Low Prevalence of Transmitted HIV Type 1 Drug Resistance Among Antiretroviral-Naive Adults in a Rural HIV Clinic in Kenya

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Abstract

Low levels of HIV-1 transmitted drug resistance (TDR) have previously been reported from many parts of sub-Saharan Africa (sSA). However, recent data, mostly from urban settings, suggest an increase in the prevalence of HIV-1 TDR. Our objective was to determine the prevalence of TDR mutations among HIV-1-infected, antiretroviral (ARV)-naive adults enrolling for care in a rural HIV clinic in Kenya. Two cross-sectional studies were carried out between July 2008 and June 2010. Plasma samples from ARV-naive adults (>15 years old) at the time of registering for care after HIV diagnosis and before starting ARVs were used. A portion of the *pol* subgenomic region of the virus containing the protease and part of the reverse transcriptase genes was amplified and sequenced. TDR mutations were identified and interpreted using the Stanford HIV drug resistance database and the WHO list for surveillance of drug resistance strains. Overall, samples from 182 ARV-naive adults [mean age (95% CI): 34.9 (33.3–36.4) years] were successfully amplified and sequenced. Two TDR mutations to nucleoside reverse transcriptase inhibitors [$n=1$ (T215D)] and protease inhibitors [$n=1$ (M46L)] were identified, giving an overall TDR prevalence of 1.1% (95% CI: 0.1–3.9). Despite reports of an increase in the prevalence of HIV-1 TDR in some urban settings in sSA, we report a prevalence of HIV-1 TDR of less than 5% at a rural HIV clinic in coastal Kenya. Continued broader surveillance is needed to monitor the extent of TDR in sSA.

Introduction

THE WORLD HEALTH ORGANIZATION (WHO) estimates that from 2003 to 2009, there was a 13-fold increase in people receiving antiretroviral (ARV) therapy in low- and middle-income countries.¹ While the scale-up of ARV has resulted to substantial declines in reported HIV/AIDS-related morbidity and mortality,² various public health challenges have emerged. In particular, the limited range of available ARVs coupled with intermittent drug supplies, drug stock-outs, suboptimal prescription habits, and poor monitoring may accelerate the emergence and spread of HIV-1 drug-resistant strains in sub-Saharan Africa (sSA).^{3–5}

HIV-1 primary or transmitted drug-resistant (TDR) strains have been shown to compromise the effectiveness of standard first-line ARV regimens.^{6–8} Increasing levels of TDR therefore

have the potential to reverse the gains made from the scale-up of ARV, especially in areas where treatment options are limited.

The prevalence of TDR in sSA has previously been reported to be <5%.^{3,9–15} However, recent data suggest an increase in the prevalence of TDR in some settings. In Kampala, Uganda, the prevalence of TDR increased from 0% (2006–2007) to 8.6% (2009–2010).^{11,16} Albeit in small numbers, the IAVI Early infection cohort conducted among the most at risk populations in East and Central Africa has reported an increase in the prevalence of TDR in Zambia, from 0% (2005) to 16% (2009).¹⁷ A multisite, cross-sectional study conducted between 2007 and 2009 at 11 sites in sSA reported a 38% increase in risk of TDR with each additional year from the start of the local ARV roll-out.¹⁸ In Yaounde, Cameroon, a steady increase in the prevalence of TDR has also been observed, from 0%

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(1996–1999) to 12.3% (2007). Interestingly, the same study reports a TDR prevalence of 4.8% (2006–2007) in rural areas of Cameroon.¹⁹

In Kenya, a handful of studies have been done to assess the prevalence of TDR. A cross-sectional study in Nairobi in 2005 found 4/53 (7.5%) new clients had TDR.²⁰ The IAVI early infection cohort reported an overall TDR prevalence of 3.1% from three sites in Kenya.¹⁷ The multisite cross-sectional study from the PASER group, conducted between 2007 to 2009, reports TDR frequencies of 9/200 (4.5%) in Mombasa and 10/204 (4.9%) in Nairobi.¹⁸ Importantly and more recently, a cross-sectional survey among newly diagnosed ARV-naïve adults attending four VCT centers from Mombasa in 2009–2010 reported an overall TDR prevalence of 13.2%.²¹

Thus, most reports of increasing TDR are from urban settings with a longer history of ARV use,^{16,18,19} or among high-risk populations.¹⁷ These findings may therefore not necessarily be extrapolated to rural settings, which comprise the majority of those affected with HIV in sSA. However, a paucity of TDR data from rural sSA exists. We analyzed samples from ARV-naïve HIV-1-infected adults with an aim of determining the prevalence of TDR from a rural HIV clinic in Kenya.

Materials and Methods

Study site

The study was carried out at the Comprehensive Care and Research Clinic (CCRC) within Kilifi District Hospital (KDH) in Kenya. The hospital is located in a rural setting along the coastal line of Kenya, approximately 60 km north of Mombasa. The prevalence of HIV-1 infection among adults (15–49 years) in Kenya is estimated to be 6.3%,²² with approximately 1.7 million people living with HIV/AIDS by the end of 2011.

Like many other resource-limited settings, Kenya has adopted the public health approach in their provision of ARV. The country rolled out its ARV program to the public health sector in 2003. The recommended first line therapy comprises two nucleoside reverse transcriptase inhibitors (NRTIs) and one nonnucleoside reverse transcriptase inhibitor (NNRTI). Clients failing first line therapy are switched to a combination of two NRTIs and a boosted protease inhibitor (PI) as the recommended second line of choice.²³

The CCRC started providing ARVs to eligible HIV-infected clients in 2004. By the end of 2011, approximately half of the 7000 clients who had ever registered in the clinic had been started on first line therapy. Of the clients on treatment, less than 2% had been switched to a second line PI-based regimen.

Study design

This study was nested in a cohort that was set up in 2008 with the aim of describing long-term outcomes of HIV-infected adults enrolling for care at the CCRC. The Kenyan national AIDS and STI control guidelines recommend routine immunological monitoring at registration into HIV care and every 6 months (or when clinically indicated) thereafter.²³ Remnant blood from routine CD4 count blood draw at registration into care was separated to obtain plasma that was archived at –80°C in a repository for subsequent viral load quantification and genotyping. These tests were neither

routinely available in the clinic nor recommended by the national guidelines at the time of the study.

Two sets of cross-sectional samples among ARV-naïve HIV-infected adults (>15 years) collected at registration into HIV care and prior to ARV initiation were retrieved from the repository. In the first cross section, samples available from clients enrolling for care between July 2008 and June 2009 were retrieved and analyzed. Samples from this cross section have been used to describe subtype diversity of HIV-1 infection in our setting.²⁴

In the second cross section, clients who had been on ARV for 6–18 months and attending care at the CCRC between January and March 2011 were identified. Their baseline samples (immediately after enrolling for care and prior to ARV initiation) were retrieved and analyzed. The second cross section was carried out to increase the sample size and power to detect primary HIV-1 drug resistance in our setting. All eligible samples were shipped and analyzed at the Virus Reference Laboratory in the Health Protection Agency, London.

Sources of data

This has been described elsewhere.²⁵ In brief, socio-demographic, clinical, and immunological data are routinely captured in an electronic data system. Upon registration, data on sociodemographic characteristics of all clients are collected using standardized questionnaires by trained counselors. Clinical data are captured on standardized forms by trained clinicians at registration and all subsequent visits. Laboratory profiles including CD4 count results are also captured from routine investigations. These data are entered into an electronic data system by a trained data clerk.

HIV-1 subtyping, phylogenetic analysis, and resistance interpretation

Genotypic resistance testing was done using an in-house assay.^{26,27} In brief, the assay is designed to amplify and sequence a portion of the *pol* subgenomic region containing the protease and part of the reverse transcriptase genes. Sequences obtained were imported, manually edited, and assembled against a reference HIV sequence using the sequencer software (GeneCodes, version 4.1). *Pol* region subtype classification and recombinant patterns were determined using the Subtype Classification Using Evolutionary ALgorithm (SCUEAL) tool.²⁸

To determine the presence of resistance-associated mutations, sequences were submitted to the Stanford HIV drug resistance database using the calibrated population resistance tool. Surveillance drug resistance mutations were identified according to the WHO list for surveillance of genotypic transmitted drug resistance.²⁹

Statistical analysis

Our analysis is based on samples from a longitudinal repository obtained from remnant blood used in routine laboratory investigations. We did a *post-hoc* sample size calculation to determine whether the observed prevalence of TDR in the number of samples available would provide sufficient statistical precision for our findings. Generally, previous data from sSA report a TDR prevalence of <5%.³ Assuming a TDR prevalence of 2.5% in our setting, the risk of

ARV-naive adults registering for HIV care with TDR for a size of 180 patients will be estimated with a precision of $\pm 2.5\%$ at a 95% confidence interval.

Categorical data were presented using frequencies (column percentages) and compared using the chi-square test. Normally distributed continuous data were presented using means [95% confidence intervals (95% CI)] and compared using the Student's *t*-test. Data that were not normally distributed were presented using medians [interquartile ranges (IQR)] and compared using the Kruskal–Wallis non-parametric test. The overall TDR prevalence and its 95% binomial confidence intervals were determined as the number of sequences with TDR mutations identified from the total number of sequences included in the final analysis. All analyses were conducted using STATA version 11.0 (StataCorp, College Station, TX).

Ethical considerations

Scientific and ethical approval was granted by the Kenya Medical Research Institute, Scientific Steering Committee, National Ethical Review Committee (No. 1341). Participants provided written informed consent for their data and samples to be collected, archived, and used for research purposes.

Results

Cohort characteristics

Overall, 213 eligible samples from ARV-naive HIV-infected adults were retrieved and processed. All the clients were registered for HIV care and the samples collected between July 2008 and June 2010. The median time from registration into care and sample collection was 0.4 (IQR: 0.0–0.9) months. Thirty-one of the 213 samples failed to amplify, while 182 (85%) were successfully amplified, sequenced, and included in the analysis.

Samples from 91 (50%) clients were from the first cross section. At enrollment into care, the majority of the clients were female [$n=138$ (76%)], married in a monogamous relationship [$n=90$ (50%)], of Christian faith [$n=114$ (63%)], and had achieved primary level education [$n=94$ (52%)]. Clients from the second cross section were substantially older, more frequently referred for care from VCT centers, less well nourished, and had more advanced disease [mean CD4 count (95% CI), *p*-value: 127 (107–146) vs. 542 (503–580), $p<0.001$] compared to clients from the first cross section (Table 1).

HIV-1 subtypes

HIV-1 subtype A1 was the most common [$n=94$ (52%)], followed by HIV-1 subtype D [$n=21$ (12%)] and C [$n=8$ (4.4%)]. HIV-1 subtype G [$n=1$ (0.5%)] and A2 [$n=1$ (0.5%)] were also reported. Intersubtype recombinants were observed in 57 (31%) sequences, with A1/D being the most prevalent [$n=11$ (6.0%)]. Complex recombinants comprised 13 (7.1%) of the samples analyzed. There were no substantial differences in the distribution of HIV-1 subtypes between samples from the first and the second cross section ($p=0.214$).

Transmitted drug resistance

Of the 182 samples, only two were found to have primary resistance mutations, giving an overall TDR prevalence of 1.1%

(95% CI, 0.1–3.9). These mutations were T215D in reverse transcriptase from a client in the second cross section and M46L in protease from another client in the first cross section. Both clients were females, infected with HIV-1 subtype A1, and aged less than 25 years old. The minor drug resistance mutation V179T in reverse transcriptase was also observed from eight patients (Table 2). Major multiple drug resistance mutations within individual samples were not observed.

Discussion

Among ARV-naive adults registering for HIV care at a rural HIV clinic in Kenya, less than 5% were infected with a virus that contained primary drug resistance mutations. These findings are consistent with findings from many other SSA settings reporting low levels of TDR.^{3,10,11} However, while our data suggest a low TDR prevalence, a recent multisite study from Mombasa, the second capital of Kenya located barely 60 km south of Kilifi, has shown a considerably higher overall TDR prevalence of 13.2% (95% CI, 6.2–23.6).²¹ The confidence intervals in the prevalence of TDR from the two sites do not overlap, which excludes randomness of these observations. Taken together, this suggests that even within a small geographic location, the extent and profile of TDR may vary substantially. A similar variation has been observed in Cameroon where a TDR prevalence of 12.3% was reported from their capital city, compared to 4.8% observed in adjacent rural areas.¹⁹ The IAVI early infection cohort also reports a significantly higher prevalence of TDR in urban centers compared to rural centers.¹⁷

The emergence of TDR in communities has been largely attributed to the longer availability of ARVs.^{18,30} It is therefore possible that ARVs were made available in established private health care institutions, mostly located in urban areas, long before they were rolled out to the public health sector. It has also been stipulated that the higher the ARV coverage, the higher the risks of the emergence and spread of TDR.^{31–33} Unlike in rural areas, urban areas have adequate infrastructure to ensure optimal coverage of ARV to those in need. This may explain, to some extent, the variations in the prevalence of TDR observed between rural and urban areas.

The two TDR mutations observed, T215D and M46L, are associated with resistance to NRTIs and PIs, respectively. It is striking that no NNRTI mutations were seen, despite the extensive use of nevirapine and efavirenz as first line therapy in this population. It is also interesting that we found a primary PI mutation, especially given that <2% of those on treatment have ever been started on second line regimen in our setting. These data may therefore suggest the introduction of HIV-1 drug-resistant strains, complementing findings from a previous study suggesting multiple introductions of HIV-1 subtypes into this area.²⁴ The V179T mutation observed in some clients is associated with reduced response to etravirine, but is likely present as a naturally occurring polymorphism.

HIV-1 subtype A1 was the most prevalent subtype observed, with intersubtype recombinants comprising almost a third of the sampled population. The high proportion of intersubtype recombinants is similar to that previously reported for this area.²⁴ It is also consistent with data from other recent studies done along the coastal parts of East Africa^{21,34} and in Nairobi, the capital of Kenya.³⁵ It has been suggested that the high proportions of intersubtype and complex recombinants

TABLE 1. DISTRIBUTION OF BASELINE CHARACTERISTICS OF ANTIRETROVIRAL-NAIVE HIV-INFECTED ADULTS REGISTERED FOR ROUTINE HIV CARE AND PRIOR TO STARTING ANTIRETROVIRALS IN A RURAL DISTRICT HOSPITAL IN KENYA [FREQUENCY (COLUMN %), N=182]

Risk factor	Categories	Cross sections			p-value ^a
		Total (n=182)	Section 1 (n=91)	Section 2 (n=91)	
Gender	Male	44 [24.2]	19 [20.9]	25 [27.5]	0.299
	Female	138 [75.8]	72 [79.1]	66 [72.5]	
Age (years) ^b	Mean	34.9	31.3	38.5	<0.001
	[95% CI]	[33.3–36.4]	[29.3–33.3]	[36.4–40.6]	
Age group (years)	15.0–24.9	32 [17.6]	25 [27.5]	7 [7.7]	<0.001
	25.0–34.9	65 [35.7]	38 [41.8]	27 [29.7]	
	≥ 35.0	85 [46.7]	28 [30.8]	57 [62.6]	
Marital status	Single	14 [7.7]	8 [8.8]	6 [6.6]	0.705
	Married monogamous	90 [49.5]	48 [52.8]	42 [46.2]	
	Married polygamous	26 [14.3]	13 [14.3]	13 [14.3]	
	Separated/divorced	31 [17.0]	14 [15.4]	17 [18.7]	
	Widowed	21 [11.5]	8 [8.8]	13 [14.3]	
Entry point	In-patient wards	21 [11.5]	17 [18.7]	4 [4.4]	0.003
	Out-patient	96 [52.8]	49 [53.9]	47 [51.7]	
	VCT centers	65 [35.7]	25 [27.5]	40 [43.9]	
Religion	Christian	114 [62.6]	60 [65.9]	54 [59.3]	0.811
	Muslim	36 [19.8]	17 [18.7]	19 [20.9]	
	Other religions	5 [2.8]	2 [2.2]	3 [3.3]	
	No religion	27 [14.8]	12 [13.2]	15 [16.5]	
Education status	No schooling	55 [30.2]	26 [28.8]	29 [31.9]	0.911
	Primary schooling	94 [51.7]	49 [53.9]	45 [49.5]	
	Secondary schooling	28 [15.4]	14 [15.4]	14 [15.4]	
	Higher education	5 [2.8]	2 [2.2]	3 [3.3]	
WHO staging	Stage I/II	149 [81.9]	83 [91.2]	66 [72.5]	0.001
	Stage III/IV	33 [18.1]	8 [8.8]	25 [27.5]	
BMI (kg/m ²) ^b	Mean	20.8	21.4	20.1	0.032
	[95% CI]	[20.2–21.4]	[20.7–22.1]	[19.2–21.1]	
BMI groups (kg/m ²)	< 18.5	54 [29.7]	17 [18.7]	37 [40.7]	0.003
	18.5–25.0	104 [57.2]	64 [70.3]	40 [44.0]	
	> 25.0	17 [9.3]	8 [8.8]	9 [9.9]	
	Missing	7 [3.9]	2 [2.2]	5 [5.5]	
MUAC (cm) ^b	Mean	24.8	25.4	24.1	0.014
	[95% CI]	[24.2–25.3]	[24.8–26.1]	[23.2–25.0]	
MUAC groups (cm)	< 18.5	5 [2.8]	1 [1.0]	4 [4.4]	0.076
	18.5–25.0	96 [52.8]	42 [46.2]	54 [59.3]	
	> 25.0	76 [41.8]	46 [50.6]	30 [33.0]	
	Missing	5 [2.8]	2 [2.2]	3 [3.3]	
CD4 count ^b (cells/μl)	Mean	336.5	541.7	126.6	<0.001
	[95% CI]	[299–374]	[503–580]	[107–146]	
CD4 groups (cells/μl)	< 350	88 [48.4]	1 [1.1]	87 [95.6]	<0.001
	351–500	45 [24.7]	44 [48.4]	1 [1.1]	
	> 500	45 [24.7]	45 [49.5]	0 [0.0]	
	Missing	4 [2.2]	1 [1.1]	3 [3.3]	

^aChi-squared test *p*-values for categorical data and paired Student's *t*-test *p*-values for continuous data.

^bMean [95% confidence intervals (CI)] for continuous variables.

WHO staging, BMI, MUAC and CD4 count at time of sampling.

BMI, body mass index; MUAC, mid-upper arm circumference; VCT, voluntary counseling and testing; WHO, World Health Organization.

observed may have resulted from multiple introductions of HIV-1 subtypes, which has been attributed to the diverse transport networks linking Mombasa and its environs, including Kilifi, to other parts of Africa.²⁴ The extent to which the prevalence and diversity of HIV-1 subtypes might affect the emergence and spread of HIV-1 drug resistance in sSA remains uncertain, although weak evidence of reduced

risk for TDR among clients with intersubtype recombinant (A/G) when compared to a pure subtype (A) has been reported.¹⁸

To our knowledge, this is among the first TDR surveillance studies to analyze sufficiently large numbers of routine samples from ARV-naïve HIV-1-infected clients prior to ARV initiation from a rural HIV clinic in sSA. This may be regarded

TABLE 2. DISTRIBUTION OF HIV-1 DRUG-RESISTANT MUTATIONS DETECTED AMONG ANTIRETROVIRAL-NAIVE ADULTS ENROLLING FOR CARE IN A RURAL HIV CLINIC IN KENYA (N=182)

Number	Cross section	Gender	Date	Age (years)	Staging	CD4 count	BMI	HIV-1 subtype	Primary resistance mutations		
									NRTI	NNRTI	PI
RS09000869	1	Female	19 Feb 2009	18.7	1	610	17.6	A1		V179T	
RS09000881	1	Female	20 Apr 2009	21.9	2	558	21.2	A1		V179T	
RS09000918	1	Female	30 Apr 2009	28.9	1	416	20.0	A1		V179T	
RS09000923	1	Female	18 May 2009	29.9	1	557	20.7	A1		V179T	
RS09000926	1	Female	20 May 2009	23.8	2	486	27.2	C		E138A	
RS09000933	1	Female	27 May 2009	22.9	1	535	20.9	A1			M46L ^a
RS09000941	1	Male	10 Jun 2009	37.0	3	437	14.8	A1	T69S		
RS09000953	1	Female	07 Aug 2008	27.2	1	506	23.1	Complex		V90IV	
RS09000969	1	Female	07 Aug 2008	30.2	1	551	17.3	A1		V179T	
RS11000235	2	Female	06 Feb 2009	41.7	3	105	18.4	D	T69NT		
RS11000248	2	Female	12 Aug 2008	29.2	3	110	21.0	A1		E138A	
RS11000258	2	Female	09 Oct 2008	42.3	2	238	20.3	A1		V179T	
RS11000262	2	Female	08 Apr 2009	31.8	2	150	20.0	A1			I54IL
RS11000275	2	Female	04 Dec 2008	40.5	2	161	16.3	A1		K103R	
RS11000277	2	Female	20 Nov 2008	29.4	3	253	19.3	A1		E138A	
RS11000278	2	Male	21 Jan 2009	30.6	2	3	19.7	A1		E138A	
RS11000288	2	Female	06 Jan 2009	37.6	1	168	27.1	A1		V179T	
RS11000294	2	Male	08 Jan 2009	41.6	2	199	17.7	Complex	T69ST		
RS11000306	2	Female	05 Jun 2009	37.0	3	(n/a)	15.3	D		V90I	
RS11000315	2	Male	24 Mar 2009	48.8	4	18	13.5	D		E138G	
RS11000335	2	Female	29 Sep 2009	46.3	2	145	26.9	A1		V179T	
RS11000342	2	Female	08 Oct 2009	16.4	2	10	17.2	A1	T215D ^a		
RS11000359	2	Female	11 May 2010	30.9	2	27	20.8	D		V90IV	

^aTransmitted drug resistance (TDR) mutations as identified from the WHO updated list of surveillance drug resistance mutations.

Date, age, staging, CD4 count, and BMI (body mass index) at point of sampling.

HIV-1 subtype using 'Subtype Classification Using Evolutionary ALgorithm (SCUEAL)' tool.

NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitors; PI, protease inhibitors; (n/a), not available.

as a strength of this study. However, our findings have to be interpreted in light of several limitations.

First, our study differed slightly from the WHO recommended HIV drug resistance threshold survey (HIVDR-TS).³ While the HIVDR-TS approach is resource minimized, it may not be feasible to many ARV programs in sSA where the majority of sites are too small to recruit sufficient numbers of clients meeting the stringent criteria recommended. Indeed, only 32 clients were less than 25 years old in our study. If we had used a "modified" HIVDR-TS context, the two primary resistance mutations observed would each yield a TDR prevalence of 3.1% (95% CI: 0.1–16.2) for the NRTI and PI groups of ARVs. This is still considered low-level prevalence TDR for these classes of ARVs, albeit with wide confidence intervals. In addition, recent data from Botswana's national HIVDR-TS have shown that the recommended WHO criteria performed poorly in identifying recent HIV-1 infections when compared to two laboratory-based methods.¹⁰

Second, it can be argued that our study population may have included clients with chronic HIV infection, which may have provided an opportunity for reversion of mutant variants to wild-type virus. This may result in underestimation in the true prevalence of TDR in our population. However, it has also been shown that transmitted resistant variants may persist for long periods of time in ARV-naïve individuals.^{30,36–38} In addition, and although we were underpowered, no difference in the prevalence of TDR was observed among samples from the second cross section of "chronic" clients compared to those from the first cross section.

Lastly, samples retrieved for analysis were from clients who reported never having taken ARV for treatment or prophylaxis prior to enrollment into care. There remains the possibility that the two clients with TDR had undisclosed previous exposure to ARV. However, this is less likely for the T215D mutation since this is known to be an indicator of transmitted resistance following transmission by back mutation from the standard NRTI mutation T215Y.³⁹

In conclusion, while recent data, mostly from urban settings, suggest an increase in the prevalence of HIV-1 TDR in sSA, we report low levels of primary HIV-1 drug resistance from a rural HIV clinic in Kenya. It is also emerging that even within a small geographic region, substantial variations in the extent and profiles of HIV-1 TDR may be observed in a resource-limited setting. As programs scale up the coverage of ARV, and as communities become more exposed to ARVs with time, the emergence and spread of TDR in sSA are inevitable. More surveillance data from diverse geographic regions are needed to describe the extent and profiles of TDR with an aim of making informed public health decisions to influence treatment guidelines and drug choices in sSA.

Sequence Data

The sequences reported in this article have accession numbers HQ441597–HQ441717 and JQ698340–JQ698430.

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Author Disclosure Statement

The authors declare no competing financial interests.

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