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Methods of gestational age assessment influence the observed association between antiretroviral therapy exposure, preterm delivery and small-for-gestational age infants: a prospective study in Cape Town, South Africa

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Abstract

Purpose—Heterogeneous findings exist on antiretroviral treatment (ART) use in pregnancy and preterm delivery (PTD) or infants born small-for-gestational age (SGA). Whether reported differences may be explained by methods used to ascertain gestational age (GA) has not been explored.

Methods—We enrolled consecutive pregnant women attending a large primary care antenatal clinic in South Africa. Public-sector midwives assessed GA by last menstrual period (LMP) and symphysis-fundal height (SFH). Separately, if clinical GA was <24 weeks, ultrasound (US) was performed by a research sonographer blinded to midwife assessments. In analysis, the impact of measurement error on the association between HIV/ART status and birth outcome by GA method was assessed, and factors associated with clinical GA under- or over-estimation identified.

Results—In 1787 women included overall, estimated PTD incidence was 36% by LMP, 17% by SFH and 11% by US. PTD risk was higher for HIV-infected than HIV-uninfected women using US-GA (adjusted odds ratio (aOR) 1.95; 95% CI 1.10–3.46); for LMP/SFH-GA the associations were smaller and not significant. These findings persisted after adjustment for age, parity, height and previous PTD. PTD risk did not vary by timing of ART initiation (before or during pregnancy) for any method. Elevated BMI and older age were associated with decreased risk of under-

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estimation by both LMP and SFH; HIV status and obesity were associated with increased risk of over-estimation by SFH. There were no differences in SGA incidence across GA methods.

Conclusions—Findings for an association between HIV/ART and birth outcomes are substantially influenced by GA assessment method. With growing public health interest in this association, future research efforts should seek to standardize optimal measures of gestation.

Background

Increasing numbers of HIV-infected pregnant women use lifelong antiretroviral therapy (ART); in high prevalence HIV countries¹ ART may be the most common drug exposure in pregnancy.² While ART is invaluable for the health of HIV-infected women and their infants, some studies have reported associations between ART use in pregnancy and adverse birth outcomes such as preterm delivery (PTD) and small-for-gestational age (SGA) infants.^{3–5} PTD and SGA substantially increase the risk of infant mortality, with PTD accounting for the highest proportion of deaths in the critical neonatal period.⁶ Thus in high HIV prevalence settings it is essential to understand how ART use may influence the risk of preterm delivery. Findings on the association between ART use and adverse birth outcomes, including PTD, are conflicting^{7,8} and possibly associated with ART regimen used, timing of initiation and ART eligibility criteria.

Studies investigating ART use in pregnancy are heterogeneous in their gestational age (GA) assessment method. GA assessment is challenging in resource-limited settings where ultrasonography is usually unavailable, and instead routinely based on clinical assessment via dating the last menstrual period (LMP) and/or measurement of the symphysis-fundal height (SFH). Although generally considered most reliable, ultrasound (US) is less accurate for pregnancy dating if carried out after 24 weeks, when biological variability of fetal biometry increases.^{9,10} The reliability of LMP-GA is limited by errors related to irregular menstrual cycles of varying duration or accurate recall of LMP dates, and variation in timing of ovulation.^{11,12} SFH-GA assessment is difficult early in pregnancy (<12 weeks); and even when measurable its accuracy can be diminished by multiple gestations, high body mass index (BMI), intrauterine growth restriction and other factors.^{13,14}

Differences in GA assessment could provide an important methodological explanation for the observed heterogeneity in findings, but has not so far been evaluated. Measurement error of GA could lead to biased estimates of the association between HIV/ART and the GA-based outcomes PTD and SGA, with the severity and nature of this bias depending on the form of the error.

We set out to examine the impact of GA assessment method, and any measurement error therein, on the association between HIV/ART status and adverse birth outcomes among a population of pregnant women undergoing routine antenatal care at a large public sector primary care facility in Cape Town, South Africa.

Methods

Between April 2014 and October 2016, consecutive pregnant women (aged 18 years) were enrolled in a prospective cohort (n=3972). All women seeking antenatal care (ANC) at a large public sector primary care facility serving a low-income, high HIV prevalence sub-district of Cape Town South Africa¹⁶. Eligibility was regardless of their HIV status and included HIV-infected women who conceived while on ART continued their current regimen, and those newly diagnosed or not on ART were initiated on the WHO recommended first-line regimen. Women clinically assessed (LMP and/or SFH) during routine ANC services to be 24 weeks gestation were referred for a same day ultrasound by a research sonographer using standardized assessment protocols and blinded to the midwife GA assessment. The study was reviewed and approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee and the University of Southampton Faculty of Medicine Ethics Committee. Written informed consent was obtained for study participation, including data abstraction from routine clinical records through the pregnancy and post-partum period.

Following their first ANC visit, enrolled women had data abstracted on HIV status, pregnancy history (previous and index pregnancies), medications prescribed during pregnancy and any maternal diagnoses during pregnancy. Obstetric outcomes, including date of delivery and birthweight, were abstracted following delivery. HIV status was categorized as HIV-uninfected or HIV-infected; ART status was categorized as initiation before or during pregnancy. The primary outcome was GA at delivery based on completed weeks by LMP, SFH and US. PTD was defined as delivery at <37 weeks gestation, late to moderate preterm as delivery 32 to 37 weeks and post-term as delivery at >42 weeks.¹⁷ Using the gender-specific Intergrowth Standards-21st project standards,¹⁸ infants with birthweights <10th percentile for GA were classified as SGA, between 10th and 90th percentile as appropriate-for-gestational age (AGA), and >90th percentile as large-for-gestational age (LGA).

Statistical analyses were performed using STATA version 14.0 (Stata Corporation, College Station, TX, USA). Analyses focused on two comparisons: HIV-infected vs HIV-uninfected women; and among HIV-infected women ART initiation before pregnancy vs initiation during pregnancy. Outcome variables (PTD and SGA) were created for each assessment method (LMP, SFH and US), using GA at booking and date of delivery. Analyses compared GA estimated by each assessment method in live singleton births. Further analyses were restricted to women with GA estimated by all three assessment methods and to women with both a clinical GA estimate (LMP and/or SFH) and an ultrasound GA estimate. Comparisons of proportions were based on chi-squared tests and rank-sum tests.

In women with GA by all three assessment methods the associations between HIV/ART status and PTD were examined by each assessment method using logistic regression. Results were presented as odds ratios (OR) with 95% confidence intervals (CI), and adjusted for age, parity, BMI and previous PTD based on their association with adverse birth outcomes.^{3,5} BMI, assessed at the first ANC visit, was classified as underweight (< 18.5 kg/m²), normal (18.6–24.9 kg/m²), overweight (25.0–29.9 kg/m²), or obese (> 30 kg/m²).¹⁹ To inform

understanding of GA under- or over-estimation by LMP and SFH compared to US, multinomial logistic regression was conducted. Concordance was defined as <7 days difference which is deemed to be clinically relevant; however for US conducted in the second trimester LMP concordance was defined according to American College of Obstetricians and Gynaecologists recommendations: <7days between 14–15weeks GA, <10 days between 16–21weeks and <14days between 22–27weeks.²⁰ Results were presented as risk ratios (RR) with 95% CI, and adjusted for age, parity, previous PTD, BMI and ART regimen (in HIV-infected only comparisons).

Results

A total of 1787 women with live singleton births were included: 1014 HIV-uninfected (57%) and 773 HIV-infected (43%), of whom 368 (48%) initiated ART before pregnancy and 405 (52%) during pregnancy. In line with local and WHO treatment guidelines, all enrolled HIV-infected women were on ART, and most (94%) were on a regimen of two NRTIs [tenofovir (TDF) and emtricitabine (FTC)], with the NNRTI efavirenz (EFV). HIV-infected women were older and less likely to be primigravid than HIV-uninfected women (Table 1). HIV-infected women who initiated ART before pregnancy were older and less likely to be primigravid than those who initiated during pregnancy. Overall 28% of women were overweight and 46% obese; there were no significant differences by HIV status or timing of ART initiation.

In total, 88% (n=1585) of women had an LMP-based GA, 68% (n=1220) SFH-based, and 67% (n=1204) US-based, with estimated median GA at enrolment varying by assessment method (Table 1). Of the ultrasounds 719 (60%) were conducted in the second trimester with 140 resulting in dating of >24 weeks GA. The estimated incidence of PTD using LMP-GA was 36% (95% CI 33–39%), 17% (95% CI 15–19%) by SFH and 11% (95% CI 9–13%) by US; with no significant difference by HIV status using LMP-GA (37% vs 36%) or SFH-GA (18% vs 17%). However, US-GA estimated PTD incidence was significantly higher in HIV-infected than HIV-uninfected women (14% vs 8%) (Table 2; Figure 1a). No significant differences in PTD incidence were observed between HIV-infected women initiating ART before or during pregnancy by LMP-GA (35% vs 39%), SFH-GA (18% vs 17%) and US-GA (12% vs 15%) (Table 2; Figure 1a).

Women with GA assessed by all three methods (Restricted Group)

Among the 35% (n=629) of women with GA assessed by all three methods, estimated median (IQR) GA at enrolment was 17 weeks (14–21) by LMP, 20 weeks (16–23) by SFH and 19 weeks (15–23) by US (Supp. Table 1a). HIV status was the only variable associated with having been assessed by all three methods, with increased odds for HIV-infected women (OR 1.54; 95% CI 1.22 – 1.84). In this group, estimated PTD incidence was 42% (95% CI 38–46%) using LMP-GA, 14% (95% CI 12–17%) using SFH-GA and 10% (95% CI 7–12%) using US-GA (Supp. Table 2).

As before for the whole group, estimated PTD incidence was significantly higher in HIV-infected than HIV-uninfected women using US-GA (12% vs 7%) but not significantly different using LMP-GA (40% vs 44%) or SFH-GA (16% vs 12%) (Supp. Table 2; Figure

1b). In HIV-infected women (n=320), no significant differences were observed between women initiating ART before or during pregnancy for LMP-GA (39% vs 41%), SFH-GA (17% vs 15%) or US-GA (10% vs 15%) (Supp. Table 2; Figure 1b).

Adjusting for age, parity, BMI and previous PTD, HIV-infected women had increased odds of US-GA PTD (aOR 1.98, 95% CI 1.12–3.52) compared to HIV-uninfected women. This was also seen with SFH-GA although not statistically significant (aOR 1.34, 95% CI 0.83–2.15); associations seen with LMP-GA were small and not significant (Table 3). In HIV-infected women, the odds of PTD did not vary appreciably by timing of ART initiation across the three assessment methods (Table 3). Similar results were seen in the overall cohort (Supp. Table 3).

Factors associated with under- and over-estimation of GA

In women with both an US estimate and a clinical estimate LMP (n=1057) or SFH (n=732), the estimated concordance with US-GA estimates was 29% (n=308) for LMP and 36% (n=261) for SFH. LMP under-estimated US-GA in 484 cases (46%), and over-estimated in 265 cases (25%). The percentages of under- and over-estimation for SFH were 21% (n=153) and 43% (n=318) respectively.

Comparing LMP-GA to US-GA, HIV status was not significantly associated with under-estimating (risk ratio (RR) 0.96, 95% CI 0.71–1.27), but was associated with over-estimating (RR 1.33, 95% CI 0.95–1.64) GA, although not statistically significant. These associations persisted in adjusted models (Table 4). No other factors were associated with under- or over-estimating by LMP-GA.

In HIV-infected women, timing of ART initiation was not significantly associated with either under- (RR 0.87, 95% CI 0.53–1.45) or over-estimating (RR 1.34, 95% CI 0.78–2.31) GA by LMP and this persisted in the adjusted models (Table 4).

Comparing SFH-GA to US-GA, there was no association with HIV status for either under- (RR 1.21, 95% CI 0.80–1.83) or over-estimating (RR 1.33, 95% CI 0.94–1.87) GA. In the adjusted model, obese women (aRR 1.72, 95% CI 1.09–2.73) were at increased risk of over-estimation by SFH; while being older (>30yrs) was associated with decreased risk of under-estimation by SFH (aRR 0.58, 95% CI 0.32–1.03).

Among HIV-infected women there was no association with timing of ART initiation for either under- or over-estimating GA by SFH and this persisted with adjustment (Table 4).

Small for gestational age deliveries

The incidence of SGA was similar according to LMP, SFH and US (10% vs 13% vs 11%); however differences were observed by assessment method for AGA and LGA, with much wider GA estimate variations when using LMP-GA and SFH-GA than using US-GA (Figure 2). The incidence of AGA was significantly higher with SFH-GA (73%) and US-GA (80%) than with LMP-GA (59%). Correspondingly, the incidence of LGA was significantly higher

when LMP-GA was used (32%) than when SFH-GA (14%) and US-GA (9%) was used (Table 2; Figure 2).

When the incidence of SGA was compared according to HIV status, SGA incidence was higher for HIV-infected than HIV-uninfected women across all three assessment methods. Using LMP-GA or US-GA, SGA estimated did not differ by timing of ART initiation. However, using SFH-GA, women initiating before pregnancy appeared to have a lower SGA incidence than those initiating during pregnancy (11% vs 19%) (Table 2). In women with GA by all three assessment methods, overall SGA was slightly higher when SFH-GA (14%) was used compared to LMP-GA (9%) and US-GA (11%). Similar patterns to those seen overall were seen in this restricted group with the incidence of AGA and LGA.

By all three assessment measures, SGA incidence was higher in HIV-infected than HIV-uninfected women. No differences were observed according to timing of ART initiation for LMP-GA and US-GA, however a lower incidence of SGA was observed in women initiating before pregnancy for SFH-GA (Supp. Table 2).

Discussion

In this prospective cohort of pregnant women seeking ANC at a large public sector primary care facility in South Africa, we found that estimated GA varied by the measurement employed and that differences in GA resulted in significant differences in the occurrence of PTD. Where analysis of safety of ART use in pregnancy by timing of ART initiation is focused on outcomes such as PTD or SGA, our work highlights the need for standardized GA ascertainment methods.

Using US-GA, PTD risk was associated with maternal HIV infection, with HIV-infected women, all on ART, almost twice as likely to deliver preterm than HIV-uninfected women. A weaker association was observed with SFH-based GA; and minimal differences with LMP-GA.. We did not find any appreciable differences in PTD risk for HIV-infected women by timing of ART initiation by all three assessment methods. Our findings (in both the overall cohort and in women with all three assessments) suggest that GA ascertainment methods could partially explain the heterogeneity of findings from previous studies on the association between ART use and adverse birth outcomes, suggesting that care should be taken when interpreting results from such studies.

Our finding for a higher PTD incidence in HIV-infected than HIV-uninfected women when using US-GA is consistent with several studies, using different assessment methods, across different settings with the incidence of any PTD (<37 weeks) ranging from 13% to 37%.^{3,7,8,21,22} Using US-GA we found an incidence of 11% (95% CI 9–13%) among HIV-infected women overall, similar to findings from a Nigerian study;²² but significantly lower than findings from other studies.^{3,7,8,21,22} In addition to differences in assessment methods, this could also be due to underlying differences in maternal (HIV disease stage and clinical characteristics) and behavioural risk factors (alcohol and substance abuse).

Using LMP-GA and SFH-GA, we found estimated PTD incidence to be higher than with US-GA, and similar to findings from previous studies, which primarily used LMP-based

measures. However, in adjusted analyses the difference in PTD risk by HIV status was marginal and not significant using either method. The most plausible explanation for the discrepancy in findings between US and LMP/SFH measures is random measurement error. When stratified by HIV and BMI status, the proportions of PTD observed by each assessment method remained similar to the overall, pointing to non-differential misclassification of PTD status due to random measurement error in the GA estimated by LMP and SFH. In previous studies, GA measurement error resulted in an over-estimation of preterm and post-term delivery incidence,^{9, 12, 23} consistent with our findings of over-estimation by both LMP-GA and SFH-GA compared to US-GA. Our findings show that PTD over-estimation by LMP/SFH was driven primarily by the inflation of the tails of the GA distribution, where estimates were more extreme than the likely true-data estimates.

In our setting, LMP-GA estimates varied widely with measurement error resulting predominantly in under-estimated GA contributing to the substantially higher PTD incidence, in contrast over-estimation would have led to higher frequencies of post-term delivery classification. While we expected some differences between LMP-GA and US-GA,²⁴ the degree of discrepancy was greater than previous reports from high-income countries and other resource-limited settings;^{24,25} it was, however, in keeping with previous South African data.^{26,27} These inconsistencies suggest the degree and type of error in LMP-GA may depend on demographic characteristics of the study population and data collection methods. For example, errors in GA estimates due to delayed ovulation usually lead to GA over-estimation, whereas GA errors due to recall issues can lead to errors in either direction. Additionally, women with non-normal BMI (<19 or $>29\text{kg/m}^2$) may misreport their LMP due to menstrual irregularities.^{28,29} In our study population, numerous factors could have played a part in the under- and over-estimation of LMP-based PTD frequencies (relative to US). In addition to BMI, other socio-demographic factors, known to be associated with reduced accuracy of LMP-GA, are prevalent in our setting. This includes high level of injectable contraception use,³⁰ long-term use of which affects return to menses and fertility, limiting LMP reliability.³¹ Additionally younger, primigravid women and women with lower educational levels are more likely to misreport LMP.^{12,28} Since HIV-uninfected women were more likely to be younger and primigravid it is possible they misreported their LMP more than HIV-infected women, which could explain why the expected association by HIV status was not observed with LMP-GA.

In contrast to LMP-GA, in our study SFH-GA was predominantly over-estimated, possibly a reflection of study participants BMI profile. Similar to a previous South African study, SFH-GA performed better than LMP-GA, with accuracy worsening with increasing BMI presumably due to increased maternal adiposity interfering with SFH measurement.²⁶ This is consistent with reports that high BMI affects the accuracy of SFH-GA estimates;^{13,32} a Mozambican study demonstrated that among women of the same GA, the fundal height of obese women was higher than that of normal-weight women resulting in GA over-estimation.³² Accuracy in overweight and obese women is compromised because GA calculation is based on fundal height growth curves derived from normal weight women.³³ SFH measurements are also subject to intra- and inter-observer errors, a particular problem with single measurements.³³ Multiple SFH measurement models have been shown to have a higher level of accuracy when repeated measurements are used.³⁴ While the association

between HIV status and PTD was in the expected direction, attenuation by BMI-related measurement errors could have resulted in non-significant associations.

In HIV-infected women, we did not observe any significant association between timing of ART initiation and PTD by any of the assessment methods. It is possible that our sample size did not enable detection of small to moderate differences between these groups of women, however our results are consistent with findings from a previous South African cohort,²¹ and findings from other settings.³⁵ This suggests that in our study population there might be minimal differences across the comparison group and that any GA measurement errors were equally distributed.

Overall, SGA incidence did not differ greatly by assessment method, which was surprising given GA differences observed according to assessment method. In contrast, LGA estimates differed by assessment methods, with larger than expected proportions of LGA by LMP-GA and SFH-GA, which would misclassification due to inaccurate GA. These results suggest that in our setting, in the absence of ultrasound facilities, SFH-GA is substantially more reliable than LMP-GA.

A major strength of our study was the prospective data collection and GA assessment by a research sonographer, who was blinded to other measures, ensuring a high-quality measure of gestation. Further, US was conducted in women < 24 weeks, when US is highly reproducible and accurate to date pregnancy duration.³⁶ While there is a possibility that our US-GA estimates may be biased due to systematic under-estimation of GA for smaller fetuses, these errors have been shown to be relatively small compared to the larger errors seen when using LMP- and SFH-based measures for GA estimation.¹² While we did not directly measure LMP and/or SFH as part of study procedures and relied instead on data abstraction from routine records, we believe any measurement errors were random rather than systematic. Study limitations include the absence of data on pre-pregnancy BMI and other factors that can influence GA estimate accuracy, such as maternal education which is known to impact LMP-GA estimate accuracy. In addition to measurement error, selection bias is also likely to have contributed to the attenuation of associations in the group of women with three measures. Moreover, given that clinical estimates, which we showed are subject to substantial measurement error, were used to determine which women were referred for ultrasounds there is a possibility of selection bias which could have led to inaccurate estimate of the true association. Additionally, while it was intended that the sonographer be blinded, this was only partial since by study protocol only women with < 24 weeks GA were referred for ultrasound assessment.

The relationship between ART and adverse birth outcomes is complex: it is well established that ART can improve birth and child health outcomes by improving maternal health, but ART may also contribute to worsening outcomes through mechanisms that have not been fully elucidated. With widespread use of ART in pregnancy and growing public health interest in this association, understanding and addressing adverse birth outcomes is vitally important. This study makes an important contribution to this emerging science by demonstrating that inaccurate GA measurement can distort associations between maternal characteristics, including HIV/ART, and adverse birth outcomes. In settings where LMP and

SFH-based measures are widely used, bias due to GA ascertainment methods employed should be considered as an alternative explanation for observed associations or null findings. Additionally, given that there appear to be systematic differences in GA estimates according to BMI status, future research efforts should consider the BMI profile of study populations and seek to standardize optimal measures of GA. While early US should be encouraged whenever possible, simple and novel methods that accurately measure GA are required particularly in settings where ultrasound is unavailable; and this should include postnatal assessments to confirm GA based on antenatal determination.

Furthermore, quantitative bias analytic techniques that account for measurement error in GA and/or missing data should be considered in future analyses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1: Incidence of preterm deliveries (<37weeks) by HIV/ART Status, **A** in all enrolled women (n=1787), and **B** in all HIV-infected women (n=773), **C** in women assessed by all three methods (n=629), and **D** in HIV-infected women assessed by all three methods (n=320)

Figure 2:

Birthweight and gestational age according to GA assessment method (boys and girls separately) SGA – small for gestational age; LGA – large for gestational age; * Superimposed smoothed 10th (SGA) and 90th (LGA) centile curves for birthweight according to gestational age and gender

Table 1:Description of enrolled women (n=1787) at 1st ANC Visit

	Total n=1787 (%)	HIV-uninfected n=1014 (%)	HIV-infected n=773 (%)	HIV-infected	
				Initiated before pregnancy n=368 (%)	Initiated during pregnancy n=405 (%)
Maternal Characteristics					
Age, years					
<24	536 (30)	398 (39)	138 (18)	43 (12)	95 (23)
25–29	500 (28)	291 (29)	209 (27)	76 (21)	133 (33)
>30	751 (42)	325 (32)	426 (55)	249 (67)	177 (44)
Median	28 (24–32)	26 (22–31)	30 (26–33)	32 (28–35)	29 (25–32)
Height, cm					
155	500 (28)	270 (27)	230 (30)	108 (29)	122 (30)
156–161	651 (36)	375 (37)	276 (36)	143 (39)	133 (33)
162	636 (36)	369 (36)	267 (35)	117 (32)	150 (37)
Median	158 (155–163)	159 (155–163)	158 (154–163)	158 (154–162)	158 (154–163)
Gestation at enrolment, weeks					
Determined by LMP	1585	896	689	368	405
Median	17 (12–22)	18 (13–24)	15 (11–21)	14 (11–19)	16 (12–22)
Determined by SFH	1219	709	510	215	295
Median	23 (18–28)	24 (19–28)	20 (16–26)	19 (14–25)	21 (17–27)
Determined by US	1204	603	601	279	322
Median	16 (12–21)	17 (12–22)	15 (11–20)	14 (11–18)	16 (12–20)
Body Mass Index					
Underweight	16 (1)	10 (1)	6 (1)	5 (1)	1 (0.3)
Normal	329 (18)	188 (19)	141 (18)	66 (18)	75 (19)
Overweight	494 (28)	282 (28)	213 (18)	104 (28)	110 (27)
Obese	823 (46)	467 (46)	356 (46)	166 (45)	189 (47)
Gravidity					
1	383 (21)	263 (26)	120 (16)	41 (11)	79 (20)
2	639 (36)	372 (37)	267 (35)	119 (32)	148 (37)
3	765 (43)	379 (37)	386 (50)	208 (57)	178 (44)
Median	2 (2–3)	2 (1–3)	2 (2–3)	2 (2–3)	2 (2–3)
Parity					
0	472 (26)	303 (30)	169 (22)	67 (18)	102 (25)
1	700 (39)	397 (39)	303 (39)	135 (37)	168 (42)
2	615 (35)	314 (31)	301 (39)	166 (45)	135 (33)
Median	1 (0–2)	1 (0–2)	1 (0–2)	1 (1–2)	1 (1–2)
Previous Preterm *					
Yes	127 (9)	58 (8)	69 (11)	37 (11)	32 (10)

	Total n=1787 (%)	HIV-uninfected n=1014 (%)	HIV-infected n=773 (%)	HIV-infected	
				Initiated before pregnancy n=368 (%)	Initiated during pregnancy n=405 (%)
Haemoglobin g/dl					
Normal (11.0)	792 (44)	496 (49)	296 (46)	171 (46)	125 (31)
Mild Anaemia (9–10.9)	613 (34)	337 (33)	276 (43)	111 (30)	165 (41)
Moderate Anaemia (7–8.9)	122 (7)	54 (5)	68 (11)	25 (7)	43 (11)
Severe Anaemia (<7)	3 (0.2)	3 (0.3)	0	0	0
ART Regimen					
TDF-3TC-EFV	691 (39)	-	691 (94)	298 (89)	393 (98)
TDF-3TC-NVP	4 (0.2)	-	4 (1)	2 (1)	2 (0.5)
Other NNRTI	20 (1)	-	20 (2)	14 (4)	6 (1)
PI regimen	22 (1)	-	22 (3)	20 (6)	2 (0.5)

* among women with a previous pregnancy

All variables, with the exception of height, BMI and haemoglobin had <1% missing data. For height, 6% (n=111) of data was missing and for BMI, 7% (n=126) was missing, for both there were similar proportions across all comparison groups. For Haemoglobin, 14% (n=57) of data was missing with similar proportions across all comparison groups. For ART status 2% (n=36) were missing regimen data

Table 2:

Birth outcomes in overall cohort (n=1787)

	Total	HIV-uninfected	HIV-infected	HIV-infected	
				Initiated before pregnancy	Initiated during pregnancy
Gestational Age (weeks) LMP	n=1585	n=896	n=689	n=336	n=353
Post-term >42	115 (7)	57 (6)	58 (8)	33 (10)	25 (7)
Term (37–42)	896 (57)	522 (58)	374 (55)	185 (55)	189 (54)
Any Preterm (< 37) *	574 (36)	317 (36)	257 (37)	118 (35)	139 (39)
<i>Late to Mod Preterm (32–37)</i>	<i>418 (73)</i>	<i>238 (75)</i>	<i>180 (70)</i>	<i>86 (73)</i>	<i>94 (68)</i>
<i>Very Preterm (28–32)</i>	<i>156 (27)</i>	<i>79 (25)</i>	<i>77 (30)</i>	<i>32 (27)</i>	<i>45 (32)</i>
Gestational Age (weeks) SFH	n=1219	n=709	n=510	n=215	n=295
Post-term >42	97 (8)	59 (8)	38 (7)	15 (7)	23 (8)
Term (37–42)	914 (75)	532 (75)	382 (75)	161 (75)	221 (75)
Any Preterm (< 37) *	208 (17)	118 (17)	90 (18)	39 (18)	51 (17)
<i>Late to Mod Preterm (32–37)</i>	<i>171 (82)</i>	<i>98 (83)</i>	<i>73 (81)</i>	<i>33 (85)</i>	<i>40 (78)</i>
<i>Very Preterm (28–32)</i>	<i>37 (18)</i>	<i>20 (17)</i>	<i>17 (19)</i>	<i>6 (15)</i>	<i>11 (22)</i>
Gestational Age (weeks) US	n=1204	n=603	n=601	n=279	n=322
Post-term >42	6 (1)	6 (10)	0	0	0
Term (37–42)	1065 (88)	547 (91)	518 (86)	244 (88)	274 (85)
Any Preterm (< 37) *	133 (11)	50 (8)	83 (14)	35 (12)	48 (15)
<i>Late to Mod Preterm (32–37)</i>	<i>114 (86)</i>	<i>47 (94)</i>	<i>67 (81)</i>	<i>28 (80)</i>	<i>39 (81)</i>
<i>Very Preterm (28–32)</i>	<i>19 (14)</i>	<i>3 (6)</i>	<i>16 (19)</i>	<i>7 (20)</i>	<i>9 (19)</i>
Size for Gestational Age LMP	n=1441	n=825	n=616	n=294	n=322
Small (SGA)	137 (10)	61 (7)	76 (12)	36 (12)	40 (13)
Appropriate (AGA)	847 (59)	498 (60)	349 (57)	181 (62)	168 (52)
Large (LGA)	457 (32)	266 (32)	191 (31)	77 (26)	114 (35)
Size for Gestational Age SFH	n=1113	n=649	n=464	n=195	n=269
Small (SGA)	145 (13)	72 (11)	73 (16)	22 (11)	51 (19)
Appropriate (AGA)	814 (73)	481 (74)	333 (72)	150 (77)	183 (68)
Large (LGA)	154 (14)	96 (15)	58 (13)	23 (12)	35 (13)
Size for Gestational Age US	n=1184	n=596	n=588	n=273	n=315
Small (SGA)	131 (11)	49 (8)	82 (14)	39 (14)	43 (14)
Appropriate (AGA)	944 (80)	492 (83)	452 (77)	213 (78)	239 (76)
Large (LGA)	109 (9)	55 (9)	54 (9)	21 (8)	33 (10)

* Any Preterm consists of the subset of Late Preterm, Moderately Preterm and Very Preterm deliveries

For Size for GA data missing for women whose GA is not within Intergrowth standard limits (<24w and >42w) and those missing either GA or birthweight

Table 3:

Adjusted association between HIV/ART status and preterm delivery in women with all three assessment methods (n=629)

Assessment Method	HIV-infected vs HIV-uninfected (Ref)			Initiated before pregnancy (Ref) vs Initiated during pregnancy		
		aOR (95% CI)	P-value		aOR (95% CI)	P-value
Last Menstrual Period (LMP)	HIV-infected	0.89 (0.63–1.24)	0.483	During pregnancy	0.94 (0.56–1.60)	0.802
Symphysis Fundal Height (SFH)	HIV-infected	1.34 (0.83–2.15)	0.225	During pregnancy	1.42 (0.76–2.84)	0.290
Ultrasound (US)	HIV-infected	1.98 (1.12–3.53)	0.020	During pregnancy	0.69 (0.33–1.47)	0.335

* Adjusted for age, parity, BMI and previous PTD

** Adjusted for age, parity, BMI, previous PTD and ART regimen

Adjusted association between HIV/ART status and concordance with US-based measures in women with both an US estimate and a clinical estimate

Table 4:

Assessment Method	HIV-infected vs HIV-uninfected (Ref) [*]			Initiated before pregnancy (Ref) vs Initiated during pregnancy ^{**}		
	Ref category: No difference	ARR (95% CI)	P-value	Ref category: No difference	ARR (95% CI)	P-value
Last menstrual period (LMP) (n=1057)	Under-estimate	0.96 (0.71–1.27)	0.764	Under-estimate	0.87 (0.53–1.45)	0.609
	HIV-infected Over-estimate	1.33 (0.95–1.64)	0.098	During pregnancy Over-estimate	1.34 (0.78–2.31)	0.288
Symphysis Fundal Height (SFH) (n=732)	Under-estimate	1.21 (0.80–1.83)	0.364	Under-estimate	1.23 (0.66–2.30)	0.518
	HIV-infected Over-estimate	1.33 (0.94–1.87)	0.102	During pregnancy Over-estimate	0.91 (0.54–1.52)	0.711

^{*} Adjusted for age, parity, BMI

^{**} Adjusted for age, parity, BMI and ART regimen