STUDY PROTOCOL

Viral and antiretroviral dynamics in HIV mother-to-child transmission fluids (VADICT) – Protocol and data analysis plan for a cohort study [version 1; referees: awaiting peer review]

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Abstract

Background: Pregnancy and polymorphisms in drug disposition genes alter the clearance of key antiretrovirals used as part of regimens for prevention of mother-to-child transmission of HIV (PMTCT). The clinical significance of these in women initiating therapy late in pregnancy has not been investigated. The primary objective of the Viral and Antiretroviral Dynamics in HIV Mother-To-Child Transmission Fluids (VADICT) study is to investigate viral and antiretroviral dynamics in matrices associated with mother-to-child transmission (MTCT) (plasma, genital fluid and breastmilk) in women (stratified by CYP2B6 genotypes) who initiate antiretroviral therapy (ART) before or early in pregnancy versus late in pregnancy or early postpartum.

Methods: A cohort of HIV-1 infected women who initiated ART containing 600 mg efavirenz before or early in pregnancy (n = 120), during the third trimester (n = 60), or early postpartum (n = 60) will be studied. Eligible patients will be recruited from four hospitals in Benue State, North Central Nigeria and followed until the end of breastfeeding. Procedures at follow up visits will include sample collection for drug quantification and HIV-1 RNA and DNA in plasma, genital fluid and breastmilk; adherence monitoring; and newborn and infant assessment. Using newborn exposure to maternal efavirenz at birth for validation, prenatal pharmacogenetics of efavirenz will be explored using physiologically-based pharmacokinetic modelling. Three integrated methods will be used to monitor patterns and correlates of adherence across pregnancy and the breastfeeding period. A population pharmacokinetic-pharmacodynamic model will be developed to describe the observed data and simulate what to expect in women initiating ART containing 400 mg efavirenz (recently approved for non-pregnant adults) late in pregnancy or early postpartum.

Discussion: This study will help in understanding residual MTCT in women...
receiving ART and reasons for the rise in MTCT risk during the breastfeeding period.

**Trial registration**: ClinicalTrials.gov: NCT03284645 (15/09/2017)

**Keywords**
antiretroviral therapy, pregnancy, human immunodeficiency virus, mother-to-child transmission, efavirenz, pharmacogenetics, pharmacokinetics-pharmacodynamics, cervicovaginal fluid

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Introduction

Current national and international guidelines for prevention of mother-to-child transmission (PMTCT) of HIV recommend urgent initiation of lifelong antiretroviral therapy (ART) in all HIV-infected pregnant and breastfeeding women. HIV-exposed infants receive daily nevirapine or dual nevirapine and zidovudine prophylaxis from within 72 hours of birth until 4–12 weeks of age. This approach has reduced mother-to-child transmission (MTCT) of HIV to less than 5% from the baseline 25–40% without intervention. ART prevents MTCT by reducing viral load in transmission fluids: plasma, genital fluids and breastmilk. However, about 150,000 infants are still infected every year (17 per hour). Data from the Malawi PMTCT programme in 2016 indicated rates of 1.4, 3.9, 4.3 and 13.3% in 4–12 week old infants when ART was started before pregnancy, in the second trimester, third trimester and postpartum, respectively.

Several factors likely contribute to these residual transmissions, including suboptimal adherence, late initiation of ART (as a result of late diagnosis of HIV), acute maternal infection during pregnancy or breastfeeding, and the presence of baseline resistance, which result in detectable HIV RNA at third trimester and delivery. In addition, mixed feeding and abrupt weaning cause elevated HIV-1 in breastmilk. These exacerbate MTCT risk. Evidence of additional risk of MTCT as a result of maternal co-infections with herpes simplex virus type 2, cytomegalovirus, and malaria in pregnancy have been reported.

The relationship between antiretroviral drug concentrations and adequate virological suppression is well documented; however, pregnancy and genetic polymorphisms in drug disposition genes alter the pharmacokinetics of many drugs, including key antiretrovirals. For instance, compared with postpartum women efavirenz area under the concentration-time curve (AUC) was 50.6% lower (p = 0.0013) and minimum plasma concentration (Cmin) was 61.6% lower (p = 0.0027) in pregnant women classified as extensive metabolisers (CYP2B6 516GG genotype). The median (range) Cmin was 592 ng/mL (429–917) in pregnant women compared with 1540 ng/mL (867–2310) in postpartum women. In a parallel study, nevirapine Cmin was also below target in 50–67% of pregnant women in a pooled analysis and when stratified into three CYP2B6 genotype groups. Similar findings have been reported by other authors, and for other antiretrovirals, including those used along with efavirenz as part of first-line regimens in pregnant women. The influence of these changes on achieving undetectable viral load in plasma, genital fluid and breastmilk in women initiating therapy late in pregnancy is not known (Figure 1). The effect of polymorphisms in efavirenz disposition genes on prenatal exposure has also not been investigated.

Major HIV treatment guidelines now include the 400 mg reduced dose of efavirenz as part of alternative first-line options based on evidence of its non-inferiority to the 600 mg dose and significantly less neuropsychiatric side effects in non-pregnant adults, except in pregnant women and patients receiving anti-tuberculosis drugs. Uncertainly about plasma concentration achievable during pregnancy have limited investigations of the 400 mg dose in pregnancy to small cohorts of virologically suppressed patients. Superior efficacy and safety of the integrase strand transfer inhibitor dolutegravir compared with an efavirenz-based regimen has been demonstrated. However, preliminary data of increased neural tube defects in infants born to women who received dolutegravir at the time of conception have raised concerns. Hence, it is not currently recommended for use in individuals of childbearing age not using effective contraception, those contemplating pregnancy, or within 12 weeks post-conception; efavirenz remains the preferred option in these groups. Therefore, there is still need for data to guide evidence-based recommendation on the use of the 400 mg reduced dose of efavirenz in viremic pregnant women.

Study objectives

Primary objectives:

1. To determine the trends in viral load changes in plasma, genital fluid, and breastmilk following early versus late ART initiation in pregnancy and postpartum;
2. To assess the relationship between viral load changes and efavirenz pharmacogenetics in plasma, genital fluid and breastmilk during pregnancy and postpartum;
3. To evaluate prenatal pharmacogenetics of efavirenz using both clinical pharmacokinetics study and mechanistic physiologically-based pharmacokinetic (PBPK) modelling.

Secondary objective: The pattern and correlates of adherence to ART in HIV infected women from pregnancy to the end of the breastfeeding period will be investigated as a secondary objective.

Exploratory objective: Patterns of viral load changes in plasma, genital fluid, and breastmilk during pregnancy and postpartum at the standard 600 mg versus 400 mg reduced dose (recently approved only in non-pregnant adults) in virtual women initiating therapy late in pregnancy and early postpartum will be compared as an exploratory objective using a pharmacokinetic-pharmacodynamic (PKPD) modelling.

Protocol

Study design. This is a cohort study of pregnant or recently postpartum HIV-infected women receiving a WHO recommended efavirenz-based, first-line ART regimen. Eligible patients will be recruited from four Nigerian hospitals: Federal Medical Centre, Makurdi; Bishop Murray Medical Centre, Makurdi; St. Monica’s Hospital, Adikpo; and St. Thomas’ Hospital, Ilugh. Inclusion criteria: receiving efavirenz-based regimen, ≥ 18 years old, planned exclusive breastfeeding until 6 months of age, able to understand study information and comply with follow-up schedule. Exclusion criteria: severe maternal or infant illness, planned exclusive formula feeding, taking drugs or herbal medication with known or uncertain interaction with study drugs.
**Viral load changes in plasma, female genital tract, and breastmilk.** Plasma, vaginal swabs, and breastmilk samples will be collected for HIV-1 RNA quantification as per the schedule of evaluations and documentation at follow-up visits (Table 1). Plasma will be obtained from whole blood collected in vacutainer EDTA tubes by centrifugation at 3000 x g for 10 minutes. Cervicovaginal swabs will be collected by the attending antenatal clinic nurse using flocked swabs (Copan, Murrieta, CA, USA). Breastmilk samples will be self-collected by patients from both breasts mid-feed by manual expression and the aliquot for viral load assay will be centrifuged at 1500 x g for 12 minutes at 4°C. The top cream layer, aqueous interface, and milk cell pellet will be stored in separate cryovials. Cell pellets will be used for HIV-1 DNA isolation and quantification while the cell-free aqueous milk fraction will be used for HIV-1 RNA isolation and quantification. All samples will be stored at -80°C at the Federal Medical Centre, Makurdi. Samples will be transported to the Translational Pharmacokinetics Research Laboratory at Obafemi Awolowo University, Ile-Ife using Arctic Express® Dry Shipper (Thermo Scientific, Waltham, MA, USA) for storage at -80°C until analysis.

**Pharmacogenetics of efavirenz in plasma, genital fluid, and breastmilk.** Up to five paired plasma and cervicovaginal swab samples (during pregnancy), and up to five paired plasma and breastmilk samples (postpartum) will be collected per subject for drug quantification (Table 1). Genomic DNA will be extracted using E.Z.N.A.® Blood DNA Mini Kit (Omega Bio-Tek,
### Table 1. Schedule of evaluations and documentation at follow-up visits.

<table>
<thead>
<tr>
<th>Evaluation &amp; Documentation (CRF Page to Complete)</th>
<th>Enrolment (1 visit)</th>
<th>Third Trimester (3 Visits)</th>
<th>Labour/Delivery (1 visit)</th>
<th>14-21 Days Postpartum (1 visit)</th>
<th>6 Weeks-18 Months Postpartum (5 visits)</th>
</tr>
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<tbody>
<tr>
<td><strong>CLINICAL</strong></td>
<td></td>
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<td>Concomitant Medications (2)</td>
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<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Eligibility (3–6)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Demographics (7–8)</td>
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<td>X</td>
<td>X</td>
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<tr>
<td>Gestational History (9)</td>
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<tr>
<td>Physical Examination (10)</td>
<td>X</td>
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<tr>
<td>Newborn Assessment at Delivery (24)</td>
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<td>X</td>
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<tr>
<td>Postpartum Infant Assessment (25)</td>
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<td><strong>LABORATORY</strong></td>
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<tr>
<td>Haematology (11, if recent &amp; available in patient record)</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Biochemistry (12, if recent &amp; available in patient record)</td>
<td>X</td>
<td>-</td>
<td>-</td>
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<td></td>
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<tr>
<td>Whole Blood for Genetics</td>
<td>1mL</td>
<td>-</td>
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<tr>
<td>Plasma for HIV RNA (16)</td>
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<td>2mL x 2</td>
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<td>2mL x 2</td>
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<tr>
<td>Plasma for PK (20–21)</td>
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<td>1mL x 1</td>
<td>1mL x 1</td>
<td>-</td>
<td>1mL x 1</td>
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<tr>
<td>Breastmilk for HIV RNA &amp; DNA (17)</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>5mL x 1</td>
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<td>Breastmilk for PK (22)</td>
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<td>5mL x 1</td>
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<td><strong>ANTENATAL/MATERNITY</strong></td>
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<tr>
<td>Vaginal Swab for HIV RNA (19)</td>
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<td>1 swab</td>
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<tr>
<td>Vaginal Swab for PK (19)</td>
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<td>1 swab</td>
<td>1 swab</td>
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<tr>
<td>Cord DBS &amp; Cord Plasma for PK (23)</td>
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<td>-</td>
<td>5 spots &amp; 2mL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Newborn/Infant DBS for EID &amp; PK (24)</td>
<td>-</td>
<td>-</td>
<td>5 spots (kid)</td>
<td>5 spots (kid)</td>
<td>5 spots (kid)</td>
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<tr>
<td><strong>PHARMACY</strong></td>
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<tr>
<td>Adherence Questionnaire, Pill Count &amp; DBS for TDM (14-15)</td>
<td>X (5 spots)</td>
<td>X (5 spots)</td>
<td>-</td>
<td>-</td>
<td>X (5 spots)</td>
</tr>
<tr>
<td>ART Dispensing Record (26)</td>
<td>X</td>
<td>X</td>
<td>-</td>
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<td>X</td>
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</tbody>
</table>


Inc, Norcross, GA). Polymorphisms in drug disposition genes previously associated with exposure to efavirenz will be determined by real-time PCR using TaqMan genotyping assays for single nucleotide polymorphisms (SNPs), including CYP2B6 516G>T (rs3745274, product ID: C_7817765_60) and CYP2B6 983T>C (rs28399499, product ID: C_60732328_20) which will be used to classify patients as slow, intermediate and fast metabolisers of efavirenz, based on the number of variant alleles. Additional SNPs could be explored based on emerging data in the literature.

**Prenatal pharmacogenomics of efavirenz.** At delivery, umbilical cord blood samples will be collected as dried blood spots (DBS) on Whatman 903 Protein Saver cards from the foetal side immediately after cord clamping. Additional DBS samples will be collected from both mother (using finger prick) and baby (using heel prick) as soon as possible after delivery before breastfeeding starts. Genomic DNA from newborn DBS will be extracted and genotyping for functional SNPs will be conducted as previously described for the mother.

Efavirenz quantification in plasma and breastmilk will be performed at the GCP Bioanalytical Facility of the University of Liverpool, Liverpool, UK, using previously validated liquid chromatography-mass spectrometry (LC-MS) methods. A new LC-MS method will be developed for efavirenz quantification in
cervicovaginal swab samples and validated in line with FDA and EMA guidelines\textsuperscript{44,45}.

**Adherence monitoring.** Adherence to antiretroviral medication will be assessed once every trimester throughout pregnancy and then 6-monthly throughout the breastfeeding period. This will be conducted using three methods: adherence questionnaire (see case report form under Extended Data\textsuperscript{39}), pill count and therapeutic drug monitoring with DBS. Adherence data from the questionnaire and pill count will be graded (poor, 0–75%; partial, 75–95%; and near perfect, >95%). Therapeutic drug monitoring using intracellular tenofovir diphosphate and emtricitabine triphosphate concentrations in DBS will be used for post-hoc validation of these two approaches in selected patients at different adherence levels (all cases of suboptimal adherence matched 1:2 with adherent patients).

**Outcomes**

The primary outcomes are the comparisons of the proportions of women in the three CYP2B6 genotype groups (slow, intermediate and fast metabolisers) with undetectable HIV-1 viral load in: (1) plasma versus genital fluid at delivery when ART is started early versus late in pregnancy, and (2) plasma versus breastmilk at 6 weeks postpartum when ART is started early or late in pregnancy versus postpartum.

Secondary outcomes will be the correlation between HIV-1 viral load in plasma and genital fluid (pregnancy), plasma and breastmilk (postpartum); comparisons of female genital tract efavirenz penetration and foetal/newborn efavirenz exposure in the three CYP2B6 genotype groups; the level of agreement between self-reported and pharmacological measure of adherence; changes in adherence pattern between pregnancy and the postpartum periods; comparison of newborn assessment scores and infant HIV status at the end of breastfeeding in the three study groups.

**Pharmacokinetic analyses**

**Pharmacokinetic-pharmacodynamic (PKPD) model of antiretrovirals in plasma, genital fluid and breastmilk.** A multi-compartment population pharmacokinetic model will be developed using nonlinear mixed effects methods to describe the plasma pharmacokinetics and distribution into female genital tract and breastmilk. Covariate effects, including genetics, demographics and clinical characteristics will be evaluated to determine their impact on variability in pharmacokinetic parameters and transfer of drug into female genital tract and breastmilk. Pharmacokinetic parameters derived from the model will be used to drive the pharmacodynamic analysis. Virological endpoints will be analysed using nonlinear mixed effects through a linked pharmacokinetic-viral dynamics model or utilising alternative statistical methods (e.g. logistic regression). Model evaluation will be achieved through internal and external validation processes. The internal evaluation will involve visual predictive check and bootstrap analysis. For external model evaluation, the predictive performance of the model will be assessed by using the model to predict the observed data from another study cohort (previously published or unpublished data accessible to the investigators).

**Mechanistic modelling of prenatal exposure to efavirenz.** A maternofoetal PBPK model integrating whole-body maternal and multi-compartmental foetal units\textsuperscript{7} will be developed using SimBiology® (MATLAB® version 2017b or later). Processes governing maternal drug disposition and maternal-foetal physiology, along with associated gestational age-dependent changes, will be described using differential equations with key system and drug-specific parameters obtained from the literature (e.g.\textsuperscript{46}). Transplacental drug transfer will be modelled as bidirectional passive diffusion. In the absence of a specific parameter, sensitivity analysis will be used to assess the impact uncertainty may have on the simulation outcome. Model refinement and verification will be based on comparison between predicted and clinically observed values of maximum concentrations ($C_{\text{max}}$), time to maximum concentration ($T_{\text{max}}$), clearance (CL), AUC, and newborn-to-maternal drug concentration ratio at delivery. The acceptance threshold will be set at 50% difference between predicted and observed values. The percentage of observed datapoints that fit within the predictive interval will be evaluated using visual predictive checks of overlays of predicted and observed pharmacokinetic profiles. The verified maternofoetal PBPK model will be used to predict foetal plasma and organ exposure to efavirenz at different stages of pregnancy. A similar model will be developed for a known teratogen (e.g. thalidomide) to obtain foetal exposure indices to use as a benchmark in evaluating potential application of this model (e.g. as part of a quantitative systems pharmacology model) in evaluating drug safety in pregnancy.

**Statistical analysis**

Correlation coefficient will be used to evaluate the degree of relationship between continuous variables (Pearson’s for normally distributed and Spearman’s for skewed data). Associations between continuous outcome measures (e.g. newborn efavirenz concentration) and explanatory variables (e.g. CYP2B6 SNPs) will be quantified using regression. Variables with p values <0.20 in the univariable analysis will be included in the multivariable model, adjusting for gestational age, maternal age, body weight, and time after dose. Linearity, homoscedasticity and other model assumptions will be investigated by plotting residuals against each explanatory variable and the fitted values. Repeated outcome measurements that are likely to be correlated in individuals (e.g. viral load measurements in genital tract during pregnancy, or in breastmilk during lactation) will be analysed using generalised estimating equations with a working assumption of an exchangeable correlation matrix.

**Sample Size Estimation**

The sample size estimate and power calculation (Stata\textsuperscript{®} v14.1, College Station, TX, USA) for achieving these objectives were based on a range of odds ratios (OR, 2.0–4.0) for the likelihood of detectable viral load in pregnant and postpartum women who initiated ART <4 months versus ≥4 months before delivery.
Bobrow et al. reported an OR of 3.98 and about 70% women with undetectable viral load at delivery\textsuperscript{49}. Therefore, based on a conservative OR of 3.0 for >0.80 power, about 240 pregnant women (including 10% dropout provision) will be recruited in three groups based on the time of ART initiation before delivery (n = 120, ≥ 4 months; n = 60, <4 months; n = 60, postpartum). This will also give > 0.80 power to detect the influence of genetic factors on antiretroviral drug exposure in the three matrices. Using recent enrolment data from the study sites, the total sample size of 240 can be recruited within 18 months. Additionally, more eligible patients are anticipated because of ongoing efforts to increase diagnosis and treatment coverage among pregnant women in Nigeria\textsuperscript{50,51}.

About 145 of these (including provision for 20% dropout) will be sufficient to evaluate the influence of maternal and infant genetics on prenatal exposure to efavirenz. This is based on a conservative estimate that a multivariate linear regression model that includes SNPs in drug disposition genes along with relevant demographic variables will explain at least 10% of observed variability in foetal exposure to maternal antiretroviral drugs as determined by infant-to-maternal and cord-to-maternal plasma concentration ratios (G\textsuperscript{4}Power) For instance, only CYP2B6 516G>T alone has been reported to explain up to 30% of observed variability in efavirenz plasma concentration in a cohort of HIV-infected adults in Serbia\textsuperscript{51}. At an α of 0.01 and an estimate of 5 predictors, a sample size of 121 will give a power of > 0.80. For a McNemar’s test for proportion in two dependent groups with α of 0.05 and 30% discordant pairs, a sample size of 240 will give more than 80% power to detect a 2-fold change in adherence between pregnancy and the postpartum period.

Ethics approval and participants’ consent
Ethics approval for the conduct of the study was obtained from the National Health Research and Ethics Committee, Abuja, (NHREC/01/01/2007-05/06/2017) and the Research Ethics Committees of participating hospitals. Participation will be entirely voluntary, and patients will be allowed to withdraw their consent at any stage. In addition, not consenting will not affect care in any way. All samples will be anonymised and no clinical data traceable to individual patients will be kept or passed on to a third party. Obafemi Awolowo University is the study sponsor. Material Transfer Agreements (MTA) will be executed between Obafemi Awolowo University and each of the institutions where laboratory analysis will be conducted.

In line with the national policy on the participation of children in clinical research, consent of both parents will be sought for the inclusion of the babies born to enrolled mothers. In cases of non-disclosure to partners, consent of the mother alone will be considered adequate if she has the primary responsibility for the child at the time of participation in the study. Considering the emotional distress that often accompanies the diagnosis of HIV during pregnancy and the early postpartum period, the following measures will be taken to avoid further aggravation and to ensure adequate protection of participants: (1) existing structures at study sites will be used to offer additional psychological support in connection with the enrolment process; (2) patients will be given ample time to consider study information and decide on whether to participate; (3) only those considered to be emotionally stable by the counsellor and the primary care provider will be enrolled.

This study was registered with ClinicalTrials.gov (NCT03284645) on the 15\textsuperscript{th} September 2017.

Dissemination of data and material
The completely anonymised data from VADICT will be published on Wellcome Open Research (or similar platform) after a two-year embargo starting from study termination. This will allow the investigators to publish study findings in peer-reviewed journals.

Study status
This study is currently recruiting and following up participants.

Data availability
Underlying data
No data is associated with this article.

Extended data
Open Science Framework: Viral and Antiretroviral Dynamics in HIV Mother-To-Child Transmission Fluids (VADICT) – Case Report Form. https://doi.org/10.17605/OSF.IO/V987T\textsuperscript{46}

This project contains the following Extended data:

- Case report form - CRF_v09112017.V2.docx

Data is available under a CC0 1.0 Universal Public Domain Dedication.

Grant information
This work was supported by the Wellcome Trust through a Training Fellowship to AO [204776].

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