HIV-positive adults who had used ART for at least 12 months but who had never been exposed to stavudine were screened for neuropathy at POKDISUS HIV Care Clinic, Cipto Mangunkusumo Hospital, Jakarta, Indonesia. Patients with any history of another condition that might be associated with a neuropathy, or any condition preventing the patient from being able to provide informed consent were

excluded. Neuropathy was assessed using the AIDS Clinical Trials Group Brief Peripheral Neuropathy Screen (ACTG-BPNS), and defined as present if the individual had one or more of the lower limb neuropathic symptoms (pain, aching or burning, pins and needles or numbness), plus absent ankle reflexes or reduced vibration sense at the great toe (vibration of a 128-Hz tuning fork felt for 10 seconds or less). We did not diagnose neuropathy in patients with only asymptomatic neuropathic signs, as the presence of both symptoms and signs on the ACTG-BPNS tool better associated with impaired peripheral nerve function and pathology.[8] BPNS and NCS were assessed by a neurologist (FO) and a trained general practitioner (DDS).

Patient height and weight were measured during the study assessment. Laboratory, clinical, and demographic data were collected from the medical file. Plasma HIV RNA was measured using a Cobas Amplicor Monitor (Roche Molecular Diagnostics, Pleasanton, CA). The study was approved by the Ethics Committee of the Faculty of Medicine, University of Indonesia (579/UN2.F1/ETIK/2014). All participants gave written informed consent.

Statistical analyses were performed using SPSS version 20.0. Demographic, clinical and treatment details of patients with and without neuropathy were compared using χ^2 tests (dichotomous variables), Mann-Whitney tests [non-normally distributed continuous variables, described using median (range)] or unpaired *t* tests (normally distributed continuous variables, described using mean $\pm$ SD). Demographic and clinical details in cohorts surveyed in 2006 and 2016 were also compared using these methods. Multivariate analyses of associations with neuropathy in 2016 were performed using multiple logistic regression modeling including all factors with $p < 0.25$ on univariate analyses, followed by a stepwise removal process. Odds ratios (OR) and 95% confidence intervals (95% CI) are presented.

Results and Discussion

After screening 2596 patients in 2016, 2399 patients were excluded due to either prior use of stavudine (684 patients), <12 months on ART (831 patients), withdrawal from ART (119 patients), diabetes mellitus (8 patients), history of stroke (8 patients), schizophrenia (3 patients), vasculitis (22 patients), deafness (2 patients), blindness (7 patients), hyperthyroid state (3 patients), systemic lupus erythematous (4 patients), cytomegalovirus radiculopathy (1 patient), a history of cancer

chemotherapy (6 patients) or refusal to participate (701 patients). 197 patients were tested for neuropathy. Demographic and clinical details are described in Table 1.

Twenty-eight patients (14.2%) were assessed as having neuropathy based on the ACTG-BPNS. Most were male (71%) and of Malay ethnicity (95%). Lamivudine was used by all patients, and zidovudine and nevirapine were used by more than 50% of patients, but choice of antiretroviral regimen was not associated with HIV-SN. Over 500 copies HIV RNA/ml and a nadir $CD4^+$ T-cell count below 200 cells/ul were associated with HIV-SN on univariate analyses. Whilst one might expect these parameters may mark the same people, patients with and without >500 copies HIV RNA/ml had similar nadir CD4 T-cell counts ($p = 0.31$). Before the era of combination ART, HIV-SN was associated with lower CD4 T-cell counts and higher plasma HIV RNA.[11] When patients receive effective therapy, CD4 T-cell counts typically improve and may not register as a risk factor,[12] but a lower nadir CD4 T-cell count has been described in patients on ART with HIV-SN,[13] as observed here.

Neither height, BMI, ethnicity, mode of HIV transmission, ART duration (Table 1), occupation nor educational status (not shown) associated with HIV-SN. Age, current CD4 T-cell count, isoniazid/pyridoxine therapy (consequent to tuberculosis) [9] and hepatitis C antibody were weakly associated with HIV-SN ($0.05 < p < 0.10$). We also assessed CMV antibodies as CMV is implicated in vascular pathology and antibodies may be used as a metric of the burden of CMV.[10] However levels of CMV antibodies were not associated with HIV-SN. All factors associating even weakly with HIV-SN ($p < 0.2$ on univariate analyses; age, nadir <200 CD4 T-cells, current CD4 T-cells, Hepatitis C antibody, HIV RNA and Isoniazid/Pyridoxine) were included in logistic regression modeling in a multivariate analysis, followed by a stepwise removal procedure. Carriage of >500 copies HIV RNA/ml [p=0.01, OR 5.2, 95% CI (1.5-18)] and increasing age [p=0.06, OR 1.1, 95% CI (0.9-1.1)] independently associated with the HIV-SN (model p=0.001, pseudo R^2=0.06). The association with HIV RNA levels is consistent with data from the pre-antiretroviral era[11,14] and suggests HIV-SN observed in these patients may have developed before ART.

We then compared the data with that obtained in the same clinic when 96 patients receiving stavudine were assessed using the ACTG-BPNS in 2006.[4] Compared with 2006, in 2016 the proportion of females was higher [13.5% in 2006 *vs* 28.9% in 2016; p = 0.003, OR 2.6, 95%CI (1.3-5)], fewer patients had

tuberculosis and received isoniazid/pyridoxine [58.3% in 2006 *vs* 45.2% in 2016; p = 0.05, OR 0.6, 95%CI (0.4-1)], the patients were older [30.2 ± 7.1 years in 2006 *vs* 35.7 ± 5.9 in 2016; p<0.001, mean difference 5.4, 95%CI (3.7-7) years] and shorter [167 ± 7.2 cm in 2006 *vs* 165 ± 7.7 cm in 2016; p=0.03, mean difference 2.1, 95%CI (0.3-3.9)], fewer patients had nadir counts <200 CD4 T-cells [85% in 2006 *vs* 67% in 2016; p = 0.001, OR 0.4, 95%CI (0.2-0.7)], fewer patients had current counts <200 CD4 T-cells [40% in 2006 *vs* 8.6% in 2016; p<0.001, OR 0.1, 95%CI (0.1-0.3)] and the prevalence of HIV-SN was lower [34% in 2006 *vs* 14.2% in 2016; p<0.001, OR 0.3, 95%CI (0.2-0.6)]. In 2006, age [p=0.04, OR 1.1, 95% CI (1-1.1)] and height [p=0.01, OR 1.1, 95% CI (1-1.2)] independently associated with the HIV-SN (model p=0.008, pseudo R^2= 0.08).

The lower observed prevalence of HIV-SN among HIV patients in our clinic who had never used stavudine compared with stavudine-exposed patients in 2006 is striking, as the 2016 cohort was older and included more females than the cohort studied in 2006, when female gender associated with resistance to HIV-SN. It is therefore likely that lack of stavudine exposure is central to the lower neuropathy prevalence observed in the current study.

Fewer patients in the current cohort had been treated for TB, compared with 2006, and the lower rate of isoniazid exposure may have impacted neuropathy prevalence. TB patients were prescribed standard therapy, including pyridoxine supplementation (25mg/day) throughout isoniazid use. Neuropathy was recognized as a side effect of isoniazid in the 1970's and has been described in 0.2%-10% of patients receiving this drug.[9] Although use of isoniazid was not associated with HIV-SN in 2006, it was weakly associated with HIV-SN in 2016. However, this was not independent of other observed associations, and the lower frequency of isoniazid use in the current cohort is unlikely to explain the lower observed HIV-SN prevalence.

Overall we show that HIV-SN remains common, affecting >14% of those treated for HIV in our clinic even though patients no longer use stavudine. Increasing age is known risk factor for HIV-SN.[5,7] Here HIV-SN patients were marginally older that those without neuropathy in both 2006 and 2016. Whilst links between HIV-SN and patient age on ART have been attributed to improved life expectancy, prolonged exposure to neurotoxic effects of stavudine and other potentially neurotoxic ART complicates these analyses[1,12]. Here other factors associated with HIV-SN in patients never exposed to

stavudine (2016 cohort) mirror associations with neuropathy among HIV patients in the pre-ART era, namely HIV replication and low nadir CD4 T-cell count,[11] highlighting the need for longitudinal studies as patients begin ART. Future work is needed to clarify the degree to which earlier initiation of treatment, as recommended by the WHO,[15] may prevent the development of HIV-SN. As even mild levels of HIV-SN impair quality of life, work is needed to understand why so many patients are still affected, and to inform efforts to prevent HIV-SN.

Table 1. Univariate analyses of risk factors for HIV-SN in 2016 and 2006

	2016			2006		
	SN free (n=169)	SN (n=28)	p	SN free (n=64)	SN (n=32)	p
Female gender[a]	28%	32%	0.66	19%	3%	0.03
Age, years[b]	35.3 ± 5.8	37.6 ± 6.5	0.06	29.4 ± 6.7	32 ± 7.6	0.12
Height, cm[b]	165 ± 8	165 ± 6	0.81	166 ± 7	170 ± 8	0.02
BMI[b]	22.2 ± 3.3	22.8 ± 3.7	0.39	20.7 ± 2.9	21.3 ± 2.7	0.17
Malay ethnicity	95%	91%	0.84	70%	58%	0.65
IVDU risk[a]	56%	54%	0.44	73%	67%	0.54
Hepatitis C antibody[a]	33%	50%	0.06	48%	58%	0.35
Isoniazid/Pyridoxine[a]	43%	60%	0.06	52%	64%	0.29
>500 copies HIV RNA /ml [a]	4%	18%	0.02	*Not done*	*Not done*	
Nadir <200 CD4 T-cells/ul[a]	65%	82%	0.05	86%	96%	0.43
Nadir CD4 T-cells/ul	122 (103-729)	65 (11-428)	0.25	53 (2-633)	33 (1-466)	0.2
Current <200 CD4 T-cells/ul[a]	8%	14%	0.2	32%	56%	0.02
Current CD4 T-cells/ul	447 (21-1166)	397 (103-729)	0.08	265 (11-1014)	187 (33-639)	0.11
CMV antibody ($AUx10^{-3}$)	27.7(0.52-365)	27.3(3.8-182)	0.7	*Not done*	*Not done*	
ART						
Stavudine	0	0		100%	100%	
Lamivudine	100%	100%		98%	100%	0.67
Zidovudine	85%	93%	0.20	60%	40%	0.82
Nevirapine	70%	79%	0.24	89%	61%	0.78
Efavirenz	47%	40%	0.29	49%	40%	0.65
Tenofovir	34%	36%	0.50	0	0	
Lopinavir/ Ritonavir	12%	14%	0.49	16%	10%	0.51
Emtricitabine	9%	7%	0.55	0	0	
ART duration, years[c]	4.1 (1 – 12.7)	5.4 (1 – 10.9)	0.33	1.3 (0.2-3.5)	1.2(0.1-3.3)	0.36

[a] categorical data assessed with Fischer's exact tests,

[b] mean ± standard deviation, assessed with Student's t tests (parametric data)

[c] median (range), assessed with Mann-Whitney tests (non-parametric data)

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