Associations of human gene *EPB41L3* DNA methylation and cervical intraepithelial neoplasia in women living with HIV-1 in Africa

Running title: *EPB41L3* DNA methylation among women with HIV-1

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Competing interest:

The authors have no conflicts of interest to disclose.

ABSTRACT

Objectives: To evaluate associations of DNA methylation of the human tumour suppressor gene *EPB41L3* with high-grade cervical intraepithelial neoplasia (CIN2+) and HIV-related factors among women living with HIV-1 (WLHIV) in Burkina Faso (BF) and South Africa (SA).

Design: Case-control study of WLHIV aged 25-50 with histology-determined CIN2+ (cases, N=152) and ≤CIN1 (controls, N=210).

Methods: *EPB41L3* methylation was measured by pyrosequencing of bisulfite converted DNA from exfoliated cervical specimens at baseline and 16 months later. Median methylation levels were compared across CIN grades using the Mann-Whitney test and Cuzick test for trend. *EPB41L3* methylation levels were dichotomized into 'high' and 'low' using the 66.7 percentile point of the distribution in the controls. Associations of *EPB41L3* methylation with HIV-related factors were estimated by logistic regression.

Results: Among 94 WLHIV in BF and 268 in SA, median methylation levels at baseline for *EPB41L3* increased with increasing CIN grade in both countries (p-trend<0.001).

'High' methylation was more frequent among women with a longer time since HIV diagnosis in BF (>5 years vs. ≤5 years; adjusted Odds Ratio [aOR]=4.15, 95%CI:1.09-15.83, adjusted for age, CD4+ count, HR-HPV and CIN status), with low CD4+ count in both countries (CD4+ ≤200 vs. ≥350 cells/mm^3: aOR=7.14, 95%CI:1.44-35.37 in BF; aOR=2.55, 95%CI:1.07-6.07 in SA), and with prolonged ART use in SA (ART >2 years vs. ART-naïve: aOR=2.40, 95%CI:1.23-4.69).

Conclusion: Methylation of *EPB41L3* DNA is elevated among WLHIV with CIN2+ and independently associated with lower CD4+ count and ART use.

Key words: DNA Methylation, *EPB41L3*, HIV, Africa, cervical intraepithelial neoplasia, CD4+ cell count, antiretroviral therapy

INTRODUCTION

Invasive cervical cancer (ICC) is one of the most common cancers in low and middle-income countries (LMIC), were 85% of the estimated 500,000 global annual cases occur[1] and is the leading cause of cancer deaths among African women[2]. ICC incidence and mortality remain high in LMIC, as organised screening and HPV vaccination programmes are limited. In sub-Saharan Africa, the prevalence of both ICC and HIV are high[3, 4]. Women living with HIV (WLHIV) are living longer due to increased availability of antiretroviral therapy (ART) and many retain a high risk of infection with HPV and high risk of progressing to cervical and other genital cancers[5, 6].

The performance of currently available screening tests for high-grade cervical intraepithelial neoplasia grade 2 or worse (CIN2+) among WLHIV is not optimal. Cervical cytology and visual inspection using acetic acid (VIA) or Lugol's iodine (VILI) are more frequently used in Sub-Saharan Africa, but also have poor accuracy and reproducibility[7-9]. HPV DNA based screening has high sensitivity for CIN2+, but as it detects many transient infections it has low specificity[10]. This has important implications for screening WLHIV among whom the prevalence of HR-HPV is high[11]. Novel methods are required that are sensitive enough to detect clinically relevant HR-HPV needing colposcopy referral and treatment but with high specificity to rule out HPV-positive women without evidence of disease, thereby avoiding the need for repeat testing which can result in substantial losses to follow-up[12, 13], and unnecessary referrals for colposcopy which increase costs and burden to the service.

While HR-HPV is necessary for the development of CIN2+[14], other molecular changes occur during HR-HPV infection that lead to alterations in the functions of gene products regulating tumour suppression and cell cycle progression. Some precancerous tissue changes are the result of DNA methylation of human genes and HPV virus[15]. Aberrant DNA methylation is a

potentially good early indicator of underlying disease and may help distinguish non-progressive HPV infections from those that will progress to cancer[16]. A combined panel of human tumour suppressor genes DNA methylation markers *CADM1, MAL* and *MiR* has shown good performance for CIN2+ detection among WLHIV in Kenya[17] and South Africa[18]. DNA methylation of another human tumour suppressor gene *EPB41L3*, alone or combined with HPV16/18/31/33, has shown good performance for CIN2+ detection in the general population of women in the UK[19], the Netherlands[20] and Canada[21]. However, there are no studies evaluating this marker among WLHIV to date.

We conducted a prospective study of cervical cancer screening in a cohort of WLHIV in Burkina Faso (BF) and South Africa (SA)[6]. In this paper, we sought to evaluate associations of *EPB41L3* DNA methylation with prevalent, incident and persistent CIN2/3; and with HIV-related factors such as CD4+ cell count and antiretroviral therapy (ART) use, and to evaluate the performance of *EPB41L3* DNA methylation testing for the detection of prevalent CIN2+.

MATERIALS AND METHODS

Study population

We used DNA isolated from stored cervical swabs collected as part of an evaluation study of cervical cancer screening strategies in Ouagadougou (BF), and Johannesburg (SA), as previously described[6]. In brief, women were included in the HARP (HPV in Africa Research Partnership) study if they were HIV-1 seropositive, aged 25-50 years and resident in the study city; and excluded if they had a history of prior treatment for cervical cancer, previous hysterectomy, or were pregnant or less than 8 weeks postpartum. Enrolment was stratified in a 2:1 ratio of ART-users: ART-naïve WLHIV. Participants were followed-up every 6 months for CD4+ T-lymphocytes cell count and up to month 18 when procedures similar to baseline were repeated.

As part of this nested case-control study, all cases of histologically confirmed CIN2+ detected at baseline (prevalent) with sufficient cervical DNA (BF: 87.5% [28/32]; SA: 96.1% [124/129]) were included and matched with at least one control without CIN (≤CIN1) by (i) age group (<35 years and ≥35 years) and (ii) country of recruitment (BF: 66; SA: 144; **Figure 1**). Given the low numbers of CIN2+ cases in BF, more than one age-matched controls were recruited from this site. HR-HPV infection was not considered as part of the matching criteria given the high prevalence in this population (59% in BF and 79% in SA)[6].

HR-HPV genotyping and *EPB41L3* DNA methylation assay

Cervical samples were collected using a Digene cervical sampler (Qiagen, Courtaboeuf, France) for HR-HPV DNA testing and genotyping and DNA methylation assays. HR-HPV testing was performed at baseline and endline at the University of Montpellier virology laboratory using genotyping with the INNO-LiPA HPV genotyping Extra® assay (Innogenetics, Courtaboeuf, France)[22]. The *EPB41L3* methylation assays (CpG sites: 438, 427 and 425) were performed at baseline and endline using DNA isolation, bisulfite conversion and quantification of methylation using pyrosequencing (PSQ) assays in single separate reactions, as previously described[23]. Primers were designed using PyroMark Assay Design software version 2.0.1.15 (Qiagen; **Supplementary Table 1**). A non-CpG cytosine was included to provide the internal control for total bisulfite conversion by the PSQ assay. PCR assays were performed as previously described[24]. All runs included standard curves as positive controls, of 0%, 50% and 100% methylated human DNA and a non-methylated control.

Visual inspection, cytology and histology

An additional cervical brush was collected from the ecto- and endocervix for cytological reading using the Papanicolaou method and read at the Pathology department at CHU-Yalgado in Ouagadougou and the National Health Laboratory Service (NHLS), Johannesburg

according to the Bethesda classification system[25]. All participants were assessed clinically using visual inspection with acetic acid or Lugol's iodine (VIA/VILI) by trained nurses/midwives. All participants were referred for colposcopy performed by trained colposcopists. Systematic 4-quadrant cervical biopsy, including directed biopsy of any suspicious lesions, was performed for participants who had abnormalities detected by cytology, VIA/VILI or colposcopy, or who were HR-HPV DNA positive using Digene HC-II (Qiagen).

Cervical biopsies were processed at the local pathology laboratories and read using the 3-tier CIN classification system[26]. Histology was classified as 'negative' (≤CIN1) or 'positive' (CIN2+) based on the highest reading across all findings from the 4-quadrant biopsies and endocervical curettage if collected. All histological slides from women with a local diagnosis of CIN2+ and approximately 10% of slides from women with ≤CIN1 histological findings were reviewed by the HARP Endpoint Committee of five pathologists, for consensus classification, which showed high agreement[27].

The same genital sampling and examination procedures were repeated at the endline visit.

Statistical analyses

The three CpG sites for *EPB41L3* were highly correlated (**Supplementary Table 2**) justifying the use of the average of the three CpG sites. The median methylation values of the average of the CpG sites for *EPB41L3* were compared across histological grades using the non-parametric Mann-Whitney U test[28] as values were not normally distributed. The Cuzick test for trend was used to evaluate trend in methylation levels by CIN grade[29].

EPB41L3 methylation values were dichotomized into 'high' and 'low' methylation levels, with 'high' defined as values above the second tertile (66.7 percentile point) of the distribution of values in the control (≤CIN1) samples, as previously used[30] and this cut-off was country-

specific. Logistic regression was used, combining cases and controls, to obtain Odds Ratios (OR) and 95% Confidence Intervals (CI) for 'high' methylation associated with socio-demographic, behavioural and HIV-related factors[31]. Multivariable analyses were adjusted for factors which were independently associated with 'high' methylation in univariate analyses ($p<0.10$) for each country.

Receiver operating characteristic (ROC) curves were plotted for the outcome of CIN2+. The area under the ROC curve (AUC) was used to evaluate the sensitivity and specificity of the *EPB41L3* methylation assay for the detection of CIN2+ and CIN3+. McNemar's test was used to compare the sensitivity and specificity of the *EPB41L3* methylation assay with current standard-of-screening in Burkina Faso (visual inspection) and South Africa (cytology)[32]. Data were analysed using Stata version 14 (Stata Statistical Software, College Station. TX: Stata Corporation).

Ethics

Ethical approval for the HARP study was granted from the Ministry of Health in Burkina Faso, the University of Witwatersrand in South Africa, and the London School of Hygiene and Tropical Medicine in the UK.

RESULTS

Study population

A full description of the HARP Study cohort (BF=615; SA=623) is provided elsewhere[6]. For this study, the median age of 94 participants in BF (28 CIN2+ cases, 66 ≤CIN1 controls) was 39 (IQR, 35-43) years and 33 (IQR, 30-38) among 268 participants in SA (124 CIN2+ cases, 144 ≤CIN1 controls), similar to the overall HARP cohort. The median CD4+ count at baseline was 453 cell/mm^3 (IQR, 303-594) in BF and 403 cell/mm^3 (IQR, 283-551) in SA, similar to the HARP cohort. There was a higher proportion of women taking ART in BF compared to in SA (85.1%

vs. 60.5%, p<0.001). The median time since HIV diagnosis was 6 years (IQR: 2-9) in BF and 4 years (IQR: 2-7) in SA.

Among 161 CIN2+ cases detected in the HARP study at baseline, cervical samples were unavailable for nine participants and *EPB41L3* methylation was performed for 152 women with CIN2+ (CIN2: BF=17; SA=73; CIN3+: BF=11; SA=51) and 210 women with ≤CIN1 (BF: 66; SA: 144; **Figure 1**). A single invasive cancer case was identified in BF. Among the 362 women selected for the case-control study, prevalence of HR-HPV was higher in the cases than the controls (BF: 100.0% vs 75.8%, p=0.005; SA: 90.7% vs. 84.0%, p=0.127).

***EPB41L3* methylation at baseline and associations with prevalent CIN2+**

At baseline, the median methylation percentage of the average of three CpG sites for *EBP41L3* increased with increasing CIN grade (Cuzick p-trend p<0.001 for countries combined; **Figure 2, Supplementary Table 3**). Methylation levels were significantly higher among women with CIN2+ compared to women with ≤CIN1 in both countries (in BF: CIN2+ median=7.05, IQR: 1.72-17.68 vs. ≤CIN1 median=1.15, IQR: 0.0-2.93, Mann-Whitney p<0.001; in SA: CIN2+ median=1.77, IQR: 0.0-10.62 vs. ≤CIN1 median=0.0, IQR: 0.0-1.53, Mann-Whitney p<0.001, data not shown).

***EPB41L3* methylation at baseline and endline and associations with longitudinal CIN outcomes**

Among the 210 women with ≤CIN1 at baseline, 185 (BF: 57; SA: 128; **Figure 1**) had histology results at endline which occurred a median 16 months later (IQR: 14.5-16.7). There were 26 incident CIN2/3: 22 CIN2 (BF: 3; SA: 19) and 4 CIN3 (BF: 1; SA: 3). The median methylation levels for *EBP41L3* at baseline were not significantly higher among women who developed incident CIN2/3 compared to those who remained ≤CIN1 in either country (incident CIN2/3 vs. ≤CIN1: Mann-Whitney p=0.42 for BF, p=0.68 for SA; **Supplementary Table 4**). However, the endline

median methylation levels were higher in both countries among the women with incident CIN2/3 compared to women with ≤CIN1 at endline (BF: p=0.05; SA=0.07).

An analysis of the baseline methylation values of 36 women in SA with prevalent CIN2/3 who could not be treated before their endline visit (**Figure 1**), show that women with persistent CIN3, or CIN2 which progressed to CIN3 (i.e, CIN2/3 persistence) had higher baseline methylation levels compared to women who remained ≤CIN1 (CIN2/3 persistence: median methylation=15.67, IQR: 3.13-24.70; ≤CIN1 at both time points: median methylation=0.0, IQR: 0.00-1.43; Mann-Whitney p<0.001, **Supplementary Table 5; Supplementary Figure 1**), and compared to women with spontaneous regression to ≤CIN1 (regression to ≤CIN1: median methylation=0.0, IQR: 0.0-3.83; Mann-Whitney p=0.016; data not shown).

Associations of HIV-related factors with 'high' *EPB41L3* methylation

The proportion of women with 'high' methylation at baseline was 41.5% (39/94) in BF and 45.9% (123/268) in SA (**Table 1**). In BF, 'high' methylation was more frequent among women with a longer time since HIV diagnosis (45.1% among women with HIV diagnosis of >5 years compared to 37.2% among women with HIV diagnosis ≤5 years; adjusted Odds Ratio [aOR]=4.15, 95%CI: 1.09-15.83, adjusted for age, CD4+ count, HR-HPV and CIN status at baseline, **Table 1**).

The proportion of women with 'high' methylation was higher among those with low CD4+ count in both countries (BF: 66.7% among women with CD4+ count ≤200 cells/mm^3 vs. 34.9% among women with CD4+ count ≥350 cells/mm^3: aOR=7.14, 95%CI: 1.44-35.37; in SA: 68.8% vs. 39.4%; aOR=2.55, 95%CI: 1.07-6.07).

In both countries, women taking ART were more likely to have 'high' methylation compared to ART-naïve women, but following adjustment, this association remained significant among

women taking ART for prolonged duration in SA only (ART >2 years vs. ART-naïve: 54.4% vs. 35.6%; aOR=2.40, 95%CI: 1.23-4.69).

Performance of *EPB41L3* DNA methylation for detection of prevalent CIN2+

The ROC curve analysis for detection of prevalent CIN2+ relative to ≤CIN1 generated an AUC of 0.77 (95%CI: 0.65-0.89) in BF (**Figure 3A**) and 0.68 (95%CI: 0.62-0.74) in SA (**Figure 3B**). The current cervical cancer screening method in BF is VIA/VILI, which had a sensitivity of 60.7% and specificity of 69.7% for CIN2+ detection in BF (**Figure 3A**). When setting the sensitivity of *EPB41L3* assay at 60.7%, there was a significant difference in the specificity between the tests (VIA/VILI: 69.7% vs. *EPB41L3*: 86.4%; McNemar's p=0.0116).

The current cervical cancer screening method in SA is cytology with cut-off of low-grade SIL and greater (LSIL+), which had a sensitivity of 97.5% and specificity of 9.2% for detection of CIN2+, while a cut-off of high-grade SIL and greater (HSIL+) had a sensitivity of 69.7% and specificity of 78.9% (**Figure 3B**). When setting the sensitivity of *EPB41L3* similar to that of cytology HSIL+, there was a significant difference in specificity between the tests (HSIL+: 78.9% vs. *EPB41L3*: 66.2%; McNemar's p=0.0201), as well as for LSIL+ (LSIL+: 9.2% vs. *EPB41L3*: 97.9%; McNemar's p<0.001).

The AUC did not vary when considering CIN3+ alone as an outcome measure relative to ≤CIN1 (BF: 0.77, 95%CI: 0.57-0.97; SA: 0.70, 95%CI: 0.62-0.78).

DISCUSSION

In this study conducted among African women living with HIV-1, we report that the methylation of the human tumour suppressor gene *EPB41L3* increased with increasing severity of CIN at baseline and with progression of CIN lesions over 16 months. Similar to studies among HIV-uninfected women[19-21], our data suggest that *EPB41L3* methylation may be involved in cervical carcinogenesis and could be useful in identifying women at risk of persistent or progressive high-grade CIN.

Our finding that *EPB41L3* methylation levels increased with increasing severity of CIN lesion over time is similar to what has been reported for other host gene markers *CADM1, MAL*[33] and *FAM19A4*[34] in the Netherlands. In our study, women who had not been treated in time before endline and who had persistent CIN3, or CIN2 which progressed to CIN3 had significantly higher baseline *EPB41L3* methylation levels compared to women who spontaneously regressed to ≤CIN1, and women with non-progressing CIN2 over 16 months. Although the number of outcomes was small, our results suggest that *EPB41L3* methylation assay may be useful in distinguishing women with CIN2+ that progress from those that regress. Given that over half of untreated women in this study with CIN2/3 at baseline had spontaneous regression to ≤CIN1 over 16 months, such a diagnostic tool would be useful to reduce over-referral with unnecessary colposcopies and overtreatment, which are problematic in limited resources settings.

We found higher levels of *EPB41L3* methylation among women with low CD4+ count (≤200 cells/mm^3) compared to women with high CD4+ count (>350 cells/mm^3). It is unclear from the literature whether CD4+ T-lymphocytes are directly involved in regulating tumour suppressor genes such as *EPB41L3*, but CD4+ T-lymphocytes can regulate tumour cells indirectly through the action of cytokines [35-37]**.** The inverse correlation of methylation levels with CD4+ cell count

may also reflect the association of HR-HPV with CD4+ cell count, as CD4+ cell count has been shown to a strong predictor of HR-HPV prevalence and persistence [5, 6, 38-40]. HPV oncogenes E6 and E7 can activate DNA methyltransferases, which are responsible for initiating and maintaining tumour suppressor gene methylation and are overexpressed in several cancers[41]. While the association of CD4+ cell count with *EPB41L3* methylation was independent of HR-HPV status, unmeasured HR-HPV particularly in integrated form (E6/E7 integrated in host DNA) cannot be ruled out.

Chronic inflammation as a result of persistent or recurrent genital infections caused by *Trichomonas vaginalis*, *Herpes simplex virus*-2 (HSV-2) and bacterial vaginosis, also known to be linked to low CD4+ cell counts and which were prevalent in this population, can promote the initiation or progression of high-grade cervical lesions[6]. Elevated levels of nitric oxide associated with *Trichomonas vaginalis*, HSV-2 and bacterial vaginosis may result in DNA damage, silencing of tumour suppressor genes and cell transformation [42, 43].

Higher methylation of *EPB41L3* was found among ART users compared to ART-naïve women in this study and this was independent of CD4+ cell count, HR-HPV and CIN status. The mechanisms behind this finding are unclear. In the HARP study, women on prolonged duration ART (>2 years) in SA had higher median CD4+ cell count and correspondingly lower CIN2+ prevalence at baseline compared to ART-naïve women, or women on short-duration ART (≤2 years)[6]. It is possible that women on ART are of more advanced ageing process than those not on ART, especially if they have been living longer with HIV, as has been shown among other HIV infected populations[44, 45]. In this study, the median time since HIV diagnosis was 5 years (IQR: 3-8) among women taking ART and 2 years (IQR: 1-4) among the ART-naïve women. Alternatively, the effects may be due to ART genetoxicity and the long term effects of ART drugs. Animal studies have shown that antiretroviral nucleoside analogue drugs, such as zidovudine are incorporated into host DNA, where it causes mutations of genes associated with

gene expression, cell cycle arrest and chromosomal aberrations[46]. Others have shown that zidovudine, or azidothymidine (AZT) can induce site-specific hypermethylation[47].

In Burkina Faso, *EPB41L3* methylation values increased across all grades of CIN, and the *EPB41L3* methylation assay had a sensitivity and specificity comparable to the studies conducted among HIV-negative women[19, 21, 24]. The performance of *EPB41L3* methylation assay appears slightly better than the current cervical cancer screening method of VIA/VILI in Burkina Faso. In South Africa, *EPB41L3* methylation assay had lower specificity compared to cytology HSIL+, but was more specific than cytology LSIL+ which is used as a cut-off for colposcopy referral according to national guidelines. These findings suggest that *EPB41L3* could be comparable, or not far from the performance of the current morphological screening method, and its performance could be enhanced by the combination of other existing human gene markers and/or HR-HPV and as a triage test, as shown by others[19, 20].

The study was constrained by its small sample size and its post-hoc case-control design nested into the HARP parent study, although we attempted to match by age and to have at least one control for each case, particularly in Burkina Faso. While 94% of prevalent cases and 96% of incident CIN2+ cases from the parent HARP study were included, only a proportion of the controls (BF: 66/583 (11%), and in SA: 144/494 (29%)) were sampled. Therefore, the data might not be entirely generalizable to all women with ≤CIN1 in the HARP cohort. Other studies have restricted measurement of DNA methylation markers to HR-HPV positive women to evaluate their performance as triage tests. In this study, the *EPB41L3* DNA methylation assay was performed irrespective of HR-HPV DNA status to describe the full range of epidemiological associations. The availability of an histological verification at both baseline and endline may have introduced a bias towards positive methylation (even in the absence of true disease) as women needed to have evidence of some abnormality in order to have been biopsied. This may have reduced the chances of increased discriminatory power

expected when comparing women with disease to women with a total absence of abnormalities. There was a higher proportion of participants in SA with zero percentage methylation for *EPB41L3* compared to women in BF which may be attributed to the higher proportion of ART users in BF given their higher median *EPB41L3* methylation and there may be epigenetic differences between the two populations linked to different environmental exposures which can modify the effects of DNA methylation[48, 49].

Despite these limitations, the study had several strengths, including its longitudinal design, the availability of a rigorously validated histological endpoint for the majority of women thereby minimizing disease ascertainment bias; and the availability of methylation data at both time points. HARP is one of a few studies investigating DNA methylation of human genes and association with CIN2+ among WLHIV[17, 18] and the only one to study *EPB41L3*.

In conclusion, methylation of human gene *EPB41L3* DNA is elevated in CIN2+ cases among African WLHIV and is higher among women with low CD4+ cell count. DNA methylation assays show promise as an earlier indicator of precancer and cervical lesion progression in high risk populations, such as WLHIV, alongside existing screening tools. Given that DNA methylation markers in combination panels generate higher sensitivity and specificity compared to individual genes on their own, multiplex DNA methylation assays including a combination of human genes may have a potential as primary screening of CIN2+ among WLHIV.

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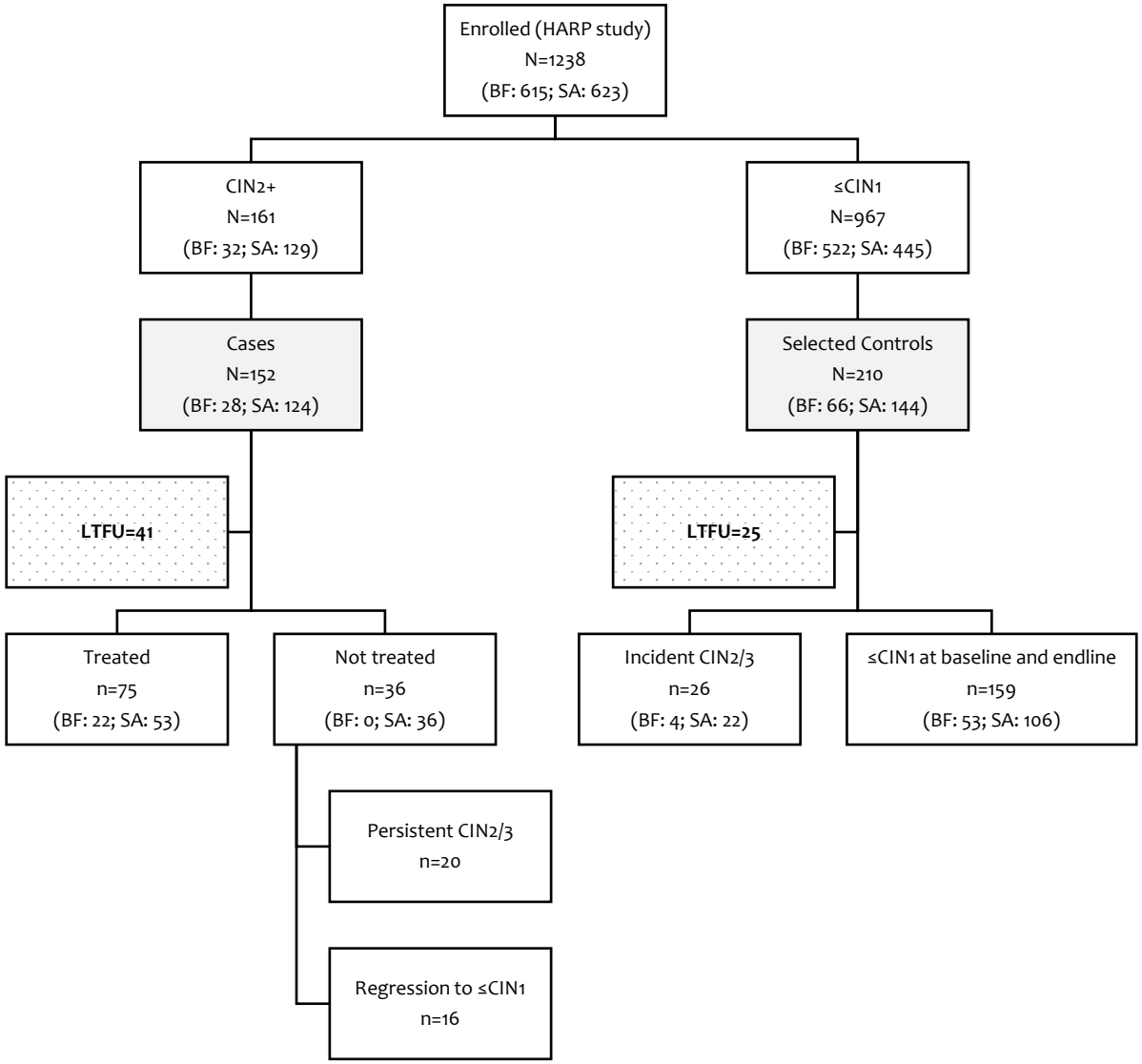
AUTHOR CONTRIBUTIONS

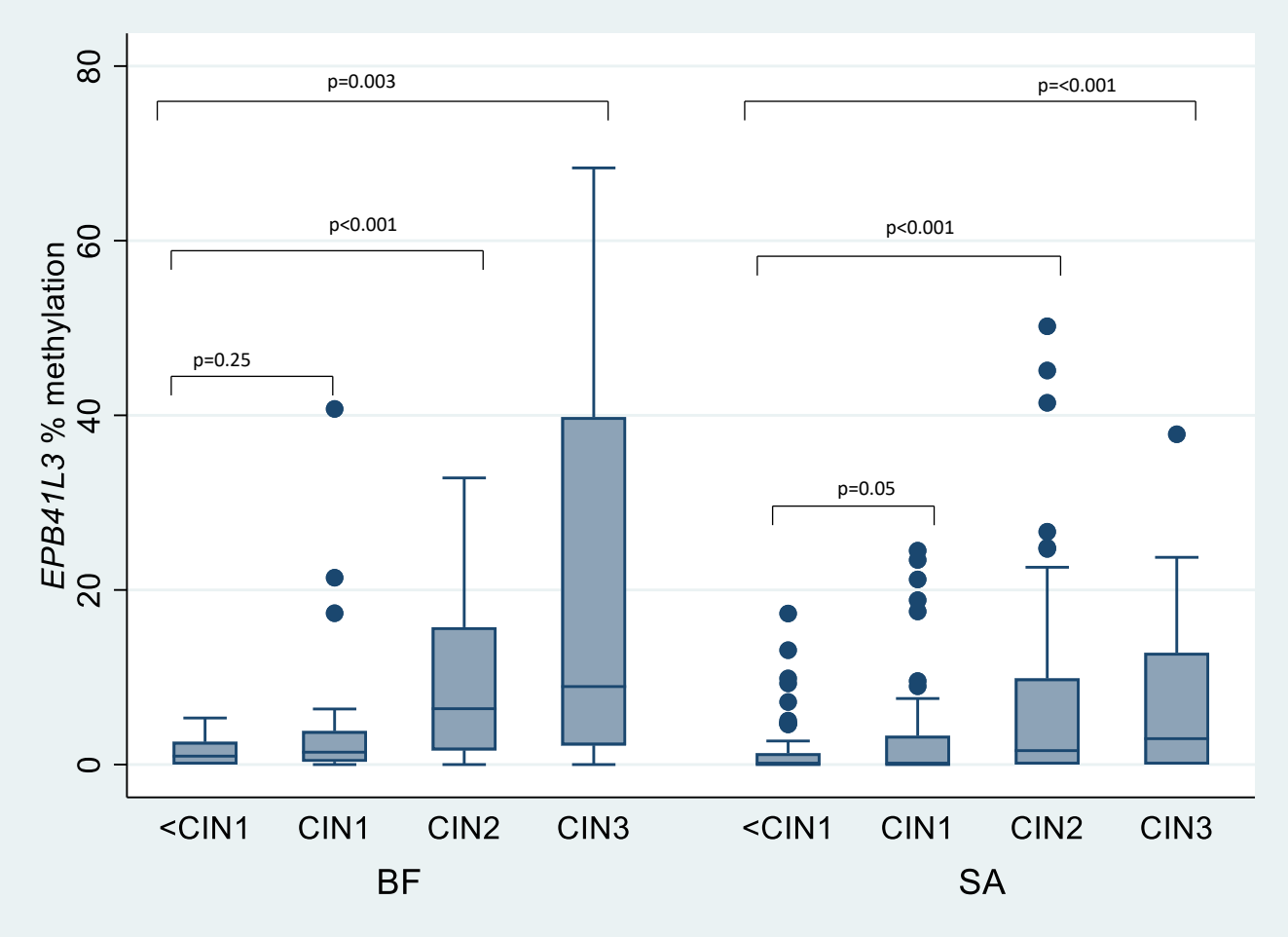
Conceived and designed the study: PM, SD, NM, HW, MS, HK, AL; Coordinated the study: HK, BS, AC, NM, SD, PM; Performed the lab testing: HK, RW, NV; Analysed the data: HK; Wrote the first draft of the manuscript: HK, PM, AL; Contributed to the writing of the manuscript: All; Criteria for authorship read and met: All; Agree with manuscript results and conclusions: all.

Figure 1. Study flowchart



LTFU=lost to follow-up

Figure 2. *EPB41L3* methylation levels (percentages) by CIN status among 94 women living with HIV-1 in Burkina Faso (BF) and 268 in South Africa (SA)



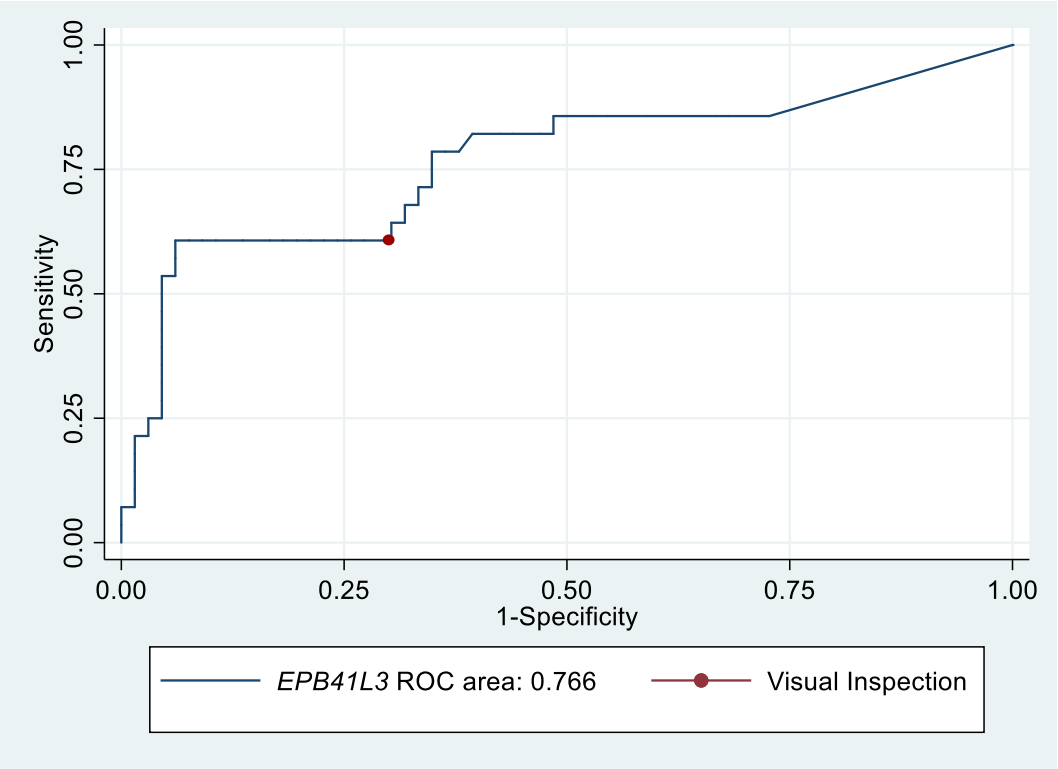
Mann-Whitney U test, p-value for difference in median values of CIN2/3 relative to ≤CIN1

Table 1. Associations of age and HIV-related factors with 'high' *EPB41L3* methylation among 94 women living with HIV-1 in Burkina Faso and 266 in South Africa†

	Burkina Faso			South Africa		
	N	**n (%)**	**aOR (95% CI) [1]**	**N**	**n (%)**	**aOR (95% CI) [1]**
All participants	94	39 (41.5)		268	123 (45.9)	
Age						
<35 years	26	12 (46.2)	1.00	156	64 (41.0)	1.00
≥35 years	**68**	27 (39.7)	0.80 (0.26-2.47)	110	59 (53.6)	**1.73 (1.01-2.96)**
Time since HIV diagnosis						
≤5 years	43	16 (37.2)	1.00	178	82 (46.1)	1.00
>5 years	51	23 (45.1)	**4.15 (1.09-15.83)**	88	41 (46.6)	1.12 (0.64-1.98)
CD4+ count (cells /mm³)						
<200	15	10 (66.7)	**7.14 (1.44-35.37)**	32	22 (68.8)	**2.55 (1.07-6.07)**
201-349	16	7 (43.8)	1.77 (0.49-6.34)	73	38 (52.1)	1.45 (0.80-2.64)
≥350	63	22 (34.9)	1.00	160	63 (39.4)	1.00
ART status						
ART-naive	14	4 (28.6)	1.00	104	37 (35.6)	1.00
≤2 years	42	20 (47.6)	2.88 (0.53-15.77)	83	43 (51.8)	1.18 (0.59-2.36)
>2 years	38	15 (39.5)	4.56 (0.78-26.71)	79	43 (54.4)	**2.40 (1.23-4.69)**
ART users						
HIV-1 viral suppression[2]						
<1000 copies/ml	64	27 (42.2)	1.00	129	68 (52.7)	1.00
≥1000 copies/ml	13	7 (53.9)	1.27 (0.24-6.63)	32	18 (56.3)	0.95 (0.39-2.32)
ART adherence[3]						
Low Adherence (<60%)	1	0 (0.0)		22	15 (68.2)	1.00
Moderate adherence (60-90%)	74	34 (46.0)		138	69 (50.0)	1.57 (0.55-4.50)

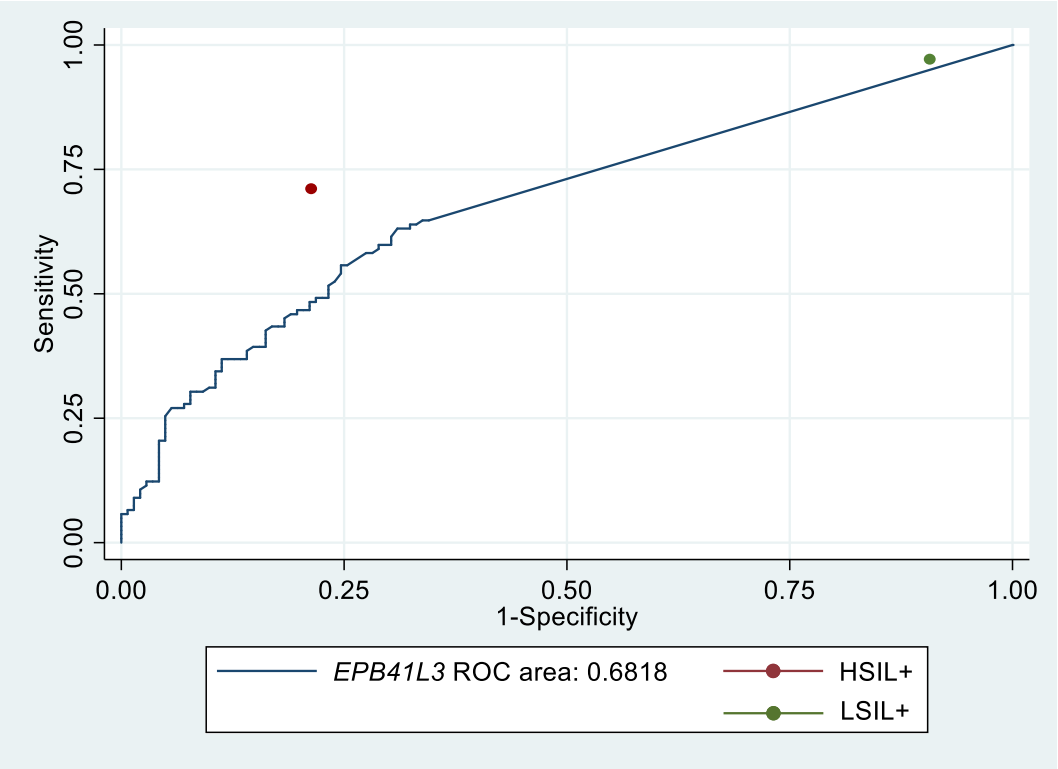
†Two women in South Africa denied being on ART at enrolment despite undetectable HIV-1 PVL and given the uncertainty of their status, these women were excluded from HIV-related factors analyses; Adjusted Odds Ratio (aOR) [1] adjusted for HR-HPV, CIN status, age and CD4+ count in both countries, in addition to *Candida albicans* in BF; [2]Three women taking ART with missing HIV-1 PVL in BF and one in SA; [3]Five participants with no data on adherence in BF and 2 in SA.

Figure 3A. ROC curve for the detection of CIN2+ among 94 women living with HIV-1 in Burkina Faso



ROC=receiver operating characteristic; Visual Inspection=abnormal for either VIA or VILI

Figure 3B. ROC curve for the detection of CIN2+ among 268 women living with HIV-1 in South Africa



ROC=receiver operating characteristic; LSIL+=low-grade squamous intraepithelial lesion, and greater; HSIL+=high-grade squamous intraepithelial lesion, and greater

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