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ORIGINAL RESEARCH

Hair Zidovudine Concentrations Predict Virologic Outcomes Among People Living with HIV/AIDS in China

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Background: Hair antiretroviral concentrations are an objective and non-invasive measure of adherence to long-term antiretroviral therapy (ART) and can further predict virologic outcomes among people living with HIV/AIDS (PLWH). Zidovudine, one of the mainstream antiretrovirals in China, has been verified to have high reliability in adherence assessment, especially for its hair concentrations. However, data are limited in its predicting virologic outcomes. Therefore, this study aimed to characterize whether hair zidovudine concentrations can predict virologic suppression among Chinese PLWH compared with hair lamivudine concentrations and two self-reported measures, the overall frequency of adherence behaviors and percentage adherence.

Methods: This cross-sectional study randomly recruited 564 PLWH currently treated with zidovudine, lamivudine, and other ART agents (efavirenz, nevirapine, or lopinavir/ritonavir) in Guangxi, China. Hair antiretroviral concentrations were determined using the LC-ESI$^+$-MS/MS method. Receiver operating characteristic (ROC) curves were used to estimate the optimal classification thresholds of hair concentrations of zidovudine and lamivudine, and the two self-reported measures. Based on those optimal classification thresholds, logistic regression was used to examine whether those four adherence measures can predict virologic suppression (HIV-1 RNA <200 copies/mL).

Results: ROC curves demonstrated good classification performance for association with virologic suppression of zidovudine with the optimal threshold at 58 pg/mg and lamivudine at 255 pg/mg but no self-reported measures. PLWH with hair zidovudine concentrations >58 pg/mg had an adjusted odds ratio (aOR) of 43.191 (95% confidence interval (CI) = 10.171–183.418, $p < 0.001$) for virologic suppression. Hair lamivudine concentrations were also associated with virologic suppression (aOR = 10.656, 95% CI = 3.670–30.943, $p < 0.001$). However, two self-reported measures did not predict virologic suppression (aORs = 1.157 and 2.488, ps >0.149).

Conclusion: Hair zidovudine concentrations can be served as an alternative tool for clinically predicting virologic suppression among PLWH in China.

Keywords: hair antiretroviral concentrations, zidovudine, virologic suppression, PLWH, LC-MS/MS

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Introduction

Antiretroviral therapy (ART) as the cornerstone of the human immunodeficiency virus (HIV) infection prevention and management can effectively reduce HIV-related morbidity, mortality, and transmission.[1–3] Actually, optimal ART highly depends on adequate adherence to ART.[4–6] Previous studies have revealed that ART adherence is a strong predictor of treatment outcomes, including virologic suppression, immune restoration, and clinical progression.[7–10] Therefore, a reliable measure of ART adherence can be used to successfully predict virologic suppression, which is vital to promote treatment outcomes among people living with HIV/AIDS (PLWH).

Traditionally, self-report with low cost and high convenience was commonly utilized for ART adherence assessment. However, as a subjective measure, it tends to represent skewed or overestimated adherence due to recall and social desirability biases.[11,12] Given the pharmacological relationship between ART adherence and antiretroviral exposure, researchers further employed pharmacologic indicators of antiretroviral exposure as objective adherence measures.[13,14] Among them, random or untimed antiretroviral concentrations in plasma and urine only reflect ART adherence within several hours or days. Meanwhile, the plasmatic and urinary measures may be susceptible to time-to-time, day-to-day and intra-individual variations,[15,16] and "white-coat" effects.[17] Moreover, antiretroviral concentrations in peripheral blood mononuclear cells or dried blood spots (DBS) can assess longer-term adherence. However, there are some difficulties in collecting and processing peripheral blood mononuclear cells.[14] DBS antiretroviral concentrations may also be impacted by some patient-specific characteristics (gender and ethnicity).[18–20] In contrast, hair antiretroviral concentrations can show stable and long-term retrospectives over weeks to months. Additionally, hair samples are easy and non-invasive to collect and can be stored and shipped at room temperature without biohazardous precautions.[16,21]

Nowadays, over twenty-five antiretrovirals in multiple classes have been approved for HIV-related clinical treatment.[22] Some studies have proved that the hair concentrations of several mainstream antiretrovirals can be used to reliably assess long-term adherence and further strongly predict virologic outcomes, such as lamivudine,[23,24] tenofovir,[25,26] efavirenz,[24,27–30] nevirapine,[27,31] ritonavir,[27,29,32,33] lopinavir,[27,29,30,33–36] and atazanavir.[27,37–39] Basically, these previous studies investigated common antiretroviral agents from ART regimens recommended by the WHO and/or the National Free Antiretroviral Treatment Program of China.[40] Notably, zidovudine combined with other antiretroviral agents as the backbone of the first-line and second-line ART regimens are widely prescribed to Chinese PLWH.[40] However, data are limited on its hair concentrations in predicting virologic outcomes in China. Moreover, as indicated by our previous findings, hair zidovudine concentrations can be a reliable measure of long-term adherence, including showing a significant difference between PLWH with high and low adherences and significant correlations with hair concentrations of other antiretrovirals.[41,42] In addition, antiretrovirals from an identical ART regimen have their individual characteristic effects in predicting virologic outcomes as evidenced by a previous study.[43] Notably, they also show significant inter-drug differences in hair concentration because of their differences in the metabolisms of the circulation system and the incorporation mechanism into the hair shaft.[44] Therefore, each antiretroviral may exhibit a unique classification threshold for predicting virologic outcomes.

Consequently, this study is intended to characterize whether hair zidovudine concentrations can predict virologic suppression and further define its optimal classification threshold among Chinese PLWH compared with the previously validated hair lamivudine concentrations and self-reported adherence measures.

Materials and Methods

Participants

Participants were from the baseline population of a longitudinal cohort study from 2017 to 2021 that aimed to explore the causal linkage between HIV-related stigma and clinical outcomes among PLWH. PLWH treated with zidovudine, lamivudine, and other agents (efavirenz, nevirapine, or lopinavir/ritonavir) were invited. With the assistance and collaboration of the Guangxi Center for Disease Prevention and Control (Guangxi CDC), a total of 564 PLWH were finally randomly recruited for this study. The sample size was calculated using the program for longitudinal study with repeated measurements (please see Supplemental Materials S1 for more details).

Sociodemographic and Clinical Characteristics

All participants reported their sociodemographic characteristics by completing a questionnaire,[24] including age, body mass index (BMI) calculated from weight and height, gender, ethnicity, education level, marital status, employment status, and average monthly income.

The clinical characteristics were extracted from their clinical records, including date of confirmed HIV diagnosis, current ART regimens, date of taking the current ART regimen, recent HIV viral loads, and recent CD4 cell counts (test results during the six months before hair sample collection). Durations of HIV diagnosis and current ART were from the date of initial diagnosis and the start date of the current ART regimen to the date of the questionnaire interview, respectively. Moreover, according to the National Free Antiretroviral Treatment Program in China, the ART regimens included both first-line regimen (lamivudine + zidovudine + efavirenz and lamivudine + zidovudine + nevirapine) and second-line regimen (lamivudine + zidovudine + lopinavir/ritonavir).[40] In addition, virologic outcomes were catalogued into virologic suppression and failure according to the criteria where virologic suppression was defined at recent viral loads <200 copies/mL and virologic failure at recent viral loads ≥200 copies/mL.[45] Recent CD4 cell counts were divided into two levels with the cut-off value at 400 cells/mm^3, which was regarded as the lowest reference value for Guangxi healthy people.[46]

Two questions were employed to measure self-reported adherence. Overall frequency of adherence behaviors (how strictly did you take your medication following doctor's advice in the last one month?) has been validated by Lu et al.[47] The response is a 5-point option (none of the time, few of the time, some of the time, most of the time, and all of the time)[47] and then quantified in 25% increments for frequency estimation (eg, none of the time = 0% and all of the time = 100%),[24,48,49] which showed good performances in predicting viral loads and CD4 cell counts.[50] Percentage adherence (percent of 30 days taken; how many days they took all needed antiretrovirals during the last 30 days) validated by Da et al[51] was also determined in the present study. The sociodemographic and clinical characteristics of all participants are listed in Table 1.

Hair Sample Collection and Analyses

The whole hair strands in the participant's posterior vertex region were cut as close to the scalp as possible after the questionnaire interview and wrapped in foil paper by labeling the segment closest to the scalp. Thereafter, the hair strands were stored in the envelope at room temperature and finally posted to our analysis laboratory at Southeast University. Only the 1-cm hair segment closest to the scalp that reflects the cumulative antiretroviral drugs' exposures during the past month before the interview and matches the timespan of questionnaire information was cut for the following analysis.

Hair samples were analyzed with the method recently developed in our lab.[42] Briefly, the collected 1-cm hair samples were rinsed twice with 2 mL methanol and then dried under pure nitrogen gas at 50°C. Thereafter, the dried samples were cut into pieces (1–2 mm) and weighed at 10 mg for analysis. The zidovudine and lamivudine in each sample were extracted via methanol and internal standard at 37°C for 16 hours. After that, the extracted solution was vortexed, separated by centrifugation, evaporated and redissolved for the following liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. The reconstituted samples were separated by 1200 high-performance liquid chromatography (Agilent, Waldbronn, Germany) with a reverse phase C18 column (5 μm, 150 mm × 4.6 mm; Dikma) and detected by 3200 Qtrap tandem mass spectrometry (ABI, Foster City, CA) using a positive electrospray ionization operating in multiple reactions monitoring mode. The linear ranges (R^2> 0.994) of standard curves were within 10–5000 pg/mg for zidovudine and 6–5000 pg/mg for lamivudine, respectively. Other method validation parameters (eg, inter- and intra-day precisions, recovery, matrix effect, and stability) also met the acceptance criteria following the US Food and Drug Administration and European Medicines Agency guidelines as previously reported.[42]

Statistical Analyses

Statistical analyses were conducted by the statistical package SPSS 24.0 for windows (SPSS Inc., Chicago, IL). Descriptive statistics were used to describe sociodemographic and clinical characteristics, hair antiretroviral concentrations, and self-reported adherence measures. Categorical variables were shown with numbers (n) and percentages (%). Shapiro–Wilk test was used to examine the distributed normality of all continuous variables. Normally distributed variables were presented as mean plus standard deviation, and non-normally distributed variables were median and

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Table 1 Descriptions, Intergroup Differences and Correlations with Virologic Outcomes and Hair Antiretroviral Concentrations for Sociodemographic and Clinical Characteristics of PLWH

	Total n = 564 (100%) [a]	Virologic Suppression n = 541 (95.9%)[a]	Virologic Failure n = 23 (4.1%)[a]	Statistic Values[b]	Correlations		
					Virologic Outcomes[c]	AZT	3TC
Age, years	39.81 ± 8.40	39.88 ± 8.42	38.17 ± 7.84	$t = -0.952$	$r = 0.032$	$r = 0.172^{**}$	$r = 0.012$
BMI, kg/m^2	21.68 ± 2.63	21.68 ± 2.65	21.65 ± 2.27	$t = -0.048$	$r = 0.007$	$r = 0.065$	$r = -0.084^{*}$
Gender				$\chi^2 = 6.130^{*}$	$r = -0.104^{*}$	$r = -0.104^{*}$	$r = -0.345^{**}$
Male	325 (57.6%)	306 (56.6%)	19 (82.6%)				
Female	239 (42.4%)	235 (43.4%)	4 (17.4%)				
Ethnicity				$\chi^2 = 0.001$	$r = -0.001$	$r = 0.001$	$r = 0.042$
Han	342 (60.6%)	328 (60.6%)	14 (60.9%)				
Non-Han	222 (39.4%)	213 (39.4%)	9 (39.1%)				
Education levels				$\chi^2 = 4.119^{*}$	$r = 0.085^{*}$	$r = -0.074^{+}$	$r = -0.013$
>9 years	183 (32.5%)	180 (33.3%)	3 (13.0%)				
≤9 years	381 (67.6%)	361 (66.7%)	20 (87.0%)				
Marital status				$\chi^2 = 4.180^{*}$	$r = 0.086^{*}$	$r = 0.014$	$r = 0.082^{+}$
Married	401 (71.1%)	389 (71.9%)	12 (52.2%)				
Others	163 (28.9%)	152 (28.1%)	11 (47.8%)				
Employment status				$\chi^2 = 0.009$	$r = 0.004$	$r = -0.011$	$r = -0.137^{**}$
Employed	470 (83.3%)	451 (83.4%)	19 (82.6%)				
Unemployed	94 (16.7%)	90 (16.6%)	4 (17.4%)				
Average monthly income, Chinese Yuan				$\chi^2 = 2.290$	$r = 0.064$	$r = -0.091^{*}$	$r = -0.019$
≥2000	354 (62.8%)	343 (63.4%)	11 (47.8%)				
<2000	210 (37.2%)	198 (36.6%)	12 (52.2%)				
HIV diagnosis duration, months	79.00 (48.25–102.75)	80.00 (50.00–104.00)	42.00 (26.00–72.00)	$Z = -4.134^{**}$	$r = 0.174^{**}$	$r = 0.002$	$r = 0.137^{**}$
Current ART duration, months	71.00 (42.00–95.00)	72.00 (42.00–95.50)	39.00 (20.00–57.00)	$Z = -3.843^{**}$	$r = 0.162^{**}$	$r = 0.016$	$r = 0.133^{**}$

Current ART regimens [d]					χ^2 = 0.983	*r* = 0.042	*r* = 0.123**	*r* = −0.164**
First-line		461 (81.7%)	444 (82.1%)	17 (73.9%)				
	3TC+AZT+EFV	223 (39.5%)	210 (38.8%)	13 (56.5%)				
	3TC+AZT+NVP	238 (42.2%)	234 (43.3%)	4 (17.4%)				
Second-line	3TC+AZT+LPV/r	103 (18.3%)	97 (17.9%)	6 (26.1%)				
Recent CD4 counts, cell/mm^3					χ^2 = 5.352*	*r* = 0.097*	*r* = −0.002	*r* = 0.066
≥400		350 (62.1%)	341 (63.0%)	9 (39.1%)				
<400		214 (37.9%)	200 (37.0%)	14 (60.9%)				
Hair AZT concentrations, pg/mg		336 (211–595)	342 (217–605)	186 (10–366)	Z = −3.699**	*r* = 0.156**	-	-
Hair 3TC concentrations, pg/mg		456 (281–654)	465 (300–659)	182 (25–343)	Z = −4.961**	*r* = 0.209**	*r* = 0.335**	-
Self-reported adherence [e], %								
Overall frequency		95.26 ± 11.19	95.29 ± 11.22	94.57 ± 10.54	Z = −0.553	*r* = 0.023	*r* = −0.005	*r* = 0.042
Percent of 30 days taken		98.72 ± 7.26	98.89 ± 6.05	94.75 ± 20.79	Z = −0.880	*r* = 0.037	*r* = 0.024	*r* = 0.002

Notes: $^+p<0.1$; $^*p<0.05$; $^{**}p<0.01$. [a]Categorical variables were exhibited as number (n) and percentages (%). Continuous variables were, respectively, expressed as median plus interquartile range for non-normally distributed variables and as mean (*M*) plus standard deviation (*SD*) for normally distributed variables. [b]Independent samples *t*-test (for normally distributed continuous variables), chi-square test (for categorical variables) and Wilcoxon rank-sum test (for non-normally distributed continuous variables) were used to compare group difference. [c]The associations of different variables with virologic outcomes were estimated using the correlations between the variables and virologic grouping where virologic suppression group was set at 1 and virologic failure group was 0. [d]The first-line ART regimen group was set at 1 and the second-line ART regimen group was 0 for statistical analysis. [e]Self-reported adherence measures were expressed with *M*±*SD* because over 75% data were 100%.

Abbreviations: AZT, zidovudine; 3TC, lamivudine; BMI, body mass index; ART, antiretroviral therapy; EFV, efavirenz; NVP, nevirapine; LPV/r, lopinavir/ritonavir.

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interquartile range. *Chi*-square test, independent samples *t*-test, and Wilcoxon rank-sum test were successively performed to assess the intergroup differences of the three types of variables. Spearman correlation analysis was used to examine the correlations of different variables with virologic outcomes and hair antiretroviral concentrations. Receiver operating characteristic (ROC) curves were employed to estimate the classification performances in predicting virologic suppression of the hair antiretroviral concentrations and self-reported measures and their optimal classification thresholds. Furthermore, the univariate logistic regression was used to evaluate the predictive utilities of the hair and self-reported measures. The multivariate logistic regression was used to further estimate their predictive utilities by adjusting sociodemographic and clinical characteristics correlated with virologic suppression or hair antiretroviral concentrations. Additionally, in those logistic regression models, participants were successively catalogued into two groups based on the classification thresholds of the four adherence measures estimated with the ROC curves.

Ethical Statement

The present study followed the Declaration of Helsinki and obtained ethical approvals from the Institutional Review Boards of Southeast University (2018ZDKYSB009), Guangxi CDC (GXIRB2016-0010-4), and the University of South Carolina (Pro00062264). In addition, all participants provided written informed consent before inclusion.

Results

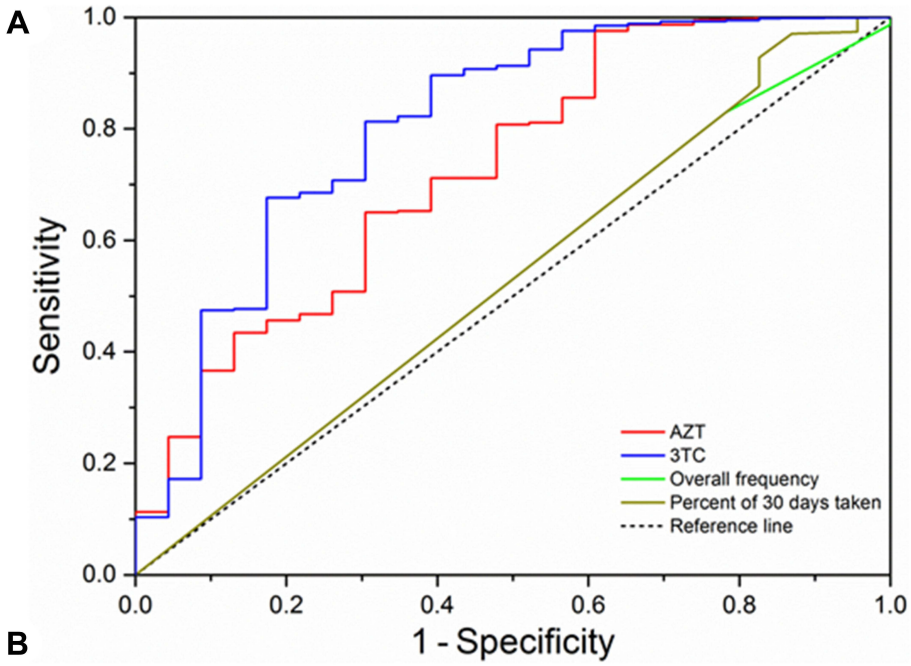
Sociodemographic and Clinical Characteristics

As listed in Table 1, 57.6% (n = 325) of participants were male and 42.4% (n = 239) were female. Among them, 39.5% (n = 223), 42.2% (n = 238), and 18.3% (n = 103) were receiving lamivudine + zidovudine + efavirenz, lamivudine + zidovudine + nevirapine and lamivudine + zidovudine + lopinavir/ritonavir, respectively. Furthermore, 62.1% achieved high CD4 counts, and 95.9% had experienced virologic suppression. The participants with virologic suppression showed a lower proportion of males than those with virologic failure (56.6% vs 82.6%) and higher proportions of higher education levels and married status (33.3% vs 13.0% and 71.9% vs 52.2%). Thus, gender, education levels, and marital status showed significant correlations with virologic suppression (rs=−0.104–0.086, ps<0.042), respectively. Similarly, the participants with virologic suppression showed a longer duration of HIV diagnosis and current ART, and especially higher recent CD4 cell counts than those with virologic failure (ps<0.021). Accordingly, there were significant and positive correlations between durations of HIV diagnosis and current ART, and recent CD4 counts with virologic suppression (rs=0.097–0.174, ps<0.021). In contrast, the rest sociodemographics and clinical characteristics were only significantly correlated with hair zidovudine concentrations (rs=−0.091–0.172, ps<0.031 for age, average monthly income, and ART regimen group) or hair lamivudine concentrations (rs=-0.164−-0.084, ps<0.046 for BMI, employment status, and ART regimen group).

The Associations of Hair Antiretroviral Concentrations and Self-Reported Measures with Virologic Outcomes

The associations of hair antiretroviral concentrations and self-reported measures with virologic outcomes were estimated by examining their differences between the virologic suppression and failure groups, and their correlations with virologic suppression. As listed in Table 1, hair zidovudine concentrations exhibited significant intergroup difference (p<0.001) and were positively correlated with virologic suppression (p<0.001). Likewise, patients with virologic suppression showed significantly higher hair lamivudine concentrations than those with virologic failure (p<0.001). Meanwhile, there was a significant correlation between hair lamivudine concentrations and virologic suppression (p<0.001). Fisher Z test revealed that there was no significant difference between the two correlation coefficients of hair antiretroviral concentrations with virologic suppression (Z=0.918, p>0.05). In contrast, the two self-reported measures presented no significant intergroup difference and correlation with virologic suppression (ps > 0.379). The detailed graphs of the correlation between four adherence measures and virologic suppression were shown in Figure S1 in Supplemental Materials. Additionally, there was a significant correlation between hair concentrations of zidovudine and lamivudine (p < 0.001) but were weak correlations of hair antiretroviral concentrations with the two self-reported measures (ps > 0.570).

A



B

	AZT	3TC	Overall frequency	Percent of 30 days taken
AROC	0.728	0.805	0.522	0.531
95% CI	0.615–0.840	0.698–0.912	0.399–0.645	0.404–0.659
Threshold	58 pg/mg	255 pg/mg	87.5%	95.0%
Sensitivity, %	97.6	81.3	83.0	92.8
Specificity, %	39.1	69.6	21.7	17.4
Youden Index, %	36.7	50.9	4.7	10.2
CC, %	95.2	80.9	80.5	89.7

Figure 1 Receiver operating characteristic curves (**A**) and its detailed descriptions (**B**) for hair antiretroviral concentrations and self-reported adherence measures.
Abbreviations: AZT, zidovudine; 3TC, lamivudine; AROC, area under receiver operating characteristic curves; CI, confidence interval; CC, classification correction.

Classification Performance in Predicting Virologic Suppression

As shown in Figure 1, the classification performance of each measure in the prediction of virologic suppression was estimated through ROC curve. Hair zidovudine concentrations showed good classification performance in predicting virologic suppression [area under the ROC curve (AROC)=0.728, 95% confidence interval (CI)=0.615–0.840, $p<0.001$], which matched with hair lamivudine concentrations (AROC = 0.805, 95% CI = 0.698–0.912, $p<0.001$). In contrast, the two self-reported measures had worse classification performances (AROC = 0.522, 95% CI = 0.399–0.645 for overall frequency and AROC = 0.531, 95% CI = 0.404–0.659 for percent of 30 days taken, $ps>0.612$).

Additionally, the optimal thresholds (that maximized the Youden Index) for predicting virologic suppression were calculated at 58 pg/mg for hair zidovudine concentrations (sensitivity = 97.6%, specificity = 39.1% and classification correction = 95.2%), at 255 pg/mg for hair lamivudine concentrations (sensitivity = 81.3%, specificity = 69.6% and classification correction = 80.9%), at 87.5% for overall frequency (sensitivity = 83.0%, specificity = 21.7% and classification correction = 80.5%) and at 95.0% for percent of 30 days taken (sensitivity = 92.8%, specificity = 17.4% and classification correction = 89.7%).

Table 2 Logistic Regression Results of Predicting Virologic Suppression with Hair Antiretroviral Concentrations and Self-Reported Measures

Measures	n (%)	Univariate		Multivariate	
		cORs	95% CI	aORs	95% CI
Hair AZT concentrations	564 (100%)	–	–	–	–
≤58 pg/mg	22 (3.9%)	1.000	ref.	1.000	ref.
>58 pg/mg	542 (96.1%)	26.110**	9.586–71.116	43.191**	10.171–183.418
Hair 3TC concentrations	564 (100%)	–	–	–	–
≤255 pg/mg	117 (20.7%)	1.000	ref.	1.000	ref.
>255 pg/mg	447 (79.3%)	9.958**	3.992–24.840	10.656**	3.670–30.943
Overall frequency	564 (100%)	–	–	–	–
≤87.5%	97 (17.2%)	1.000	ref.	1.000	ref.
>87.5%	467 (82.8%)	1.356	0.491–3.744	1.157	0.390–3.429
Percent of 30 days taken	564 (100%)	–	–	–	–
≤95.0%	43 (7.6%)	1.000	ref.	1.000	ref.
>95.0%	521 (92.4%)	2.710+	0.879–8.358	2.488	0.722–8.569

Notes: +$p < 0.1$; **$p < 0.01$.
Abbreviations: cOR, crude odds ratio; CI, confidence interval; aOR, adjusted odds ratio; AZT, zidovudine, 3TC, lamivudine.

Predictive Utility Based on Classification Thresholds

In order to further evaluate the predictive utilities of the aforementioned four measures, the univariate and multivariate logistic regression models were successively used to estimate the predictions of hair antiretroviral concentrations and self-reported measures (that were classified with the optimal thresholds calculated by ROC curves) on virologic suppression. As listed in Table 2, high hair zidovudine concentrations (>58 pg/mg) in univariate model were the strong predictor of virologic suppression [crude odds ratio (cOR)=26.110, 95% CI = 9.586–71.116, $p<0.001$]. In multivariate model, after adjusting the sociodemographic and clinical characteristics that correlated with virologic suppression and hair antiretroviral concentrations, hair zidovudine concentrations still showed the strong prediction effect on virologic suppression [adjusted odds ratio (aOR)=43.191, 95% CI = 10.171–183.418, $p<0.001$]. Similarly, hair lamivudine concentrations were the strong predictor of virologic suppression whether in univariate or multivariate models (cOR = 9.958, 95% CI = 3.992–24.840; aOR = 10.656, 95% CI = 3.670–30.943, $ps<0.001$). In contrast, each self-reported measure was not predictor of virologic outcomes in univariate model (cOR = 1.356, 95% CI = 0.491–3.744, $p=0.557$ for overall frequency and cOR = 2.710, 95% CI = 0.879–8.358, $p=0.083$ for percent of 30 days taken) and it was also true in multivariate model (aORs = 1.157 and 2.488, $ps>0.149$).

Discussion

In the present study, we reported that hair zidovudine concentrations had a similar effect on predicting virologic suppression as hair lamivudine concentrations did and were much better than self-reported measures. To the best of our knowledge, while no association between hair zidovudine concentrations and virologic outcomes was reported in our previous study with small-size participants,[24] we further characterized the strong prediction of hair zidovudine concentrations on virologic outcomes among a large-scale cohort of Chinese PLWH. The results indicated that hair zidovudine concentrations might be an alternative biomarker of virologic outcomes consistent with the verified hair lamivudine concentrations. Furthermore, we also first defined the optimal threshold of hair zidovudine concentrations at 58 pg/mg for

predicting virologic suppression. This may provide a useful measure for monitoring ART adherence and improving virologic outcomes in the clinics.

This study found that hair zidovudine concentrations are closely associated with virologic suppression as indicated by the significant intergroup difference and correlations between them. The intergroup difference was also observed in our previous study,[24] illustrating that PLWH showing high hair zidovudine concentrations due to adequate ARV adherence may have more access to virologic suppression. Furthermore, hair zidovudine concentrations gave prediction power matching with, but slightly weaker than hair lamivudine concentrations. Moreover, there was no significant difference between hair zidovudine and lamivudine concentrations in the coefficient correlation with virologic suppression. The consistency is most likely attributed to their similarity in the long-term antiretroviral exposure (medication behaviors) and the treatment utility due to the same working pathway. Additionally, hair zidovudine concentrations in this study presented stronger predictive utility (with optimal threshold at 58 pg/mg) on virologic suppression than that in our previous study.[24] This may be as a result of a larger sample size in the current study (n = 564 vs n = 67), which improved the statistical power and reliability of the result.

This study also found that hair lamivudine concentrations as a reference measure showed strong prediction on virologic outcomes and its optimal threshold was defined at 255 pg/mg in predicting virologic suppression. The results were in line with those in a previous study showing strong prediction, and optimal threshold at 260 pg/mg after PLWH with drug resistance was excluded.[23] However, the present specificity of optimal threshold was much lower than that in the previous study (69.6% vs 89.9%).[23] Similarly, hair lamivudine and zidovudine concentrations in the present study showed weaker prediction power than other antiretrovirals in a previous study.[27] Tabb et al reported that, after excluding PLWH with high-level drug resistance, hair concentrations of nevirapine, efavirenz and lopinavir showed the AROCs between 0.77 and 0.94 and the sensitivity and specificity of optimal threshold were more than 94.00% and 61.54%, respectively.[27] These results indicated that resistance mutation may limit the prediction power of hair concentrations of zidovudine and lamivudine in the present study. PLWH with drug resistance leads to more false-negatives (patients showing high hair antiretroviral concentrations while actually experiencing virologic failure) which reduced the sensitivity and specificity.[23]

This study also found that two self-reported adherence measures were not significantly correlated with virologic outcomes and hair antiretroviral concentrations. Furthermore, they showed poor prediction on virologic suppression. These results were in line with previous studies,[27,30,35,39] illustrating the limitations of self-reported measures in ART adherence assessment due to the effects of recall bias and social desirability.[11,12] In contrast, hair antiretroviral concentrations in this study showed higher utility in predicting virologic suppression. Previously, self-reported questionnaires showing simple collection and low-cost were commonly suggested in resource-limited settings. Currently, non-invasive hair measure also showed increasing acceptability in different populations and countries (approximately 60%)[38,52,53] and especially much higher (over 95%)[21,34,54] after providing appropriate education about hair collection to local staff and PLWH participants at the start of the study. The main limitation that hinders hair measure development is the assay cost using LC-MS/MS. Our group is working on developing series LC-MS/MS methods for simultaneously determining multiple antiretrovirals in hair in order to reduce the assay cost.[41,42] Another group also reported a low-cost method for the concentration determination of hair nevirapine.[55] In the future, it is believed that hair antiretroviral concentrations and/or thresholds will play an important role in assessing long-term adherence and further predicting clinical outcomes.

Although the present study verified the strong performance of hair zidovudine concentrations in predicting virologic outcomes with a large-scale cohort, there were still a few limitations in this study. Firstly, PLWH participants were only enrolled from a particular region in China. The generalizability of the present results might be impaired by participants' homogeneity to some extent. In addition, the applications of these results may limit only in China because zidovudine is widely used in China rather than other countries. Secondly, the results based on a cross-sectional design did not explore the longitudinal predictions of hair antiretroviral concentrations on virologic outcomes over time. Thirdly, PLWH with virologic failure were only a small proportion of participants in this study and did not experience the resistance mutation testing. These may reduce the prediction of hair antiretroviral concentrations on virologic suppression. Additionally, some other factors that influenced antiretroviral concentrations in hair were not considered, such as pharmacokinetic-related factors (eg, renal function and genetic factors), hair treatment (eg, washing frequency, hair dye, and cosmetics), and sunshine.

Conclusion

This study verified that hair zidovudine concentrations could predict virologic outcomes showing matched prediction power with hair lamivudine concentrations and higher prediction power than self-reported adherence measures. Furthermore, the optimal threshold of hair zidovudine concentrations at 58 pg/mg was also defined to predict virologic suppression. This may provide a reliable and useful clinical tool for preventing virologic failure among PLWH in China.

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Disclosure

The authors report no conflicts of interest in the work.

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