Maternal body mass index and proinflammatory immune markers in pregnant women living with HIV on antiretroviral treatment in Cape Town, South Africa

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PREAMBLE

DECLARATION

I, **Jessica More**, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I further declare that this work was not published prior to my registration for the degree of Master of Public Health (Epidemiology and Biostatistics).

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ABSTRACT

Background. High maternal body mass index (BMI) and the timing of antiretroviral (ART) initiation in pregnant women living with human immunodeficiency virus (HIV) (WLWH) may affect the controlled systemic inflammation during pregnancy. Proinflammatory immune markers during pregnancy and the impact of maternal BMI in WLWH initiating ART in pregnancy or preconception will be evaluated.

Methods. In this mini-dissertation is a protocol (Part A), journal formatted manuscript (Part B) and Appendices (Part C) for a study on maternal BMI and inflammation in WLWH. A subset cohort from the Prematurity Immunology in HIV-infected Mothers and their infants Study (PIMS) study had three plasma immune markers (c-reactive protein (CRP), interferon-gamma inducible protein-10 (IP-10) and serum amyloid A (SAA)) and maternal weight measured from April 2015 to October 2016, at four antenatal care visits (visit 1, 2 (two weeks post-ART initiation in those initiating in pregnancy), 3 and 4). The association with maternal BMI by ART initiation (on ART preconception or initiated during current pregnancy) was assessed.

Results. Among 526 pregnant WLWH, those on preconception ART had CRP and IP-10 levels lower compared to those who initiated ART in pregnancy. CRP was higher in obese WLWH (irrespective of timing of ART initiation) than those with a normal or overweight BMI. IP-10 was elevated in the 2nd trimester and SAA levels were highest in WLWH with a normal BMI.

Conclusion. Immune marker level elevation is dependent on timing of ART exposure and pregnancy trimester. The timing of ART initiation and maternal BMI may adversely impact systemic inflammation in pregnant WLWH.

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Lastly, I would like to thank my family for their unwavering faith in my journey.

LIST OF ABBREVIATIONS

APR	Acute-phase response
ANC	Antenatal clinic
ART	Antiretroviral therapy
BMI	Body mass index
CD4	Cluster of differentiation 4
CI	Confidence interval
CRP	C-reactive protein
CXCL-10	CXC motif chemokine ligand 10
EFV	Efavirenz
EVT	Extravillous trophoblast cell
GLMM	Generalised linear mixed model
GMOU	Gugulethu Maternal Outpatients Unit
GWG	Gestational weight gain
HIV	Human immunodeficiency virus
IRIS	Immune reconstitution inflammatory syndrome
IFN-γ	Interferon-gamma
IL-10	Interleukin-10
IP-10	Interferon-gamma inducible protein-10
IUGR	Intrauterine growth restriction
IQR	Interquartile range
3TC	Lamivudine
NNRTI	Non-nucleoside reverse transcriptase inhibitors
PI	Protease inhibitor
PIMS	The Prematurity Immunology in HIV-infected Mothers and their infants Study
PTD	Preterm delivery
PMTCT	Prevention of mother-to-child transmission
SAA	Serum amyloid A
SD	Standard deviation
SGA	Small for gestational age
Th	T-helper
TDF	Tenofovir disoproxil fumarate
WHO	World Health Organisation
WLWH	Women living with HIV

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PART A. PROTOCOL

1. INTRODUCTION

1.1 Background

During pregnancy, the maternal immune system undergoes major adaptions to protect the mother and fetus from external insults (e.g. pathogens and injury), and to prevent harmful immune responses to the allogeneic fetus [1]. Pregnancy can be described as a state of mild, controlled inflammation: a necessity to facilitate normal physiological processes during pregnancy [2]. These features of the adaptive immune response involve T cell- mediated immunity in which proinflammatory T-helper (Th) cells (like Th1 lymphocytes), which release cytokines, predominate early in pregnancy and anti-inflammatory Th cells (like Th2 lymphocytes) later in pregnancy (1). Cytokines, which can be classified as either proinflammatory (enhance and worsen inflammation) or anti-inflammatory (decrease inflammation), are major immune regulators that act as initiators and mediators of inflammation [2]. The maintenance of a normal healthy pregnancy requires inflammatory mediators at different stages of the reproductive process including implantation, placental formation, and parturition [3]. In early pregnancy, proinflammatory mediators such as the Th1 lymphocytes are required to stimulate implantation of the blastocyst and assist with remodelling of the uterine spinal arteries for placental formation . Th1 immunity, characterised by immune-inflammatory responses, becomes dominant during the peri-implantation period, and the "controlled" Th1 immunity benefits the invading trophoblasts rather than harms them. [1, 3]. Quickly after the placental implantation, the early inflammatory Th1 immunity is shifted to the Th2 anti-inflammatory immune responses. During the second trimester, anti-inflammatory mediators, and hormones such as progesterone contribute to immune responses during fetal development [4, 5]. In the third trimester, to prepare for parturition which is facilitated by proinflammatory mediators, T cell changes take place [1, 5, 6]. The predominant Th2 immunity, which overrules the Th1 immunity at the placental implantation site, protects a fetus by balancing Th1 immunity and accommodating fetal and placental development. These changes in proinflammatory and anti-inflammatory processes that occur throughout the different gestational periods of pregnancy, however necessary for a successful pregnancy, may also be associated with adverse pregnancy outcomes if there are disruptions in the balance between Th1 and Th2 responses, if they occur during implantation or placental development stages of pregnancy [3].

Maternal immunity is necessary for antimicrobial transfer to the fetus [7]. Infection with human immunodeficiency virus (HIV) in pregnancy, however, has been shown to affect immune responses as HIV alters this transfer of immunity [7, 8] and there is an increase in proinflammatory cytokines associated with pregnancy [1]. HIV infection results in dysregulation of the immune system, through the systemic destruction of immune cells, this leads to both immunodeficiency and immunosuppression [9, 10]. Some level of immune restoration occurs after the initiation of antiretroviral therapy, through the reversal of the cytokine shift from Th2 to Th1 [11]. In pregnancy, the impact of these cytokine shifts that occur because of HIV infection and subsequent initiation of ART have been hypothesised to be associated with adverse pregnancy outcomes [11]. In some cases, the ART-mediated immune restoration may be erratic, leading to acute inflammatory responses known as immune reconstitution inflammatory syndrome (IRIS) shortly after ART initiation [11]. This state of low maternal immune activation, possibly linked to an imbalanced recovery, has been shown to be associated with preterm delivery or pregnancy loss [11, 12].

Another condition in pregnancy that impacts immune functioning is obesity (body mass index (BMI) >30kg/m^2), the prevalence of which is rapidly increasing [13]. This is concerning as maternal obesity in pregnancy is associated with an increased risk of adverse maternal-fetal outcomes such as preterm labour, preeclampsia, caesarean section, prematurity, and fetal death [14, 15]. A common pathophysiology of obesity in non-pregnant individuals is chronic inflammation [16]. In pregnancy, obesity has been shown to result in an exaggeration of systemic and placental inflammatory responses [17], which has been reflected by increased circulating levels of proinflammatory cytokines [18]. Placental function is intimately associated with the control of inflammation during pregnancy [19]. Elevated BMI induced inflammation has been shown to result in placental function alterations which can negatively impact the fetus and neonatal outcomes [17,18]. These include spontaneous miscarriage, preeclampsia, preterm birth, perinatal neuroinflammation, and other post-natal conditions. Differing levels of placental cytokines and molecular inflammatory mediators also have known associations with preeclampsia and developmental outcomes [19]. Consequently given the impact of pregnancy, HIV and maternal obesity individually on the immune system, it is important to elucidate the combined effect of these three factors on the immune
system.

With an increasing population of obese pregnant women living with HIV (WLWH) in our setting, we set out to investigate the relationship between immune markers and BMI in pregnant people initiating or continuing ART at a primary care facility in Cape Town, South Africa.

1.2 Rationale

South Africa has a high burden of both HIV and obesity, particularly in the female sex of reproductive age. The HIV seroprevalence in pregnant people in South Africa is estimated at 30% [20], while approximately 42% of women >20 years are obese [21].

There is limited and inconsistent knowledge and evidence on cytokine profiles throughout the various stages of pregnancy in low-resource settings [22, 23]. Some studies have shown that these profiles may be impacted by conditions such HIV and obesity. While no single immune marker may explain immune dysregulation and the sequalae thereof, this study aims to focus on the three markers of inflammation measured in the parent study during pregnancy, namely acute phase proteins s-reactive protein (CRP) and serum amyloid A (SAA), and the chemokine interferon-gamma inducible protein-10 (IP-10) [24, 27]. CRP and SAA to a lesser extent are known to be markers of general inflammation, IP-10 plays a role in proinflammatory microenvironments particularly during early pregnancy and previous studies in non-pregnant people have shown that it may predict HIV load [25-29].

Additionally, ART initiation and the timing of the initiation in pregnancy has been associated with adverse birth outcomes [30] and is also associated with IRIS [31]. CRP is also raised in pregnancy [27]. Experimental studies have shown that SAA may be associated with placental immune responses [26]. CRP and SAA have also been shown to be positively associated with BMI in some studies [24, 25]. By assessing different groups, one which is ART naïve and those stable on ART, inflammation during gestation may provide indication on viral suppression and time points during gestation [32]. It is therefore important to understand the impact of the combined effect of HIV and obesity on cytokine levels through the measurement of immune markers that are impacted by both.

2. AIMS AND OBJECTIVES

2.1 Aim

This study aims to assess the relationship between immune markers during pregnancy and BMI in pregnant WLWH on ART at a maternal healthcare facility in Cape Town, South Africa.

2.2 Objectives:

- To assess the levels of immune markers during pregnancy in WLWH on ART
- To assess the relationship between immune markers during pregnancy and BMI in WLWH on ART

3. METHODS

3.1 Study design

The study will be an analysis of data collected from a prospective cohort of pregnant people titled: The Prematurity Immunology in HIV-infected Mothers and their infants Study (PIMS). The PIMS study has previously been described in detail [33], however the primary objective of this study was to quantify the risk of preterm delivery (PTD) and small for gestational age (SGA) infants in pregnant women at a primary healthcare facility in Cape Town, South Africa. The PIMS study enrolled all pregnant people (>18 years), regardless of their HIV status, at their first antenatal care visit between April 2015 to October 2016. Routinely collected last menstrual period (LMP)-based GA and symphysis-fundal height (SFH)-based GA were collected by public health midwives. Given that in our setting LMP has previously been shown to not be accurate, within the overall cohort, a subset of pregnant WLWH who were < 24 weeks (considered gold standard in our setting) as determined by a research sonographer, were enrolled into a prospective cohort study. They had intensive measurements throughout pregnancy until 12 months postpartum.

3.2 Study Setting

The PIMS study took place at the Gugulethu Midwife Obstetric Unit (GMOU) in Gugulethu, a low-income peri-urban area of Cape Town with an estimated HIV prevalence of 26% [34]. The GMOU is a primary level healthcare facility, run by midwives, which provides antenatal and obstetric care for pregnant people with low-risk pregnancies. Pregnant people with high-risk pregnancies or requiring specialist care are referred to Mowbray Maternity Hospital

(secondary level) or Groote Schuur hospital (tertiary level) obstetric facilities. Prevention of mother-to-child transmission (PMTCT) services are integrated into antenatal care services.

3.3 Study Population and Sampling

This proposed analysis will include all pregnant WLWH enrolled into the PIMS prospective cohort who had an ultrasound determined gestational age of < 24 weeks with data available on anthropometric measurements and plasma immune marker levels. Inclusion criteria for this cohort was:

- Age ≥ 18 years
- Confirmed HIV-infection via two finger-prick rapid tests using different HIV test kits (per routine protocol in the MOU) or self-reported HIV infection
- Confirmed pregnancy (positive urine pregnancy test, ultrasound, or clinically) < 24 weeks at visit 1 (baseline)
- Confirmed haemoglobin (point-of-care Haemocue or other method) >7.0g/dL
- Ability to provide informed consent

3.4 Data Collection

Data collected during the antenatal study visits of the PIMS study will be used for this proposed analysis; this included visits at a minimum of 3 time points: <24 weeks' gestation, 28 weeks' gestation, and 34 weeks' gestation. Pregnant WLWH initiating ART in pregnancy had an additional study visit two weeks post-ART initiation. Data collected at these study visits that will be included in this analysis will be based on standardised interviewer administered questionnaires, review of medical records, maternal anthropometric measurements, and blood draws for plasma collection and analysis (Table 1).

3.4.1 Questionnaires

In the PIMS study, the interviewer administered questionnaires collected data on maternal demographics and at enrolment (Appendix 1A); and data on ART adherence and intercurrent medical and obstetric events (Appendix 1B).

3.4.2 Medical Record Review

Data was abstracted from the patient-held maternity case record by the research team which comprised of nurses, graduate level research assistants and field workers (Appendix 1C). Data abstracted included results of routine blood tests (syphilis screening, haemoglobin, and

ABO blood grouping), HIV data including date of diagnosis, viral load enumeration and ART regimen. Following delivery, abstraction of pregnancy and obstetric data was also conducted (Appendix 1D); this data included medical and obstetric conditions experienced during pregnancy, labour details, infant outcomes, and the discharge summary.

3.4.3 Anthropometric measurements

Data from anthropometric measurements conducted during the course of the parent study will be included, namely measurement of maternal height (stadiometer (Seca, Birmingham, United Kingdom)) at enrolment, and weight (calibrated scale (Charder, Taichung City, Taiwan) at each antenatal study visit as measured by the research team (Appendix 1E) [35].

3.4.4 Phlebotomy

Maternal plasma samples collected at all antenatal study visits will be used for the measurement of the selected immune markers (Appendix 1F).

SAA levels were assessed using the human SAA enzyme-linked immunosorbent assay (ELISA) kit (Colorimetric) NBP2-68119 (Novus Biologicals, Littleton, Colorado, United States); while CRP and IP-10 were assessed using QuantikineELISA for human c-reactive protein/CRP and human CXCL10/IP-10 (R&D Systems, Minneapolis, Minnesota, United States) in line with the manufacturer's protocols. Each assay plate had intra-assay (samples repeated within the same plate) and inter-assay (samples repeated across plates) controls to assess variations.

Table 1. Variables for data analysis

Variable	Category	Scale
Age (years)	18-24, 25-29, 30-34, >35	Categorical (ordinal)
	Mean (SD) or Median (IQR)	Numerical (continuous)
Education	No school, Primary (≤7) Secondary (8-12), Tertiary (>12)	Categorical (ordinal)
Employment status	Unemployed or studying, Employed	Categorical(nominal)
Gestational age (weeks) at enrolment	Mean (SD) or Median (IQR)	Numerical (continuous)
Gravidity	1, 2, ≥3	Categorical (ordinal)
	Frequency, proportions	Numerical (discrete)
Parity	Nulliparity (0), Primiparity (1), Multiparity (≥2)	Categorical (ordinal)
	Frequency, proportions	Numerical(discrete)
Previous Pregnancies		
Miscarriages	Yes, No	Categorical (binary)
Preterm delivery	Yes, No	Categorical (binary)
Intercurrent comorbidity in current pregnancy	Yes, No	Categorical (binary)
Baseline blood pressure measured at the first ANC visit (mmHg)	Normotensive (≤139/89mmHg), Hypertensive (≥140/90mmHg)	Categorical (ordinal)
	Mean (SD) or Median (IQR)	Numerical (continuous)
Baseline haemoglobin measured at the first ANC visit (g/dl)	≤7,7.1-8.9,9-10.9, ≥11	Categorical (ordinal)
	Mean (SD) or Median (IQR)	Numerical (continuous)
TB status	None, Current, Previous	Categorical (nominal)
ART status	Initiated before pregnancy, Initiated during current pregnancy	Categorical (binary)
ART regimen	TDF-3TC-EFV, Other NNRTI-based regimen, PI-based regimen	Categorical (nominal)
Viral load (copies/ml)	Mean (SD) or Median(IQR)	Numerical (continuous)
Maternal weight (kg)	Mean (SD) or Median(IQR)	Numerical (continuous)
Maternal height (cm)	≤160,161-164, 165-169, ≥170	Categorical (ordinal)
Body mass index (kg/m^2)	Normal weight, overweight, obese	Categorical (ordinal)
	Mean (SD) or Median(IQR)	Numerical (continuous)
CRP, SAA, IP-10 (ng/mL or pg/mL)	Mean (SD) or Median(IQR)	Numerical (continuous)

3.5 Exposure of Interest

The main exposure of interest is body mass index (BMI), which will be calculated using the maternal weight (at baseline and at subsequent ANC visits) in kilograms divided by the baseline height in metres squared (kg/m^2) (13). In this analysis BMI will be categorised as:

- Normal weight (18.5-24.9 kg/m^2),
- Overweight (25-29.9 kg/m^2),
- Obese ($\geq$ 30 kg/m^2)

3.6 Outcome of Interest

The outcomes of interest will be the measured levels (nanograms or picograms per millimetre) of the three immune markers c-reactive protein (CRP), serum amyloid A (SAA) and interferon-gamma inducible protein-10 (IP-10) at the antenatal time points.

3.7 Covariates of Interest

The following covariates will be assessed (Table 1):

- Maternal demographics
  - age, education, employment status
- Obstetric history
  - gravidity, parity, current gestation age, previous miscarriages (miscarriage defined as loss of pregnancy < 20 weeks), previous preterm delivery (preterm delivery defined as delivery < 37 weeks)
- Medical history
  - Intercurrent comorbidity in current pregnancy, blood pressure (millimetres of mercury i.e., mmHg), haemoglobin (grams per decilitre), TB status
- HIV
  - ART status, ART regimen, viral load (copies per millilitre)

3.8 Data Management and Analysis Plan

3.8.1 Data Management

Data collected from the PIMS study were entered into a customised study Microsoft Access database, which is maintained in a firewall protected UCT server with automated daily backups. This data is protected by password encryption software known by the student (Jessica More) and supervisors (Thokozile Malaba and Elton Mukonda). Backups will be stored remotely. All study records contain anonymous participant identifiers with no personal participant names or identifiers record. Data to be utilised for this analysis will be stored on a password protected personal computer accessible only to the researcher.

3.8.2 Data Analysis

All statistical analyses will be conducted using software from RStudio 2020; version 1.3.1093 (RStudio, Public Benefit Corporation, Boston, Massachusetts). Descriptive statistics will be generated and summarised as mean and standard deviation or median and interquartile range for continuous variables; while categorical variable swill be summarised using proportions. The ANOVA (Analysis of variance) or Kruskal-Wallis test will be used to compare the immune markers between the BMI categories depending on the normality of continuous variables. A post-hoc analysis will be conducted.

The relationship between the levels of the markers and BMI categories across the three visits will be assessed using generalised linear mixed models (GLMM) with fixed (BMI categories, study visits (time) and other covariates) and random effects (individuals). Results will be expressed using beta (β) values with 95% confidence intervals.

Immune marker profile values (mean concentration) will be presented as geometric means with 95% confidence intervals for the assumption of normality. Statistical significance will be when $p<0.05$.

4. ETHICAL CONSIDERATIONS

The parent study has been approved by the UCT Research and Ethics Committee of Health Sciences (HREC) (739/2014) (Appendix 2A-C) and the University of Southampton Institutional Review Board (IRB) (12542 PIMS) (Appendix 2D). Provincial approval from the Western Cape Government was also granted (Appendix 2E). Ethical approval for this proposed analysis will be sought from the UCT-HREC.

4.1 Informed Consent

Informed consent was obtained from all pregnant people enrolled in the parent study. Two consent forms were completed by pregnant people enrolled in the prospective cohort – first for inclusion in the overall cohort (Appendix 3A) and then a subsequent consent form was completed for enrolment into the prospective cohort (Appendix 3B). In consenting to participate in the PIMS study pregnant individuals gave permission for abstraction of data from their routine clinical records during pregnancy. As part of this proposed analysis, pre-existing data will be utilised in accordance with the consent received from these participants, therefore no direct contact with participants will be required – consequently informed consent will not be sought for this secondary data analysis. The study will comply with the Declaration of Helsinki [36].

4.2 Privacy and Confidentiality

The parent study made the following provisions to minimise risk of confidentiality and privacy loss:

- Participant names were omitted from study forms
- No personal information or laboratory specimen had personal identifying information
- All study materials were stored in a locked filing cabinet
- Study personnel involved in routine audits only had access to study materials
- Staff collecting data were trained in confidentiality

During this proposed analysis, privacy and confidentiality of participant data will be maintained. Participant identification information will not be extracted for the purpose of this study, with only use of the anonymous identifiers assigned during the parent study. Results of this proposed analysis will not report on individual participants ensuring confidentiality.

4.3 Risks and Benefits

A description of risk and benefits, as well as reimbursements for the parent study have been previously approved (HREC REF: 739/2014). This analysis poses no more than minimal risk to the individual patients. This minimal risk is a loss of confidentiality and the parent study made provisions to minimise this risk. There is no direct benefit derived by the participants as it is an analysis of previously collected data, however there could be indirect benefit derived from the knowledge gained about the relationship between maternal BMI changes in pregnancy and the association with inflammatory immune markers in pregnancy. This could potentially assist with the development of tools to improve antenatal care in pregnancy and in pregnant WLWH for PMTCT and ART initiation. Improved maternal health in pregnancy can contribute to the prevention or early detection of possible adverse birth and delivery outcomes for pregnant WLWH and their infants.

4.4 Reporting and Implementation

Upon completion of this analysis, findings will be submitted to a peer-review journal in consultation with the various study partners. This data will also be presented at international and local conferences and to the GMOU management team.

4.5 Logistics

Table 2 provides the anticipated time frame within which this proposed analysis will be conducted.

Table 2. Study Time Frame

Month (year)	July (2021)	August-September (2021)	October-November (2021)	November-December (2021)	December-January (2021/2)	January-February (2022)
Activity						
Literature review						
Protocol development						
Protocol ethics submission						
Data analysis						
Write up dissertation						
Submit to UCT						
Prepare manuscript for publication						

4.6 Budget

This analysis will be conducted as part of an MPH degree as such no payment for the student is required.

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PART B. MANUSCRIPT

Proinflammatory immune markers in pregnant women living with HIV (WLWH) on antiretroviral treatment (ART) by maternal body mass index (BMI), in Cape Town, South Africa

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ABSTRACT

Background. Elevated body mass index (BMI) in pregnant women living with human immunodeficiency virus (HIV) (WLWH) may lead to dysregulation of the tightly controlled immune system, possibly leading to pregnancy complications. We aimed to evaluate the association between maternal BMI status on immune marker levels during pregnancy.

Methods. The Prematurity Immunology in HIV-infected Mothers and their infants Study (PIMS) enrolled pregnant WLWH investigating the epidemiology and immunology of timing of ART initiation in pregnancy. A subset aged ≥18 years had inflammatory immune markers (c-reactive protein (CRP), interferon-gamma inducible protein-10 (IP-10) and serum amyloid A (SAA)) and maternal weight measured at three (those on antiretroviral treatment (ART) preconception) or four (those initiating ART) antenatal visits (<24 weeks' gestation, 28 weeks' gestation, and 34 weeks' gestation). Associations between these markers and maternal BMI (normal weight (<24.9 kg/m^2), overweight (25–29.9 kg/m^2), obese (>30 kg/m^2)) were assessed using generalised linear mixed models.

Results. In 526 pregnant WLWH, those who initiated ART in pregnancy (53%) had the highest CRP levels two weeks post-ART initiation with obese WLWH having the highest (median 13247.85 ng/mL, IQR: 11368.02 – 17074.05, p=0.01). Mean IP-10 levels were 69.34 pg/mL (p<0.001) lower in those on ART preconception than those who initiated ART in pregnancy. Mean SAA levels were 2.26 ng/mL lower in those who initiated ART in pregnancy than preconception and 3.64 ng/mL lower for obese WLWH than those with a normal BMI.

Conclusion. Obesity and ART exposure were associated with inflammatory marker levels in pregnancy. Assessing markers of systemic inflammation may help to understand the impact of maternal BMI and immunity on placental development and adverse pregnancy outcomes among WLWH.

Keywords: BMI, maternal obesity, immune marker, inflammation, HIV.

1. INTRODUCTION

Controlled systemic inflammation is both crucial and beneficial in pregnancy [1]; inflammatory mediators play a pivotal role during implantation, placentation, and parturition [2]. To facilitate implantation, the proinflammatory environment dominates, with mediators such as the T-helper (Th) 1 lymphocytes producing cytokines [3]. These proinflammatory cytokines stimulate blastocyst implantation and assist with uterine spinal arteries remodelling for placental formation [2, 4]. They also mediate the release of proteins like C-reactive protein (CRP), an acute-phase response (APR) protein, which is an early marker of systemic inflammation or infection [5, 6]. Though CRP's function in pregnancy is not fully elucidated, *in vitro* studies show that human placental trophoblasts may secrete CRP in early pregnancy [7]. Clinically, CRP levels >10 000 ng/mL indicates inflammation above normal, and has been associated with increased cardiovascular events risk in healthy non-pregnant individuals [8]. These elevated CRP levels in early pregnancy have also been associated with preeclampsia, preterm birth, and intrauterine growth restriction [9].

Proinflammatory cytokines can also lead to an increased secretion of the APR protein, serum amyloid A (SAA) [10-12]. Recent evidence suggests that SAA may be a damage-associated molecular patterns molecule in pregnancy, released in response to placentation, assisting with implantation, parturition and pregnancy-related infections, trauma, and preterm labour [14]. Another Th1-related chemokine playing a role in proinflammatory microenvironments during early pregnancy is interferon-gamma inducible protein-10 (IP-10)/CXC motif chemokine ligand 10 (CXCL-10) which is released in response to interferon- gamma (IFN-γ) [15]. In pregnancy, IP-10 facilitates the migration of the blastocyst to the endometrial epithelium and subsequent trophoblast adhesion [17]. Additionally, because of its dual function as an anti-angiogenic and angiogenic chemokine, IP-10 has also been implicated in preeclampsia development [15].

Proinflammatory processes early in pregnancy are followed by a shift to an anti-inflammatory environment during the second and early third trimester to enable adequate fetal growth [3]. Accompanying this shift, inflammatory mediators, and hormones such as progesterone contribute to immune responses during fetal development [1, 18, 19]. Subsequently, to facilitate parturition there is a shift back to a proinflammatory environment

towards the end of the third trimester. T cell changes take place resulting in a shift from Th1 to regulatory Th2 lymphocyte-mediated inflammation as neutrophils and macrophages infiltrate the myometrium [1, 2, 4, 20]. All these immune processes are tightly regulated during a normal pregnancy, therefore dysregulation of any of these processes may contribute to pregnancy complications [2].

Pregnant people experience physiological changes necessary to maintain a healthy pregnancy. One such feature, which is inevitable during pregnancy, is changes in maternal body weight. In South Africa more than 40% of non-pregnant women are obese [25]. Obesity in pregnancy is associated with pregnancy complications; abnormal increases in proinflammatory cytokines have been shown to cause dysregulation of placental functioning in obese pregnant people [26-28]. Studies in non-pregnant individuals have shown that adipose tissue acts as an endocrine organ promoting the release of proinflammatory cytokines [10]. SAA levels have been positively associated with gestational weight gain (GWG) in pregnant people [29]. These implications could highlight potential aetiological pathways for adverse birth and pregnancy outcomes in obese pregnant individuals [10-12].

With maternal HIV infection being shown to result in cytokine shifts during pregnancy [21], this may cause increases in placental proinflammatory cytokines and altered fetal immunity [22]. In non-pregnant individuals, HIV infection has been shown to upregulate IP-10 levels due to infection-related decreases in T cells [23, 24]. With the overlapping impacts of maternal HIV infection and elevated maternal body mass index (BMI), the additive effect of these conditions in pregnancy may result in an exaggeration of systemic and placental inflammatory responses [30]. Further, obesity in pregnancy, HIV infection and ART initiation, have been associated with increased risk of adverse maternal-fetal outcomes such as preterm labour, preeclampsia, prematurity, and possibly fetal death [31 - 33]. We explored the relationship between three immune markers, which serve as proxies for various states of inflammation, by maternal ART and BMI status in a cohort of pregnant people living with HIV (WLWH) followed prospectively throughout pregnancy.

2. MATERIALS AND METHODS

2.1 Study design and population

This analysis forms part of the Prematurity Immunology in HIV-infected Mothers and their infants Study (PIMS), whose primary immunological aim was to examine proinflammatory immune response by ART status in pregnant WLWH. PIMS was a multicomponent study that enrolled consecutive pregnant WLWH accessing antenatal care between April 2015 and October 2016 at the Gugulethu Midwife Obstetric Unit (GMOU) in Cape Town, South Africa [34]. Details of the PIMS study have previously been published [34]. This analysis focused on the pregnant WLWH (<24 weeks' gestation, as determined by a research sonographer) enrolled into a sub-set cohort. Ethical approval for this study was obtained from the University of Cape Town (UCT) Faculty of Health Sciences Human Research Ethics Committee (HREC) (739/2014) (Appendix 2A-C) and the University of Southampton Institutional Review Board (12542 PIMS) (Appendix 2D).

2.2 Data collection

Pregnant WLWH who enrolled in the subset cohort underwent intensive measurements across study visits throughout pregnancy. Antenatally, phlebotomy was conducted at enrolment (visit 1: <24 weeks' gestation and pre-ART initiation for those initiating ART at first antenatal visit), visit 2 (for those initiating ART in pregnancy an additional study visits two weeks post-ART initiation), visit 3 (28 weeks' gestation) and visit 4 (34 weeks' gestation for all). Anthropometric measurements were conducted by a trained study nurse at enrolment (maternal height and weight) and at subsequent visits (maternal weight) [35]. Maternal weight was measured using a calibrated scale (Charder, Taichung City, Taiwan) accurate to within 0.5 kg; while maternal height was measured to the nearest 0.1 cm using a stadiometer (Seca, Birmingham, UK).

2.3 Immune marker assays

Blood was collected by participants at each study visits [34] and samples (one vial per participant visit) were pulled out of the centralised biorepository (-80°C) and thawed overnight on ice, in the fridge (4°C) for next day analysis. Prior to initiating experimental sample run for cytokine determination, a subset of participant samples were selected based on the highest likelihood of detectable cytokine levels to confirm the optimal dilution for plasma samples required to maximise probability of detection. Criteria used included naivety to ART and high blood HIV viremia where available. Blood plasma was assayed using enzyme-linked immunosorbent assay (ELISA) kits to measure the systemic concentration of SAA (Novus Biologicals (NBP2-68119)), CRP (R&D Systems QuantikineELISA) and IP-10 (R&D Systems QuantikineELISA). The respective reported kit sensitivities were 0.75 ng/mL for SAA, 0.022 ng/mL for CRP and 4.46 pg/mL for IP-10. A 100-fold dilution was applied for samples assayed for CRP, while samples were assayed undiluted for SAA and IP-10. Sample sets for all completed visits for each participant were batched and run on the same plate to minimise potential variation when comparing cytokine level across different visits. Quality control and biological principle of the assay are described in the Supplementary data and in the PIMS cohort study [34].

2.4 Exposures

The exposures of interest were maternal ART status and maternal BMI status. ART status was categorised by timing of ART initiation: preconception (before pregnancy) or during pregnancy (current pregnancy). Maternal BMI status was calculated using study determined maternal height and weight, and categorised based on World Health Organisation (WHO) classifications as: normal (<24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obese (30 kg/m^2) [36].

2.5 Outcome

The primary outcome of interest was the levels throughout pregnancy of the measured plasma concentrations (nanograms or picograms per millimetre) of the markers CRP, SAA, and IP-10 (chosen during the parent study).

2.6 Statistical analysis

Descriptive statistics were generated to compare variables which included include obstetric history, ART history, TB history and baseline plasma concentrations which have been highlighted in the cohort paper [34]. These data were summarised as median and interquartile range for continuous variables, and as proportions for categorical variables. Normality of the continuous variables was assessed using the Shapiro-Wilks test. Comparison of the immune markers by BMI categories was conducted using the non-parametric Kruskal-Wallis test. A post-hoc analysis was also conducted using the Dunn's test and is described in the Supplementary data.

The relationship between the immune marker concentrations and BMI categories across visits (time-dependent) was assessed using generalised linear mixed-effects models (GLMM) with random intercepts. GLMM were adjusted to assess group differences between normal, overweight, and obese participants and longitudinal changes in ART status and visit, which were decided on *a priori*. Adjusted and unadjusted GLMM analyses were run. Separate models were run for the different immune profile markers. Immune marker results were presented as mean changes. Overall, results were expressed using beta (β) values with 95% confidence intervals. Statistical significance was defined as $p<0.05$.

3. RESULTS

3.1 Baseline characteristics

Of the 552 pregnant WLWH enrolled in the PIMS subset cohort, 526 were included in this analysis with exclusions based on ineligibility for this analysis represented in Supplementary Figure 1. Those initiating ART during the current pregnancy (n=246) were more likely to be obese (54%) compared to those on ART preconception (n=278) (Table 1). The median gestational age (GA) was 14 weeks (IQR:11 – 18 weeks). Obese pregnant WLWH were more likely to be older, multigravida and to have had more previous miscarriages than those who were overweight or had a normal BMI. The proportion of pregnant WLWH initiating ART preconception (47%) and during pregnancy was similar across the BMI categories (Table 1).

3.2 Immune marker levels across study visits by BMI category

Overall, median CRP levels were elevated (>10 000 ng/mL) across all pregnancy visits (Table 2). At each visit, significant group differences in CRP ($p \leq 0.05$) were observed between obese pregnant WLWH and those who were normal or overweight at visit 1 and visit 4 (Table 2). No significant group differences in GA were observed. Differences in immune markers levels taken at each visit were evaluated by pairwise comparisons of BMI status (Supplementary Table 1).

In the overall cohort, elevated CRP levels were highest at visit 1 (median GA 14, IQR:11 – 18 weeks) and 2 (median GA 17.14, IQR:14 – 21 weeks in those who had initiated ART in pregnancy) when compared to visit 3 (median GA 27.71, IQR:27.14 – 28.04 weeks) and 4 (median GA 33.71, IQR:33.14 – 34.14 weeks) (Figure 1A (i)). No significant differences in median IP-10 levels were observed across pregnancy visits between BMI categories, however, IP-10 and SAA levels were highest in all BMI categories at visit 4 in those on ART preconception (Figure 1B (ii)). Pregnant WLWH with a normal BMI had the highest median SAA levels at visit 1 (early 2nd trimester) when compared to overweight or obese WLWH (Figure 1A & B (iii)).

3.3 Immune marker levels are associated with ART status

Differences in immune marker levels across BMI categories are shown at each visit in pregnant WLWH initiating ART (Table 3) and those who initiated ART preconception (Table 4). Differences in immune marker levels stratified by ART status are represented in Supplementary Table 2.

3.3.1 CRP

Overall, those who had initiated ART in pregnancy had significantly elevated CRP levels (>10 000 ng/mL) at visits 1 (early 2nd trimester), 2, 4 (3rd trimester) ($p \leq 0.05$), except in overweight women whose CRP was (<10 000 ng/mL) at visit 1. Obese WLWH who initiated ART preconception had higher CRP levels at baseline (median 13057.14 ng/mL, IQR: 8483.96 – 15303.84 ng/mL) and throughout pregnancy compared to those who initiated ART in pregnancy (Figure 2A). No significant group differences were observed in this group though

CRP levels showed a decreasing trend across visits when compared to those who had initiated ART in pregnancy (Table 3).

3.3.2 IP-10

Pregnant WLWH who had initiated in the current pregnancy had statistically significantly ($p<0.001$) higher IP-10 levels at visit 1 (early 2nd trimester) which is pre-ART initiation, when compared to those on ART preconception (Supplementary Table 2). IP-10 levels increased across visits in those on ART preconception (Figure 2B), though these changes were not statistically significant.

3.3.3 SAA

In those who had initiated ART in pregnancy, SAA levels were highest in those with a normal (median 13.88 ng/mL, IQR: 6.39 – 30.93) or overweight (median 13.43 ng/mL, IQR: 6.72 – 22.19) BMI at first ANC visit (Figure 2C). For those on ART preconception, SAA levels increased across pregnancy visits, and group differences were observed at visit 3 (median GA 27.57, IQR:27.14 – 28 weeks, $p=0.04$), with these differences being highest in those with a normal BMI (Table 3).

3.4 Immune marker levels show longitudinal changes during pregnancy

3.4.1 CRP

GLMM analysis showed that obese WLWH had CRP levels 1279.73 ng/mL higher compared to those with a normal BMI (Table 5). Compared to visit 1, CRP levels at visit 2 (2nd trimester) (only those who initiated ART in pregnancy) were 1523.22 ng/mL higher. No significant differences were noted by ART status. The unadjusted model is represented in Supplementary Table 3.

3.4.2 IP-10

Those who initiated ART preconception had IP-10 levels 69.34 pg/mL lower than those who initiated in the current pregnancy, $p<0.001$ (Table 5). IP-10 levels were 31.45 pg/mL lower at visit 3 (late 2nd and early 3rd trimester) than visit 1, $p<0.001$. No differences were observed in IP-10 levels by BMI status.

3.4.3 SAA

Compared to those with a normal BMI, SAA levels for obese pregnant WLWH were 3.64 ng/mL lower (Table 5). For those who initiated ART preconception, SAA levels were 2.26 ng/mL lower than for those who initiated ART in the current pregnancy. No differences were observed by visit.

4. DISCUSSION

In this cohort of pregnant WLWH accessing ANC at a public sector facility in South Africa, we found that proinflammatory cytokines increased in pregnancy depending on maternal BMI and timing of ART initiation. Overall, median CRP levels were >10 000 ng/mL (inflammation above normal) at every visit. CRP levels were significantly elevated at visit 1 (median 14, IQR:10-17 weeks) in obese WLWH compared to those with a normal and overweight BMI. ART initiators had IP-10 levels higher at visit 1 and 2 (early 1st and late 2nd trimester) when compared to those who had initiated ART preconception. SAA levels were elevated in those with a normal BMI and at later pregnancy visits 3 and 4 (late 2nd and early 3rd trimester) irrespective of ART use. These findings are significant in improving our understanding of the relationship between immune markers and systemic inflammation during pregnancy which may impact pregnancy outcomes.

We found that obese pregnant WLWH had elevated CRP levels at early pregnancy visits 1 and 2 compared to those with normal or overweight BMI, regardless of the timing of ART initiation. CRP is a known marker of systemic inflammation and may be elevated in acute infection, [5, 6] and studies in non-pregnant individuals have found that obesity is associated with chronic low-grade inflammation [37]. The release of inflammatory cytokines like interleukin-10 (IL-10) and SAAs 1 and 2 by adipocytes as well as non-fat cells further highlights the endocrine nature of adipose tissue which is abundant in obesity [10]. The implications related to this systemic inflammation in pregnancy are on the potential development of placental insufficiency, preeclampsia, and poor fetal outcomes like intrauterine growth restriction (IUGR) [2].

IP-10 levels were highest in those who initiated ART in the current pregnancy compared to those on ART preconception. As HIV is known to cause T cell depletion and low Cluster of Differentiation 4 (CD4) cell counts which is indicative of HIV disease progression, studies in WLWH have shown that HIV-related T cell depletion is associated with high IP-10 levels and can be reduced by ART initiation [23, 24]. We are unable to determine whether this is the reason for the higher IP-10 levels we observed in initiators because we did not assess changes in CD4 cells. We also found that IP-10 levels were higher in the 3rd trimester in those on ART preconception, which was like what has previously been observed in other studies investigating IP-10 levels with increasing levels also seen late in pregnancy [21]. IP-10 works by binding to the CXCR3 receptor which, although not an HIV coreceptor, together with its ligand, has been associated with increasing IP-10 levels in HIV infection [23]. A study in South Africa which observed 22 systemic cytokine profiles across pregnancy in 56 pregnant WLWH and 68 HIV uninfected woman (control group) found that IP-10 had the highest detection rate across pregnancy and remained above the levels of the control group in WLWH [21]. Though no association with small for gestational age (SGA) was seen in this study, IP-10 was noted to likely be a specific rather than general contributor to placental pathology [21]. High IP-10 levels in HIV infection have been linked to disease progression and persistently elevated levels have been correlated with ART treatment failure [24]. The implications of this in pregnancy together with IP-10's known anti- angiogenic role inhibiting angiogenesis, could lead to adverse outcomes related to pre- eclampsia and SGA infants in pregnant WLWH [15, 16, 24].

We found that obese pregnant WLWH (regardless of ART initiation status) had lower SAA levels compared to others, which contrasts a cross-sectional analysis of 671 pregnant women in Denmark, which observed a higher SAA concentration with GWG in the 3rd trimester (weeks 30 and 37) [29]. We found increasing SAA levels throughout pregnancy which is different to previously reported findings in a study comparing HIV-uninfected pregnant women with and without preeclampsia and non-pregnant women [38]. They found no differences in SAA levels in pregnancy between the groups, despite women with preeclampsia having a higher pre-pregnancy BMI [38]. Understanding the combined impact of BMI and HIV on SAA levels in pregnancy is essential because studies in non-pregnant HIV individuals have implicated SAA in vascular dysfunction and atherosclerosis [12, 39]. Consequently, this could translate to an elevated risk of poor maternal-fetal outcomes.

Notable strengths in this study are the measurement of the plasma concentration of proinflammatory cytokine levels longitudinally in large sample of pregnant WLWH. Other studies assessing immune markers in pregnancy have smaller sample sizes. Tjoa *et al.* measured CRP levels in the 1st trimester of pregnancy in 107 women and found that they were at higher risk of preeclampsia and IUGR [9]. Our study limitations include not having comparable data on HIV-uninfected pregnant people, which would enable comparisons of the contribution of ART and HIV to systemic inflammation in pregnancy. Another limitation was our inability to grade inflammation, consequently differentiation of acute, subacute, or chronic inflammation by cut-off immune marker levels was not possible. Thresholds for IP-10 and SAA were also not reported in this analysis, however, inflammation is likely different across the exposure groups. With respect to samples, variability in sample handling, kits used for analysis and laboratory protocols, make comparison of our levels with results reported in other studies difficult.

A large percentage of the women in this analysis were ART naïve, defaulters or newly diagnosed and as this study was prior to the implementation of dolutegravir (DTG) based regimens, these are not included in this analysis. Given the high burden of obesity in South Africa, a strength of the present studies analysis is the assessment of maternal BMI. The use of prepregnancy BMI to measure GWG throughout pregnancy would allow for better monitoring of pregnancy weight gain as factors such trimester and twin pregnancy are taken into consideration [33].

This study suggests that maternal BMI and the timing of ART initiation may impact proinflammatory cytokine levels in pregnant WLWH. Owing to the complexity of inflammation, the pathways of inflammation involved in elevated or reduced cytokines and in pregnancy BMI in pregnant WLWH initiating or on ART preconception cannot fully be explained by this study data. Elevated CRP levels in; WLWH, 1st and 2nd trimester and obesity, lower SAA levels in; obesity, 1st and 2nd trimester, and the association of IP-10 levels with the timing of ART initiation requires further exploration. This could aid in the diagnostic and clinical application of immune markers in the early detection and therefore prevention of adverse pregnancy and birth outcomes.

Conflicting Interests

The authors declare that there are no conflicting interests.

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Table 1. Baseline characteristics of the 526 pregnant women living with HIV (WLWH) by BMI category

Characteristic	Overall, N = 526	Normal, N = 112	Overweight, N = 146	Obese, N = 268	P-value
Demographic details					
Maternal age (years)	30 (26-34)	28 (24.75-32)	30 (26-33)	31 (27-35)	**<0.001**
Gestational age (weeks), Median (IQR)	14 (11-18)	14.5 (11-18)	15 (11.25-18)	14 (10-17)	0.2
Employment, n (%)	525	112	146	267	0.8
Yes	251 (48%)	56 (50%)	71 (49%)	124 (46%)	
Missing	1	0	0	1	
Obstetric history					
Gravidity, n (%)	519	110	144	265	**<0.001**
1	87 (17%)	29 (26%)	25 (17%)	33 (12%)	
2	177 (34%)	41 (37%)	53 (37%)	83 (31%)	
>=3	253 (49%)	40 (36%)	65 (46%)	148 (57%)	
Missing	7	2	2	3	
Parity, n (%)	518	110	144	264	**0.01**
Nulliparity	124 (24%)	38 (35%)	36 (25%)	50 (19%)	
Primiparity	213 (41%)	45 (41%)	55 (38%)	113 (43%)	
Multiparity	180(35%)	27 (25%)	52 (36%)	101 (39%)	
Missing	8	2	2	4	
Previous preterm, n (%)	421	81	118	222	0.6
0	374 (89%)	70 (86%)	106 (90%)	198 (89%)	
1	40 (9.5%)	11 (14%)	9 (7.6%)	20 (9.0%)	
2	6 (1.4%0	0 (0%)	3 (2.5%)	3 (1.4%)	
3	1 (0.2%)	0 (0%)	0 (0%)	1 (0.5%)	
Missing	105	31	28	46	
Previous miscarriage, n (%)	512	109	142	261	
0	402 (79%)	92 (84%)	117 (82%)	193 (74%)	0.07
1	97 (19%)	15 (14%)	23 (16%)	59 (23%)	
2	12 (2.3%)	2 (1.8%)	1 (0.7%)	9 (3.4%)	
3	1 (0.2%)	0 (0%)	1 (0.7%)	0 (0%)	
Missing	8	2	2	4	
ART history					
ART status, n (%)	526	112	146	268	0.4

Initiated before pregnancy (preconception)	246 (47%)	49 (44%)	75 (51%)	124 (46%)	
Initiated during current pregnancy	278 (53%)	63 (56%)	71 (49%)	144 (54%)	
ART regimen, n (%)	526	112	146	268	0.6
Other NNRTI-based regimen	22 (4.2%)	2 (1.8%)	9 (6.2%)	11 (4.1%)	
TDF-3TC-EFV	476 (90%)	106 (95%)	129 (88%)	241 (90%)	
TDF-3TC-NVP	4 (0.8%)	0 (0%)	1 (0.7%)	3(1.1%)	
PI-based regimen	24 (4.6%)	4 (3.6%)	7 (4.8%)	13 (4.9%)	
Viral load (copies/ml)	464	102	130	239 (100%)	0.068
<50	471 (100%)	102 (100%)	130 (100%)	239 (100%)	
50-1000	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
>1000	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Missing	56	11	16	29	
Haemoglobin, Hb (g/dl)	463				**<0.001**
Median (IQR)	11.10 (10.2-12.0)	10.30 (9.78-11.60)	11.00 (10.10-11.80)	11.5 (10.6-12.10)	
Missing	63	12	19	32	
TB history					
Current TB, n(%)	522	111	145	266	>0.9
Yes	1 (0.2%)	0 (0%)	0 (0%)	1 (0.4%)	
No	521 (99.8%)	110 (100%)	144 (100%)	263 (99.6%)	
Missing	4	1	1	2	
Previous TB, n (%)	510	106	144	260	>0.9
Yes	97 (19%)	21 (20%)	28 (19%)	48 (18%)	
No	413 (81%)	85 (0)%)	114 (80%)	212 (82%)	
Missing	16	6	2	8	

Abbreviations: ART, antiretroviral therapy; IQR, interquartile range; TDF, tenofovir; NVP, nevirapine; 3TC, lamivudine; EFV, efavirenz; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; Hb, haemoglobin; TB, tuberculosis; CRP, c-reactive protein; SAA, serum amyloid A; IP-10, interferon-gamma-inducible protein-10; BMI, body mass index; copies/ml' g/dl, grams per decilitre; copies per millilitre; ng/mL, nanograms per millilitre; pg/mL, picograms per millilitre. P-value <0.05=statistically significant.

Table 2. Median (IQR) plasma concentration of immune markers by BMI category across all visits in all 526 pregnant women living with HIV (WLWH)

Immune marker by Visit	Total (N)	BMI category Normal (N)	Overweight (N)	Obese (N)	P
Visit 1	**526**	**112**	**146**	**268**	
Gestational age (GA) in weeks, Median (IQR)	14 (11 – 18)	14.5 (11 – 18)	15 (11.25 – 18)	14 (10 – 17)	0.20
CRP (ng/mL)	12412.78 (7547.28 – 15158.60)	11379.73 (5887.08 – 14652.27)	10759.42 (6338.20 – 14984.85)	12779.89 (9019.13 – 1699.57)	**0.05**
IP-10 (pg/mL)	160.76 (105.46 – 259.36)	163.35 (97.37 – 267.39)	161.59 (102.29 – 258.35)	153.39 (110.92 – 258.91)	0.76
SAA (ng/mL)	8.93 (4.80 – 16.33)	9.74 (5.32 – 20.29)	9.99 (4.68 – 17.48)	8.08 (4.82 – 13.84)	0.10
Visit 2*	**223**	**46**	**62**	**115**	
Gestational age (GA) in weeks, Median (IQR)	17.14 (14 – 21)	17.5 (14.04 – 21)	18.07 (15 – 21)	16 (13.07 – 20.43)	0.18
CRP	12779.89 (8018.67 – 16586.46)	12252.19 (8018.75 – 14636.36)	10685.05 (6720.70 – 15832.97)	13427.86 (11398.02 – 17074.05)	**0.01**
IP-10 (pg/mL)	197.84 (139.13 – 274.96)	195.35 (127.56– 266.96)	193.41 (139.56 – 296.52)	199.72 (141.94 – 274.96)	0.89
SAA (ng/mL)	8.78 (5.32 – 15.12)	11.73 (6.75 – 18.70)	8.98 (4.43 – 17.81)	7.93 (5.33 – 13.61)	0.34
Visit 3	**440**	**63**	**128**	**249**	
Gestational age (GA) in weeks, Median (IQR)	27.71 (27.14 – 28.04)	27.71 (27.14 – 28.14)	27.71 (27.14 – 28)	27.71 (27.14 – 28.04)	0.92
CRP ng/mL)	12336.56 (8069.68 – 15277.87)	12252.19 (8117.95 – 14984.85)	1175.70 (7344.34 – 15158.60)	12755.55 (8567.72– 15603.18)	0.22
IP-10 (pg/mL)	148.71 (105.49– 214.39)	145.01 (100.48– 234.91)	157.82 (114.20 – 216.53)	143.84 (103.95 – 208.70)	0.40
SAA (ng/mL)	10.02 (5.12 – 16.69)	11.71 (6.95 – 19.07)	11.05 (5.80 – 19.05)	9.21 (4.72 – 15.08)	0.12
Visit 4	**415**	**49**	**116**	**250**	
CRP (ng/mL)	12306.71 (9201.07 – 14509.43)	12306.71 (9201.07 – 14509.43)	10700.84 (5560.25 – 12898.27)	12662.48 (8557.46 – 14984.85)	**0.03**

Gestational age (GA) in weeks, Median (IQR)	33.71 (33.14 – 34.14)	33.71 (33.14 – 34.14)	33.57 (33.14 – 34)	33.71 (33.29 – 34.14)	0.89
IP-10 (pg/mL)	179.98 (130.31 – 2293.21)	179.98 (130.31 – 223.21)	172.04 (130.88 – 250.99)	158.76 (123.74 – 217.23)	0.51
SAA (ng/mL)	10.61 (7.44 – 16.58)	10.61 (7.44 – 16.58)	11.07 (6.35 – 18.50)	8.55 (5.17 – 16.05)	0.33

Abbreviations: IQR, interquartile range; CRP, C-reactive protein; SAA, Serum amyloid A; IP-10, interferon-gamma-inducible protein-10, BMI, body mass index, ng/mL, nanograms per millilitre; pg/mL, picograms per millilitre; N, number.
*Visit 2 includes only those participants who initiated ART in the current pregnancy.
P-value<0.05=statistically significant.

Table 3. Median (IQR) plasma concentration of immune markers in pregnant women living with HIV (WLWH) who initiated ART in pregnancy across all 4 visits

		BMI category			
Immune marker by Visit	**Total (N)**	**Normal (N)**	**Overweight (N)**	**Obese (N)**	**P**
Visit 1	278	63	71	144	
Gestational age (GA) in weeks, Median (IQR)	14 (11 – 18)	16 (12 – 19)	15 (11.5 – 19)	14 (10 – 18)	0.10
CRP (ng/mL)	11646.94 (7112.06 – 14991.56)	10914.42 (5126.36 – 13836.90)	8829.59 (4829.10 – 13057.14)	12662.48 (9170.22 – 15699.57)	**0.01**
IP-10 (pg/mL)	226.97 (147.72 – 353.29)	237.16 (146.80 – 425.91)	255.07 (165.78 – 384.20)	211.51 (141.50 – 312.84)	0.08
SAA (ng/mL)	9.29 (4.84 – 17.38)	13.88 (6.39 – 30.93)	13.43 (6.72 – 22.19)	7.90 (4.53 – 12.36)	**0.01**
Visit 2	223	46	62	115	
Gestational age (GA) in weeks, Median (IQR)	17.14 (14 – 21)	17.5 (14.04 – 21)	18.07 (15 – 21)	16 (13.07 – 20.43)	0.18
CRP	12779.89 (8018.67 – 16586.46)	12252.19 (8018.75 – 14636.36)	10685.05 (6720.70 – 1832.97)	13247.85 (11368.02 – 17074.05)	**0.01**
IP-10 (pg/mL)	197.84 (139.13 – 274.96)	195.35 (127.56 – 266.96)	193.41 (139.56 – 296.52)	199.72 (141.94 – 274.96)	0.89
SAA (ng/mL)	8.78 (5.32 – 15.12)	11.73 (6.75 – 18.70)	8.98 (4.43 – 17.81)	7.93 (5.33 – 13.61)	0.34
Visit 3	232	36	68	128	
Gestational age (GA) in weeks, Median(IQR)	27.71 (27.29 – 28.14)	27.86 (27.25 – 28.29)	27 64 (27.14 – 28)	27.79 (27.29 – 28.14)	0.30
CRP ng/mL)	12252.19 (7983.61 – 15196.68)	12252.19 (8497.19 – 14179.92)	10416.32 (6781.30 – 13265.53)	12488.02 (8868.54 – 15695.06)	0.07
IP-10 (pg/mL)	165.23 (122.714 – 247.65)	190.39 (120.14 – 264.32)	161.80 (125.84 – 237.48)	165.23 (120.97 – 248.16)	0.47
SAA (ng/mL)	10.76 (5.69 – 18.34)	11.26 (6.83 – 14.35)	13.39 (6.20 – 20.65)	9.56 (4.75 – 15.32)	0.59
Visit 4	217	29	62	126	

Gestational age (GA) in weeks, Median (IQR)	33.86 (33.29 – 34.14)	34 (33.29 – 34.14)	33.57 (33.14 – 34.11)	33.86 (33.43 – 34.14)	0.61
CRP (ng/mL)	12090.30 (7477.11 – 14245.81)	12361.23 (9312.50 – 14489.94)	10305.215 (5217.35 – 12779.89)	12252.19 (8241.17 – 14474.74)	**0.04**
IP-10 (pg/mL)	179.88 (133.71 – 251.92)	187.65 (155.91 – 224.07)	190.62 (135.48 – 265.22)	172.17 (131.01 – 248.00)	0.61
SAA (ng/mL)	10.24 (6.08 – 17.89)	9.45 (7.17 – 18.15)	13.19 (8.53 – 20.80)	9.52 (5.41 – 15.10)	0.08

Abbreviations: IQR, interquartile range; ART, antiretroviral therapy; CRP, c-reactive protein; SAA, serum amyloid A; IP-10, interferon-gamma-inducible protein-10, BMI, body mass index, ng/mL, nanograms per millilitre; pg/mL, picograms per millilitre; N, number.

P-value<0.05=statistically significant.

Table 4. Median (IQR) plasma concentration of immune markers of pregnant women living with HIV (WLWH) who initiated ART preconception across 3 visits

		BMI category			
Immune marker by Visit	**Total (N)**	**Normal (N)**	**Overweight (N)**	**Obese (N)**	**P**
Visit 1	248	49	75	124	
Gestational age (GA) in weeks Median (IQR)	14 (11 – 17)	13 (10 – 17)	14 (11.5 – 18)	14 (11 – 16)	0.24
CRP (ng/mL)	12841.04 (7716.59 – 15168.60)	12662.48 (7272.91 – 14876.66)	12252.19 (7446.87 – 15845.11)	13057.14 (8483.96– 15303.84)	0.79
IP-10 (pg/mL)	121.05 (86.72 – 171.33)	102.07 (81.40 – 168.30)	122.13 (81.59 – 169.06)	122.94 (89.71 – 173.63)	0.65
SAA (ng/mL)	8.16 (4.67 – 15.37)	8.95 (4.16 – 14.33)	6.88 (3.46 – 14.59)	8.16 (5.12 – 15.74)	0.75
Visit 3	208	27	60	121	
Gestational age (GA) in weeks Median (IQR)	27.57 (27.14 – 28)	27.29 (27.14–27.79)	27.7 1(27.29 – 28.14)	27.43 (27.14 – 28)	0.18
CRP ng/mL)	12779.89 (8166.22 – 15277.87)	11285.71 (7411.88 – 14984.85)	12895.55 (8964.13 – 15848.11)	12791.29 (8307.36– 1158.60)	0.44
IP-10 (pg/mL)	135.55 (99.29 – 182.17)	114.65 (89.70 – 174.43)	155.46 (105.60 – 198.59)	127.62 (100.25 – 174.34)	0.83
SAA (ng/mL)	9.23 (4.87 – 16.21	13.05 (7.16 – 21.38)	9.01 (5.04 – 16.60)	8.64 (4.66 – 14.72)	**0.04**
Visit 4	198	20	54	124	
Gestational age (GA) in weeks Median (IQR)	33.57 (33.14 – 34)	33.36 (33.00 – 33.93)	33.64 (33.04 – 34)	33.57 (33.14 – 34)	0.43
CRP (ng/mL)	12335.46 (8491.51 – 14703.41)	12121.38 (8708.68 – 14299.59)	11341.58 (7092.32 – 12902.19)	12779.89 (8841.23– 15158.60)	0.31
IP-10 (pg/mL)	150.61 (116.16 – 201.20)	156.28 (108.72 – 222.89)	156.35 (123.49 – 214.20)	149.44 (115.67 – 199.03)	0.37
SAA (ng/mL)	9.25 (5.36 – 15.66)	11.64 (9.11 – 13.68)	9.83 (5.34 – 12.74)	8.28 (4.68 – 17.42)	0.94

Abbreviations: IQR, interquartile range; ART, antiretroviral therapy; CRP, c-reactive protein; SAA, serum amyloid A; IP-10, interferon-gamma inducible protein-10, BMI, body mass index; ng/mL, nanograms per millilitre; pg/mL, picograms per millilitre; N, number.P-value<0.05=statistically significant.

Table 5. Associations between immune markers and covariates for the adjusted mixed model.

	CRP (ng/mL)			IP-10 (pg/mL)			SAA (ng/mL)		
Predictors	*β*	*CI*	*P*	*β*	*CI*	*P*	*β*	*CI*	*P*
BMI category									
Normal (ref)	-	-	-	-	-	-	-	-	-
Overweight	260.36	-580.39 – 1101.12	0.55	3.23	-16.27 – 22.73	0.75	-1.47	-4.34 – 1.40	0.32
Obese	1279.74	321.45 – 2238.01	**<0.001**	-2.81	-23.29 – 17.68	0.79	-3.64	-6.39 – -0.88	**<0.001**
ART status									
Initiated in pregnancy (ref)	-	-	-	-	-	-	-	-	-
Initiated preconception	427.68	-473.51 – 1328.86	0.35	-69.35	-86.70 – -51.98	**<0.001**	-2.26	-4.43 – -0.09	**0.04**
Visit									
Visit 1 (ref)							-	-	-
Visit 2	1523.22	914.43 – 2132.01	**<0.001**	-6.97	-22.39 – 8.46	0.38	-1.95	-4.57 – 0.66	0.14
Visit 3	227.72	-249.69 – 705.14	0.35	-31.45	-43.48 – -19.41	**<0.001**	-0.12	-2.17 – 1.93	0.91
Visit 4	-210.62	702.24 –281	0.40	-12.62	-24.97 – -0.27	**0.05**	-0.47	-2.57 – 1.62	0.66

Abbreviations: β, beta coefficient; CI, confidence interval; CRP, c-reactive protein; SAA, serum amyloid A; IP-10, interferon-gamma inducible protein-10; ref, reference. P-value <0.05 = statistically significant.

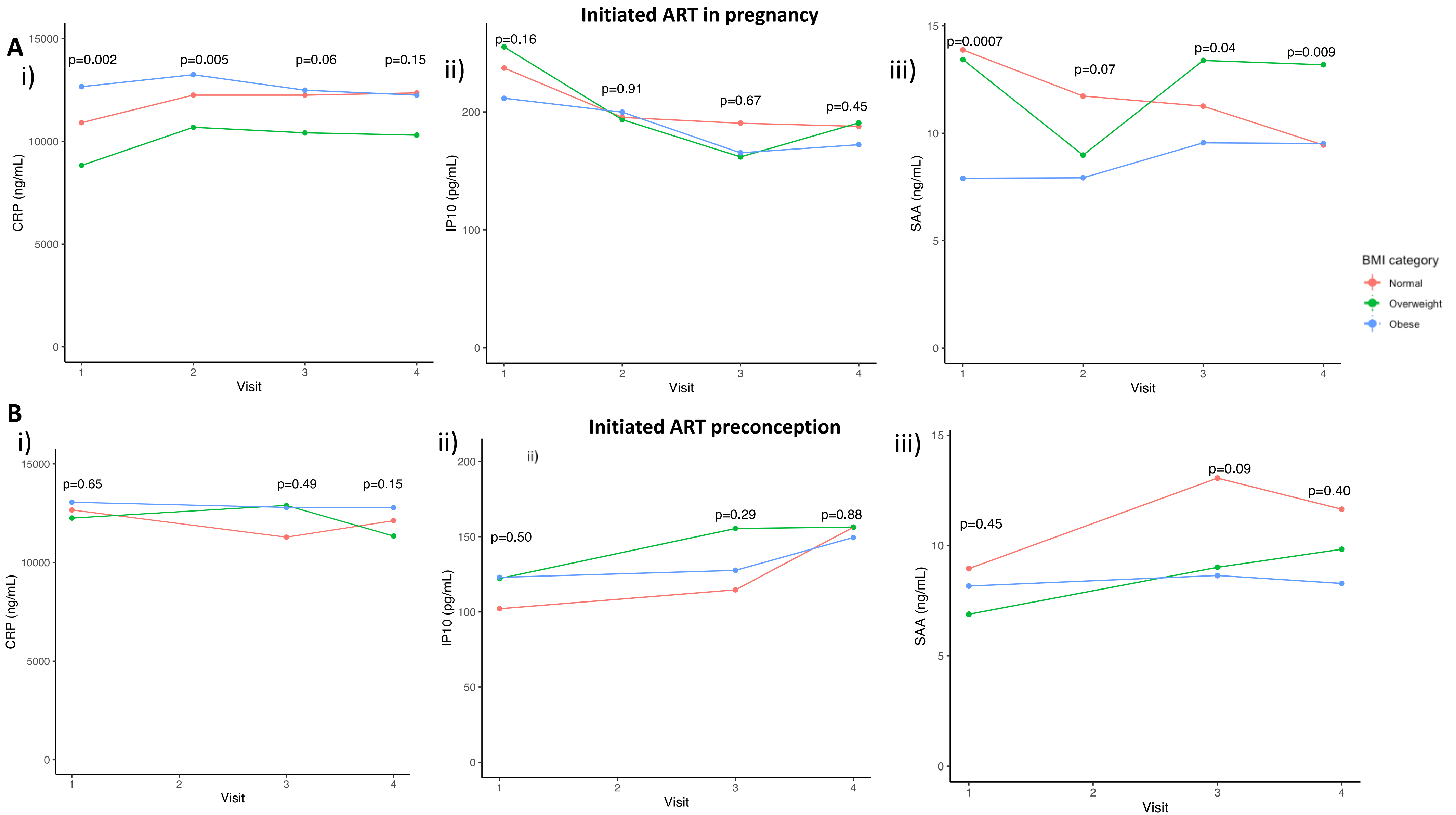

Figure 1. Median plasma concentration of immune markers at each visit by BMI category. A) Pregnant women living with HIV (WLWH) who initiated ART in pregnancy and B) those who initiated ART preconception showing the , i) CRP, ii) IP-10 and iii) SAA level for each BMI category (normal, overweight, obese). Visits shown are at baseline (1), 2 weeks post-ART initiation in those who initiated ART (2), ~ 28 weeks' gestation (3) and ~34 weeks' gestation (4). Abbreviations: ART, antiretroviral therapy; CRP, c-reactive protein; SAA, serum amyloid A; IP-10, interferon-gamma inducible protein-10, BMI, body mass index, ng/mL, nanograms per millilitre; pg/mL, picograms per millilitre. P-values <0.05 = statistically significant. P-values represented are from the non-parametric Kruskal-Wallis test.

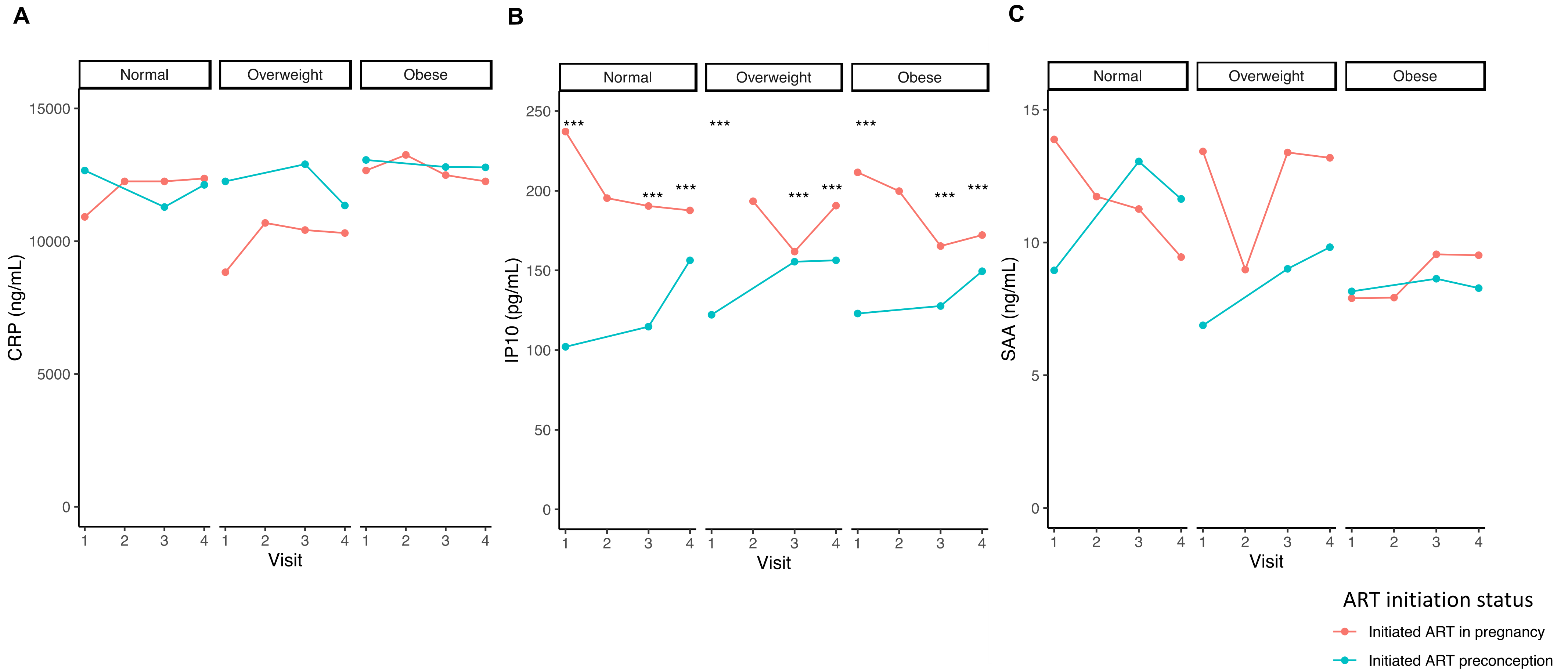

Figure 2. Median plasma concentration of immune markers and at each visit for those who initiated ART in the current pregnancy and those on ART preconception stratified by BMI. A) CRP, B) IP-10 and C) SAA level at baseline visit (1), 2 weeks post- ART initiation (for those who initiated ART in the current pregnancy (pink)) (2), ~ 28 weeks' gestation (3) and ~34 weeks' gestation (4). Those on ART preconception are represented in blue. Abbreviations: ART, antiretroviral therapy; CRP, c-reactive protein; SAA, serum amyloid A; IP-10, interferon-gamma inducible protein, BMI, body mass index; ng/mL, nanograms per millilitre; pg/mL, picograms per millilitre. P-value ≤ 0.001***, ≤ 0.01**, ≤ 0.05*. Only significant P-values are represented in the graphs.

PART C. APPENDICES

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1. QUESTIONNAIRES AND DATA ABSTRACTION FORMS

1A Maternal Demographic, Obstetric and Medical History Questionnaire

PWID: __________ - ____

MATERNAL DEMOGRAPHICS, OBSTETRIC & MEDICAL HISTORY

This CRF applies to ALL enrolled Group 2 Participants
(HIV-infected & ≤24 weeks GA as confirmed by USS)
Complete during Enrolment (≤24 weeks) Study Visit

Visit Date								
D	D	M	M	M	Y	Y	Y	Y

Visit Code	
A	1

SOCIODEMOGRAPHIC INFORMATION	
1. Mingaphi iminyaka yakho *What is your age?*	*Age:* _______ Iminyaka *years*
2. Uzelwe Nini *What is your date of birth*	______ /______ /________ DD MMM YYYY
3. Uthetha oluphi ulwimi ekhayai? *What language do you speak at home?*	☐ isiXhosa ☐ isiZulu ☐ isiBhulu *Afrikaans* ☐ isiNgesi *English* ☐ Olunye *Other* Cacisa *Specify*: ____________________
4. Leliphi elona banga liphezulu oliphumeleleyo? *What is the highest level of schooling/education that you have completed?*	☐ Umgangatho *Grade*: _______ Okanye *or* Ibanga *Standard*: _______ ☐ Imfundo enomsila *Postsecondary* : _______
5. Ngoku uyasebenza okanye uyafunda *Are you currently working and /or studying?*	☐ Ewe *Yes* ☐ Hayi *No* → **Gqithela ku Q7 SKIP to Q7**
6. Ukuba nguEwe, yeyephi kwezi zilandelayo echaza, bhetele ukuba wenza ntoni? *If yes, which of one the following best describes what you do?* **Khetha ibenye** ***/Choose one only***	☐ Ndiphangela isigxina *Employed full-time* ☐ Ndiphangela mangqaphangqapha *Employed part-time* ☐ Ndiphangela izingxungxo /ndingumatheng'ethengisa *Informal job/hawker* ☐ Uhamba isikolo/ ungumfundi *Attending school/learner* ☐ Uhamba isikolo semfundo enomsila *Attending tertiary education facility*

Initials of interviewer: _______

Question	Response
7. Uhlala kwikhaya elinjani? *What kind of home do you live in?*	☐ Ityotyombe/ uhlaliso olungahlelwanga *Shack/informal dwelling* ☐ Indlu yesitena *Formal house* ☐ Ifleti/ indlu kamaspala *Flat/council home* ☐ Olunye *Other* Cacisa *Specify*: ____________
8. Ingaba indlu yakho inazo ezi zinto zilandelayo: *Does your house have the following:* **Phendula ZONKE** *Respond to ALL*	*a.* Indlu yangasese *A toilet inside* ☐ Ewe *Yes* ☐ Hayi *No* *b.* Amanzi abalekayo empompo *Running water inside* ☐ Ewe *Yes* ☐ Hayi *No* *c.* Umbane *Electricity inside* ☐ Ewe *Yes* ☐ Hayi *No* *d.* Isikhenkcisi *A refrigerator* ☐ Ewe *Yes* ☐ Hayi *No* *e.* Umnxeba *A telephone* ☐ Ewe *Yes* ☐ Hayi *No* *f.* Umabona kude *A television* ☐ Ewe *Yes* ☐ Hayi *No*
9. Ukhulelwe kangaphi (kudibene nesi isisu)? *How many times have you been pregnant (incl. current pregnancy)?*	Inani lokukhulelwa: ______ *# of pregnancies:*
10. Bangaphi abantwana obazeleyo? *How many children have you given birth to?*	Inani labantwana: ☐ ______ ☐ None *# of children* → **Ukuba awunabo abantwana, Gqithela ku Q12** *If NONE, SKIP to Q12*
11. Bangaphi kwaba bantwana abaphilayo? *How many of these children are living?*	Inani labantwana: ☐ ______ ☐ None *# of children*
12. Uya thandana ngoku? *Are you currently in a relationship?*	☐ Ewe *Yes* ☐ Hayi *No* → **Gqithela ku Q15**
13. Ungaluchaza njani uthando lwakho? *How would you describe your current relationship?*	☐ Utshatile *Married* ☐ Anditshatanga ,ndiya hlalisana *Not married, living together* ☐ Nditshatile, asihlali kunye *Married, not living together* ☐ Anditshatanga, asihlali kunye *Not married, not living together* ☐ Olunye *Other* Cacisa *Specify*: ____________
14. Lileshe ellingakanani unobudlelwana nalomntu? *How long have you been in a relationship with this person?*	Ixesha *Duration in:* Inyanga *Months*: ______ Okanye *or* Iminyaka *Years*: ______

Initials of interviewer: ______

CLINICAL & ART HISTORY	
15. Ubuqala ukufumanisa ukuba unentsholongwa kagawulayo kolumitho okanye phambi kokuba ukhulelwe? *Did you first test HIV positive in this pregnancy or before this pregnancy?*	☐ Koku ukukhulelwa ➔ **Gqithela ku Q41** *In this pregnancy* ☐ Phambi koku ukukhulelwa *Before this pregnancy*
16. Kwakunini ukuqala kwakho ukufumanisa ukuba unentsholongwane kagawulayo? *When did you 1st test HIV-positive?*	______/_______/________ DD MMM YYYY
17. Kwakutheni ukuze oluhlolo lwenziwe? *Why was this test conducted?*	☐ Ndivavanywe ngelixesha ndikhulelweyo *Tested during pregnancy* ☐ VCT/Ndandifuna ukuvavanywe *VCT/Wanted to be tested* ☐ Ndafunyaniswa ndinesifo sephepha (TB) *Diagnosed with TB* ☐ Ndangeniswa esibhedlele *Admitted to the hospital* ☐ Olunye *Other* Cacisa *Specify*: ____________________________
18. Emva kokugqibela kwethu ukuthetha, ukhe watya iAZT? *Have you ever taken any AZT (while you were pregnant?)*	☐ Ewe *Yes* ☐ Hayi *No* ➔ **Gqithela ku Q21**
19. Kwizisu zakho ozi khulelweyo uyifumene kangaphi iAZT *For how many pregnancies have you taken AZT?*	# izisu: _______ *# pregnancies*
20. Ugqibele nini ukuyitya? *When did you last take AZT?*	______/_______/________ DD MMM YYYY
21. Emva kokugqibela kwethu ukuthetha, ukhe watya iNVP? *Have you ever taken any NVP (at delivery)?*	☐ Ewe *Yes* ☐ Hayi *No* ➔ **Gqithela ku Q24**
22. Kwizisu zakho ozi khulelweyo uyifumene kangaphi iNVP *For how many pregnancies have you taken NVP?*	# izisu: _______ *# pregnancies*
23. Ugqibele nini ukuyitya? *When did you last take NVP?*	______/_______/________ DD MMM YYYY
24. Wawuke wawathatha amachiza okuthomalalisa intsholongwane (awobomi bakho bonke) *Have you ever taken triple drug antiretroviral therapy (lifelong ART)?*	☐ Ewe *Yes* ☐ Hayi *No* ➔ **Gqithela ku Q41**

25. Ingaba wawafumana amachiza okuthomalalisa intsholongwane ukugqibela kakho? *Where did you receive ART the last time?*	Igama lekliniki: ______________________ *Name of clinic:*
26. Ukususela ukuqala kwakho ukutya amachiza, wawuke wawayeka na? *Since you first started taking ART, have you ever stopped?*	☐ Ewe *Yes* ☐ Hayi *No* ➔ **Gqithela ku Q31**
27. Mangaphi amaxesha uyeka uphinde uqalele ukutya amachiza? *How many times have you stopped and restarted ART?*	Amaxesha: ______ *# times*
28. Bekunini ukugqibela kwakho ukuqalela amachiza? *When did you restart ART the last time?*	_____/______/_______ DD MMM YYYY
29. Usawatya amachiza okuthomalalisa intsholongwane kagawulayo? *Are you still on ART?*	☐ Ewe *Yes* ➔ **Gqithela ku Q31** ☐ Hayi *No*
30. Ukuba nguHayi, uyeke nini ukuwatya amachiza okuthomalalisa intsholongwane kagawulayo? *When did you stop taking ART?*	_____/______/_______ DD MMM YYYY ➔ **Gqithela ku Q41**
31. Yintoni igama lamachiza owatyayo ngoku? *What are the names of the ARVs you are currently taking?*	☐ TDF-3TC-EFV ☐ TDF-FTC-EFV ☐ AZT-3TC-EFV ☐ AZT-3TC-NVP ☐ LPV/r ☐ Unknown ☐ Other: ________________
32. iART uzithatha kangaphi ngemini? *How many times a day do you take your ART pills?*	Amaxesha: ________ *# of times*
33. Zingaphi ipilisi ozityayo ngexesha? *How many pills do you take each time?*	# Iipilisi: ________ *# of pills*
34. Mangaphi amachiza entsholongwane ohlukeneyo owatyayo? *How many different HIV medicines do you take?*	# Amchiza: ________ *# of medicines*

Initials of interviewer: _______

ADHERENCE	
35. Njengokuba uqalile ukutya iART, ungazibeka kweliphi inqanaba lokutya ngendlela owawuyibonisiwe yokutya amachiza akho? *Since you started taking HIV medicine, how would you rate how well you usually do taking your HIV medicines in the way you are supposed to?*	☐ Kakubi kakhulu *Very poor* ☐ Kakubi *Poor* ☐ Ndiphakathi *Fair* ☐ Kakuhle *Good* ☐ Kakuhle kakhulu *Very good* ☐ Kakuhle okugqithisileyo *Excellent*
36. Ngoku cinga ngentsuku ezi-30 ezidlulileyo.yeyiphi kwezi zilandelayo echaza eyona ndlela otya ngayo amachiza akho? *Now think about the last 30 days. How would you rate how well you did taking your HIV medicines?*	☐ Kakubi kunakuqala *Worse than usual* ☐ Kakuhle kunakuqala *Better than usual* ☐ Kuyafana njengesiqhelo *About the same as usual*
37. Kwezi ntsuku eziyi-30 ezidlulileyo, zimini ezingaphi okhe walibala ukutya amchiza akho entsholongwana? *In the last 30 days, on how many days did you miss at least one dose of any of your HIV medicines?*	Iintsuku: _______*(0-30)* *# of days*
38. Kwezi ntsuku eziyi-30 ezidlulileyo, kukangaphi usitya amachiza akho entsholongwane ngendlela omele kuwatya ngayo? *In the last 30 days how often did you take your HIV medicines in the way that you were supposed to?*	☐ Zange *Never* ☐ Kumbalwa *Rarely* ☐ Ngamanye amaxesha *Sometimes* ☐ Ngesiqhelo *Usually* ☐ Malunga lonke ixesha *Almost always* ☐ Lonke ixesha *Always*
39. Kwezi ntsuku zi-30 zidlulileyo uwatye kakuhle kanjani amachiza akho entsholongwane njengohlobo omele ukuwatya ngalo? *In the last 30 days, how good a job did you do at taking your HIV medicines in the way that you were supposed to?*	☐ Kakubi kakhulu *Very poor* ☐ Kakubi *Poor* ☐ Ndiphakathi *Fair* ☐ Kakuhle *Good* ☐ Kakuhle kakhulu *Very good* ☐ Kakuhle okugqithisileyo *Excellent*
40. Kunzima kangakanani ukutya amachiza akho entsholongwana ngendlela omele kukuwatya ngayo? *How hard is it for you to take your HIV medicines in a way you are supposed to?*	☐ Kunzima kakhulu kakhulu *Extremely hard* ☐ Kunzima kakhulu *Very hard* ☐ Kunzima nje *Somewhat hard* ☐ Akunzimanga *Not very hard* ☐ Akunzimanga kwaphela *Not hard at all*

Initials of interviewer: _______

PWID: __________ - ____

TB HISTORY	
41. Kolu umitho ingaba, ugqira okanye unesi uthe une-TB? *During your current pregnancy, has a doctor or nurse told you that you have TB?*	☐ Ewe *Yes* ☐ Hayi *No* ➔ **Gqithela ku Q46**
42. Uxelelwe nini ngoku kugula? *When did you receive this diagnosis?*	_____ /_______ /_______ DD MMM YYYY
43. Uxelelwe phi ngoku kugula? *Where did you receive this diagnosis?*	Igama lekliniki : ___________ *Name of clinic*
44. Iphi emzimbeni wakho le TB? *Where in your body was the TB (eg, lungs, other location)?*	Indawo emzimbeni : _________________ *Place in body*
45. Uye wafumana unyango lwayo? *Did you receive treatment for TB?*	☐ Ewe *Yes* ☐ Hayi *No*
46. Ngaphandle koku kukhulelwa, ugqira okanye unesi bakhe bakuxelela ukuba une-TB? *Other than during this pregnancy has a doctor or nurse ever told you that you have TB?*	☐ Ewe *Yes* ☐ Hayi *No* ➔ **Gqithela ku Q52**
47. Waxelelwa nini ngoku kugula kuqala ingekuko ngoku ukhulelwe? *When did you receive this diagnosis the last time (not during this pregnancy)?*	_____ /_______ /_______ DD MMM YYYY
48. Waye walufumana unyango ngoko? *Did you receive treatment for TB the last time?*	☐ Ewe *Yes* ☐ Hayi *No* ➔ **Gqithela ku Q52**
49. Kungamaxesha amangaphi ewonke ufumana unyango lweTB? *How many times in total have you been treated for TB?*	Amaxesha: ____________ *# of times*
50. Walufumana phi unyango lwe TB? *Where did you receive your TB treatment?*	Igama lekliniki : ____________ *Name of clinic*
51. Lixesha elingakanani ufumana unyango lweTB ukugqibela kwakho ukunyangelwa yona? *How long did you receive treatment for TB the last time you were treated for TB?*	☐ 6 nyanga *6 months* ☐ 8 nyanga *8 months* ☐ 9 nyanga *9 months* ☐ Iyaqhubekeka *On-going* ☐ Amanye, cacisa: ____________ *Other, specify* ☐ Andazi *Don't know*

Initials of interviewer: ______

MEDICAL HISTORY			
52. Wakhe whacitha ubusuku esibhedlele? *Have you ever spent the night in hospital?*	☐ Ewe *Yes* ☐ Hayi *No* **➔ Gqithela ku Q54**		
53. Ukuba nguEwe, cacisa ngezantsi ulaliso ngalunye *If yes, list details for each admission below:*			
a. Isizathu *Reason for admission*	*b.* Ulaliswe nini? *Date of Admission*	*c.* Isibhedlele/kliniki *Hospital/ Clinic*	*d.* Wawukhulelwe *Were you pregnant at the time of this admission?*
i.	____/____/______ DD MMM YYYY		☐ Ewe *Yes* ☐ Hayi *No*
ii.	____/____/______ DD MMM YYYY		☐ Ewe *Yes* ☐ Hayi *No*
iii.	____/____/______ DD MMM YYYY		☐ Ewe *Yes* ☐ Hayi *No*
iv.	____/____/______ DD MMM YYYY		☐ Ewe *Yes* ☐ Hayi *No*
v.	____/____/______ DD MMM YYYY		☐ Ewe *Yes* ☐ Hayi *No*

SUBSTANCE USE

54. Ngaphambili ukhe wasebenzisa oku kulandelayo *In the past, have you used any of the following :*	Alcohol:	☐ Ewe/*Yes* ☐ Hayi/*No*
	Cigarettes:	☐ Ewe/*Yes* ☐ Hayi/*No*
	Drugs	☐ Ewe/*Yes* ☐ Hayi/*No*
55. Kwezi ntsuku ziyi 30 ukhe wasebenzisa oku kulandelayo *In the last 30 days, have you used any of the following*	Alcohol:	☐ Ewe/*Yes* ☐ Hayi/*No*
	Cigarettes:	☐ Ewe/*Yes* ☐ Hayi/*No*
	Drugs	☐ Ewe/*Yes* ☐ Hayi/*No*

Signed Interviewer completing CRF: ______________________________

Date of QC: _____ / ________ / ________ Signed Study Nurse:____________________
DD MMM YYYY

Initials of interviewer: _______

1B Medical and Obstetric Events Questionnaire

PWID:

MEDICAL & OBSTETRIC EVENTS

This CRF applies to ALL Group 2 Participants
Complete during 28 Weeks Antenatal Study Visit

Visit Date							
D		M	M	Y	Y	Y	Y

Visit Code	
A	2

Siza kubuza imibuzo ngempilo yakho: *We are going to ask you some questions about your health since we last met*	
1. Ingayonke inot isahamba kakuhle ngelixesha ukulelweyo? *Is everything still ok with your pregnancy?*	D EweYes D Hayi *No* If NO, Specify: D Delivered **Complete** <7 days **Maternal Adherence, Medical & Obstetrics Events (MAMOE Pl)** D Pregnancy Ended **Complete Study Termination Form** **Any questions Contact SC**
Sokubeleka sicela ukujonga ukuba zisemi ngendlela owawusinike yona iinkcukacha zakho: *Please can we update your locator information:*	
2, Usahlala okanye ufudukile kula ndlu ubukade uhlala kuyo ukugqibela sithetha? *Have you moved to a different home since we last spoke to you?*	☐ Ewe *Yes* **complete Locator Update (PL2)** ☐ Hayi *No*
3. Uzitshintshile inombolo zakho zomnxeba ukugqibela kwethuukuthetha? *Have you changed your cell phone number(s) since we last spoke to you?*	☐ Ewe *Yes* **complete Locator Update (PL2)** ☐ Hayi *No*
4. Ukhona omnye umntu esinoqhakamishelana naye xa kukho imfuneko? *Is there anyone else that we can contact ifwe arelooking for you in the event of an emergency?*	☐ Ewe *Yes* **complete Locator Update (PL2)** ☐ Hayi *No*

Initials of Interviewer:

MAMOEA2

PWID:

MEDICAL AND OBSTETRIC EVENTS

1. Ukugqibela kwethu ukuthetha nawe,ukhe wathunyelwa kwesinye isibhedlele kuba ugulisw a kukukhulelwa (Mowbray okanye Groote Schuur) *Since we last spoke to you, have you been referred to any other health facility for pregnancy-related care (eg, Mowbray or Groote Schuur)?*	D *EweYes* D Hayi *No* **Gqithela kuQ2**	a. Ubuthunyelwe phi? *Where were you referred?*	Igama lendawo *Location:*
		b. Wawusithini umhla wokuthunyelwa kwakho? *What was the date of the referral?*	____ /____ /____ 00 MMM yyyy
		c. Yintoni isizathu sokuthunyelwa kwakho? *What was the reason for referral?*	Isizathu *Reason*
		d. Ingaba wafumana unyango olutsha/ amayeza? ***Did you receive any new treatment or medications as a result of this** referral?*	D Ewe *Yes* Cacisa *If Yes, specify* ☐ Hayi *No*
2. Ukugqibela kwethu ukuthethanawe uye wathunyelwa kwesinye isibhedlele ngenxayokugula (GF Jooste okanye Groote Schuur) *Since we last spoke to you, have you been referred to any other health facility for !ltf1ff medical care (eg, CF Jooste or Groote Schuur)?*	D *EweYes* D Hayi *No* **Gqithela kuQ3**	a. Ubuthunyelwe phi? *Where were you referred?*	Igama lendawo *Location:*
		b. Wawusithini umhla wokuthunyelwa kwakho? *What was the date of the referral?*	____ / __ /_ 00 MMM yyyy
		c. Yintoni isizathu sokuthunyelwa kwakho? *What was reason for the referral?*	Isizathu *Reason*
		d. Ingaba wafumana unyango olutsha/amayeza? ***Did you receive any new treatment** or medications as a result of this referral?*	D Ewe *Yes* Cacisa */[Yes, specify:* D Hayi *No*

TUBERCULOSIS (TB)	
3. Ukugqibela kwethu ukuthetha ugqira okanye unesi bakhe bathi une-TB? ***Since we last spoke to you, has a doctor or nurse told you that you have TB?***	D *EweYes* 0 Hayi *No* **Gqithela ku Q8**
4. Uxelelwe nini ngoku kugula? ***When did you receive this diagnosis?***	___ / __ / _ 00 MM M YYYY
5. Uxelelwe phi ngoku kugula? ***Where did you receive this diagnosis?***	Igama lekliniki: ________ ***Name of Clinic***
6. Iphi emzimbeni wakho le TB? ***Where in your body was the TB (eg: lungs, other location)?***	Indawo emzimbeni: ________ *Place in body*
7. Uye wafumana unyango lwayo? ***Did you receive treatment for TB?***	D *EweYes* D Hayi *No*

Initials of Interviewer:

MATERNAL ART ADHERENCE

Siza kubuza imibuzo ngamachiza akho amachiza athomalalisa intsholongwane:
We are going to ask you some questions about your HIV medicine

	Question	Response
8,	Ingaba ukhona ugqirha okanye unesi otshintshe amchiza akho *Has a doctor or nurse changed anything about your HIV treatment*	☐ Ewe *Yes* Cacisa *lfYes, Reason:* ____ ☐ Hayi *No*
9,	Yintoni igama lamachiza owatyayo ngoku? *What are the names of the ARVsyau are currently taking?*	☐ TDF-3TC-EFV 0 TDF-FTC-EFV ☐ AZT-3TC-EFV 0 AZT-3TC-NVP ☐ LPV/r D Unknown ☐ Other: ____
10,	Uwafumana phi amachiza akho? *Where are you receiving your ART from*	Igama lekliniki: ____ *Name of clinic:*
11,	Uwatya kangaphi ngeminin amachiza akho? *How many times a day do you take your ART pills?*	Amaxesha: ____ *#of times*
12.	Zingaphi ipilisi ozityayo ngexesha? *How many pills doyou take each time?*	# lipilisi: ____ *#of pills*
13.	Mangaphi amachiza entsholongwane kaGawulayo ohlukeneyo owatyayo? *How many different HIV medicines do you take?*	# Amchiza: ____ *# of medicines*
14.	Ngoku cinga ngentsuku ezi-30 ezidlulileyo.yeyiphi kwezi zilandelayo echaza eyona ndlela otya ngayo amachiza akho? *Now think about the last 30 days. How would you rate how well you did taking your HIV medicines?*	D Kakubi kunakuqala *Worse than usual* D Kaku hie kunakuqala *Better than usual* D Kuyafana njengesiqhelo *Aboutthesameasusual*
15.	Kwezi ntsuku eziyi-30 ezidlulileyo, zimini ezingaphi okhe walibala ukutya amchiza akho entsholongwana? *In the last 30 days, on how many days did you miss at least one dose of any of your HIV medicines?*	Jintsuku:: D ___ *(0-30J* *#of days* ☐ Zange *Never*
16.	Kwezi ntsuku eziyi-30 ezidlulileyo, kukangaphi usitya amachiza akho entsholongwane ngendlela omele kuwatya ngayo? *In the last 30 days how often did you take your HIV medicines in the way that you were supposed to?*	D Kumbalwa *Rarely* D Ngamanye amaxesha *Sometimes* D Ngesiqhelo *Usually* D Malunga lonke ixesha *Almostalways* D Lonke ixesha *Always*
17.	Kwezi ntsuku zi-30 zidlulileyo uwatye kakuhle kanjani amachiza akho entsholongwane njengohlobo omele ukuwatya ngalo? *In the last 30 days, how good a job did you do at taking your HIV medicines in the way that you were supposed to?*	D Kakubi kakhulu *Very poor* D Kakubi *Poor* ☐ Ndiphakathi *Fair* ☐ Kakuhle *Good* ☐ Kakuhle kakhulu *Very good* D Kakuhle okugqithisileyo *Excellent*

Signed Interviewer Completing CRF: ____________________

Date of QC: __ / __ / __ Signed Study Nurse: ______________
DD MMM YYYY

Initials of Interviewer:

1C Maternity case record abstraction form

PIMS: Data Abstraction Form: Maternity Case Record
Version 1.0, May 2015

MCRA

PWID: _ _ _ _ _ _ - _ _

MATERNITY CASE RECORD ABSTRACTION FORM

This form applies to ALL enrolled Group 1 Participants
Complete after conclusion of routine Antenatal Care Booking Visit Procedures

Date of Data Abstraction:	____ / ______ / _____ (DD MMM YYYY)	**Initials of Data Abstractor:**	
Participant Full Name:		**Participant Date of Birth:**	____ / ______ / _____ (DD MMM YYYY)
Participant National ID		**Provincial Folder Number:** (specify 3 letter facility prefix; GUP or other)	G U P
Gravidity		**Parity**	
Miscarriages			

Previous Pregnancies (as per Maternity Chart)

Year	Gestation	Delivery	Weight	Sex	Outcome	Complications

Medical & General History

THIS Pregnancy **Healthy:** ☐ YES ☐ NO If NO tick all options that apply	☐ Hypertension ☐ Epilepsy ☐ Cardiac ☐ TB ☐ Other:______________________ Further details if any of the above selected: ______________________ ______________________
Previous Pregnancies ☐ N/A	☐ Twins ☐ Diabetes ☐ TB ☐ Congenital ☐ Other:______________________ Further details if any of the above selected: ______________________ ______________________

Details of Booking Examination

Date	____ / ________ / ______ (DD MMM YYYY)	BP	_____ / _____ (Systolic Diastolic)	HB	______ g/dl
Height: _________ cm		**Weight**: _______ kg		**MUAC**: _________ cm	
SFH: _________ cm		**Correlation with dates:**	Y / N	**Other::**	

RPR Result	Neg	Pos	Titre if pos: ______	Rhesus: ______	ABO Bloodgroup: _____

1st HIV Test	___ / _____ / _____ (DD MMM YYYY)	RESULT			2nd HIV Test	___ / ____ / ____ (DD MMM YYYY)	RESULT		
		POS	NEG	Decline			POS	NEG	Decline
On ART	Y	N	Initiation Date	____ / _____ / _____ (DD MMM YYYY)	Regimen				

EDD Estimation

Date of assessment #1	___ / _____ / ____	# 1: Type	Date / LNMP	SFH	USS
Gestational Age (weeks):		EDD: ____ / ____ / ____ (DD MMM YYYY)			
Date of assessment #2	___ / _____ / ____	#2: Type	Date / LNMP	SFH	USS
Gestational Age (weeks):		EDD: ____ / ____ / ____ (DD MMM YYYY)			
Date of assessment #3	___ / _____ / ____	#3: Type	Date / LNMP	SFH	USS
Gestational Age (weeks):		EDD: ____ / ____ / ____ (DD MMM YYYY)			

Additional Notes:

____

____

____

____

____

____

____

1D Obstetrics Abstraction Form

PIMS: Data Abstraction Form: Obstetric Record
Version 3.0, 19th December 2016

PWID: _ _ _ _ _ _ - _ _ **ODA**

OBSTETRICS ABSTRACTION FORM

This form applies to ALL enrolled Group 1 Participants
Complete after delivery

Date of Data Abstraction:	____ / ____ / ____ (DD MMM YYYY)	**Initials of Data Abstractor:**	
Participant Full Name:		**Participant Date of Birth:**	____ / ____ / ____ (DD MMM YYYY)
Participant National ID		**Provincial Folder Number:** specify 3 letter facility prefix; GUP or other	G U P
Source Document	☐ Maternity Case Record ☐ Delivery Register ☐ PMTCT Register (incl DISA/Trakcare) ☐ Other, Please Specify: ______		

Mothers Details

Name of clinic		**GA at 1st visit**			**Booking Date**	____ / ____ / ____ (DD MMM YYYY)	
Hb		**Rhesus**	Pos	Neg	**Syphilis**	Pos	Neg
HIV results	Pos / Neg	**HIV retest**	Pos	Neg	**ART**	Y	N
Regimen	☐ NR				**ART Start Date**	____ / ____ / ____ (DD MMM YYYY)	
Estimated Gestational Age	Weeks:______ If weeks not recorded: ☐ Term ☐ Preterm	**Gestational Age estimated by**			☐ Dates ☐ SFH ☐ Not Reported	☐ Palpitation ☐ Ultrasound Scan	

Blood Pressure Details

Any hypertension noted at any time before labour? *(Systolic Over 140 and/or Diastolic Over 90)*	☐ Yes ☐ No	**Were any hypertensives given during this period?**	☐ Yes ☐ No
Any hypertension noted during labour? *(Systolic Over 140 and/or Diastolic Over 90)*	☐ Yes ☐ No	**Were any hypertensives given during this period?**	☐ Yes ☐ No
Maximum Systolic BP (at any point in pregnancy) *Record complete measure*	____ / ____ (systolic diastolic)	**Maximum Diastolic BP (at any point in pregnancy)** *Record complete measure*	____ / ____ (systolic diastolic)
Date this BP was taken	____ / ____ / ____ (DD MMM YYYY)	**Date this BP was taken**	____ / ____ / ____ (DD MMM YYYY)

Labour Details

Delivery Date	____ / ____ / ____ (DD MMM YYYY)			**Delivery time**	____ : ____		
Fetal Heart	Present	Absent	uncertain	**Fetal Distress**	Y	N	NR
Place of Delivery	☐ GMOU ☐ MMH ☐ GSH ☐ Other, Specify: ______			**Delivery Method** *Please tick all methods used during this delivery*	☐ NVD ☐ Vacuum	☐ C/S ☐ Forceps	☐ Induction of labour ☐ BBA

If C/S, Primary indication	☐ Fetal distress ☐ Twins/Triplets ☐ APH ☐ Other, specify: ______	☐ Obstructed labour ☐ Pre-eclampsia /eclampsia ☐ Previous C/S	If by C/S, was it performed after membrane rupture?	☐ Yes, Duration:____ Mins ☐ No ☐ NR

Please tick all major medical and/or obstetric conditions the mother experienced during pregnancy and/or during delivery.	☐ Chorio amnionitis ☐ IUGR ☐ Diabetes mellitus ☐ PPH ☐ Cervical tear	☐ Sepsis ☐ Hypertension ☐ Preterm labour ☐ Prolonged ROM ☐ Perineal tear	☐ UTI / Pyelonephritis ☐ Pre-eclampsia/ eclampsia ☐ APH ☐ Prolonged labour ☐ Episiotomy
	☐ Other, Specify: ______		

Placenta method of delivery	☐ Active ☐ Spontaneous ☐ Manual	Placenta	☐ Complete ☐ Incomplete ☐ NR	Weight: ______ g

Infant Details

Infant DOB	___ / ___ / ___ (DD MMM YYYY)	Infant DOB for Twin B	___ / ___ / ___ (DD MMM YYYY)
Gender	☐ Male ☐ Female	Gender Twin B	☐ Male ☐ Female
Outcome	☐ Alive ☐ Stillborn ☐ NND	Outcome Twin B	☐ Alive ☐ Stillborn ☐ NND
Resuscitation	☐ Yes ☐ No	Resuscitation Twin B	☐ Yes ☐ No
Birthweight	______ g	Birthweight Twin B	______ g
Head circumference	______ cm	Head circumference Twin B	______ cm
Length	______ cm	Length Twin B	______ cm
APGAR Score	1 min: ____ 5 min: ____	APGAR Score Twin B	1 min: ____ 5 min: ____
Congenital Abnormalities?	☐ Yes ☐ No ☐ NR	Congenital Abnormalities Twin B	☐ Yes ☐ No ☐ NR
Polio Vaccine?	☐ Yes ☐ No ☐ NR	Polio Vaccine Twin B	☐ Yes ☐ No ☐ NR
BCG Vaccine?	☐ Yes ☐ No ☐ NR	BCG Vaccine Twin B	☐ Yes ☐ No ☐ NR
NVP at birth?	☐ Yes ☐ No ☐ NR	Twin B NVP at birth?	☐ Yes ☐ No ☐ NR
Feeding Option	☐ Breast ☐ Formula		

Discharge Summary

Family planning choice	☐ Oral contraceptive ☐ Implant	☐ Injectable ☐ Tubal ligation	☐ IUD ☐ Other: ______
Date of discharge	___ / ___ / ___ (DD MMM YYYY)		

Stage Period	Date	Time	Reading
1st ANC Visit	___/___/___	___:___	___/___
2nd ANC Visit	___/___/___	___:___	___/___
3rd ANC Visit	___/___/___	___:___	___/___
4th ANC Visit	___/___/___	___:___	___/___
5th ANC Visit	___/___/___	___:___	___/___
6th ANC Visit	___/___/___	___:___	___/___
Labour Initial	___/___/___	___:___	___/___
1st Stage	___/___/___	___:___	___/___
2nd Stage	___/___/___	___:___	___/___
3rd Stage	___/___/___	___:___	___/___
4th Stage	___/___/___	___:___	___/___
Post- delivery	___/___/___	___:___	___/___
Other Readings	___/___/___	___:___	___/___
	___/___/___	___:___	___/___
	___/___/___	___:___	___/___
	___/___/___	___:___	___/___
	___/___/___	___:___	___/___
	___/___/___	___:___	___/___
	___/___/___	___:___	___/___

1E Maternal Physical Examination Form

PIMS: Maternal Physical Examination Form A1
Version 1.0, April 2015

MPE A1

PWID: _ _ _ _ _ _ - _ _

MATERNAL PHYSICAL EXAMINATION FORM

This CRF applies to ALL enrolled Group 2 Participants
Complete during Enrolment (≤20 weeks) Study Visit

Visit Date								
D	D	M	M	M	Y	Y	Y	Y

Visit Code	
A	1

ANTHROPOMETRY	
Height	☐☐☐.☐ cm ☐ Not Measured
Weight	☐☐☐.☐ kg ☐ Not Measured
MUAC	☐☐.☐ cm ☐ Not Measured
BLOOD PRESSURE	
Reading 1	**Time of Measurement** ☐☐:☐☐ **Systolic:** ☐☐☐ mmHg **Diastolic:** ☐☐☐ mmHg
Reading 2	**Time of Measurement** ☐☐:☐☐ **Systolic:** ☐☐☐ mmHg **Diastolic:** ☐☐☐ mmHg
Reading 3	**Time of Measurement** ☐☐:☐☐ **Systolic:** ☐☐☐ mmHg **Diastolic:** ☐☐☐ mmHg
	☐ Blood Pressure Not Measured → **END** **Method:** (tick one) ☐ Manual ☐ Automated **Location:** (tick one) ☐ Left Arm ☐ Right Arm **Position:** (tick one) ☐ Sitting ☐ Supine ☐ Standing

Initials of Assessor: ______

Additional Notes:

____

Signed Assessor measurements obtained by: __________________________

Date of QC: ______ / _________ / __________ Signed Study Nurse: ________________
DD MMM YYYY

1F Specimen Collection Form

PIMS: Specimen Collection Form
Version 2.0, 5th June 2015

SC

PWID: __________ - ____

SPECIMEN COLLECTION FORM

This Form applies to ALL enrolled Group 2 Participants
Complete during ALL study visits

Visit Date								
D	D	M	M	M	Y	Y	Y	Y

Visit Code	

STORE AT ROOM TEMPERATURE UNTIL COLLECTION

Form Completed by: ______________________

Were the following Specimens Collected?		Number of tubes collected	Time Collected	Collected by (initials)	Specimen ID (SID)/Barcode
1. Sodium Heparin (Green Top) **4 x 9ml** **At 1st (A1) and 4th (P1) study visit ONLY** ------------------------- **5 x 9ml** **1 x 4ml** **At the rest of the study visits**	☐ Yes ☐ No	_____			Place bar coded sticker ☐☐☐☐☐
2. EDTA (Purple Top) **1 x 10ml** **At 1st (A1) and 4th (P1) study visit ONLY**	☐ Yes ☐ No	_____			Place bar coded sticker ☐☐☐☐☐
3. PAXGene (Clear Top) **1 x 2.5ml** **At 1st study visit (A1) ONLY**	☐ Yes ☐ No	_____	____ h ____ min		Place bar coded sticker
4. Other __________	☐ Yes ☐ No	_____	____ h ____ min		Place bar coded sticker
5. Other __________	☐ Yes ☐ No	_____	____ h ____ min		Place bar coded sticker

Page **1** of **1**

2. ETHICS APPROVAL FORMS

2A PIMS UCT Research and Ethics Committee of Health Sciences (HREC) Approval

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

30 October 2014

HREC REF: 739/2014

A/Prof L Myer
Epidemiology & Biostatistics
Public Health & Family Medicine
Falmouth Building
Level 5, entrance 5

Dear A/Prof Myer

PROJECT TITLE: ANTIRETROVIRAL THERAPY AND RISK OF PREMATURE DELIVERY: THE PREMATURITY IMMUNOLOGY IN HIV-INFECTED MOTHERS AND THEIR INFANTS STUDY (PIMS)

Thank you for your response to the Faculty of Health Sciences Human Research Ethics Committee dated 28 October 2014.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th October 2015.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

HREC 739/2014

2B UCT Research and Ethics Committee of Health Sciences (HREC) Approval

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room 45, E-52- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

17 January 2022

HREC REF: 027/2022

Dr T Malaba
Division of Epidemiology & Biostatistics
Public Health & Family Medicine
Email: thoko.malaba@uct.ac.za
Student: Mrxjes006@myuct.ac.za

Dear Dr Malaba

PROJECT TITLE: MATERNAL BODY MASS INDEX AND PRO-INFLAMMATORY IMMUNE MARKERS IN HIV-INFECTED PREGNANT WOMAN ON ANTIRETROVIRAL TREATMENT IN CAPE TOWN, SOUTH AFRICA (SUB-STUDY - 739/2014) (MASTER'S DEGREE - DR JESSICA MORE)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020: 06 July 2020 & 01 July 2021.

Approval is granted for one year until the 30 January 2023

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: -Dr Jessica More will also be involved in this study.

Please quote the HREC REF 027/2022 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

2C UCT Research and Ethics Committee of Health Sciences (HREC) Annual Approval

HUMAN RESEARCH ETHICS COMMITTEE
24 MAY 2022
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30.5.23
☐ Not approved	See attached comments		
Signature Chairperson of the HREC	[signature]	Date Signed	24/5/2022

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	20th May 2022		
HREC REF Number	739/2014	Current Ethics Approval was granted until	30/03/2022
Protocol title	Antiretroviral treatment during pregnancy: understanding the risk of premature delivery: The Prematurity Immunology in HIV-infected Mothers and their infants Study (PIMS)		
Protocol number (if applicable)	N/A		
Are there any sub-studies linked to this study?		☐ Yes ☑ No	
If yes, could you please provide the HREC Ref's for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Professor Landon Myer		
Department / Office Internal Mail Address	Level 5, School of Public Health and Family Medicine, Falmouth Building, Faculty of Health Sciences.		

1.1 Does this protocol receive US Federal funding?	☑ Yes	☐ No

21 February 2019 Page 1 of 6 FHS016

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

2D University of Southampton Faculty of Medicine Ethics Committee approval

Professor Marie-Louise Newell
Faculty of Medicine
University of Southampton

26 November 2014

Re: Project 12542 PIMS

Dear Professor Newell

Thank you for submitting your revised application relating to this study. I am pleased to inform you that full approval has now been granted by the Faculty of Medicine Ethics Committee.

Approval is valid from today until 8 January 2018 which is the end date specified in your application. We will be in touch with you again in January 2018 to confirm that your project has been completed.

Please note the following points:

- the above ethics approval number must be quoted in all correspondence relating to your research, including emails;
- if you wish to make any substantive changes to your project you must inform the Faculty of Medicine Ethics Committee as soon as possible.

Please note that this email will now constitute evidence of ethical approval. Should you require a paper signed copy of this approval, please contact the FoMEC Administrative Team via email at: Medethic@soton.ac.uk. We wish you well with your research.

Yours sincerely

Dr Catherine Hill

Faculty of Medicine Ethics Committee

Please reply to:
Mrs Anne Tarrant
Faculty of Medicine Ethics Committee, Southampton General Hospital, Mailpoint 801, South Academic Block, Tremona Road, Southampton SO16 6YD United Kingdom

University of Southampton, Highfield Campus, Southampton SO17 1BJ United Kingdom
Tel: +44 (0)23 8059 2819 Fax: +44 (0)23 8059 3131 www.southampton.ac.uk

2E Western Cape Government Provincial approval

STRATEGY & HEALTH SUPPORT
Health.Research@westerncape.gov.za
tel: +27 21 483 6857; fax: +27 21 483 9895
5th Floor, Norton Rose House., 8 Riebeek Street, Cape Town, 8001
www.capegateway.gov.za)

REFERENCE: WC_2015RP32_631
ENQUIRIES: Ms Charlene Roderick

University of Cape Town
Anzio Road
Observatory
Cape Town
7935

For attention: **Prof Landon Myer, Dr Greg Petro, Dr Max Kroon, Prof Clive Gray and Dr Mushi Matjila**

Re: PREMATUIRITY IMMUNOLOGY IN HIV-INFECTED MOTHERS AND INFANTS STUDY (PIMS)

Thank you for submitting your proposal to undertake the above-mentioned study. We are pleased to inform you that the department has granted you approval for your research.
Please contact the following people to assist you with any further enquiries in accessing the following sites:

Mowbray Maternity Hospital	**S Fawcus**	**Contact No: 021 659 5579**
Gugulethu CHC	**L Mbanga**	**Contact No: 021 637 1280**

Kindly ensure that the following are adhered to:

1. Arrangements can be made with managers, providing that normal activities at requested facilities are not interrupted.
2. Researchers, in accessing provincial health facilities, are expressing consent to provide the department with an electronic copy of the final report within six months of completion of research. This can be submitted to the provincial Research Co-ordinator (Health.Research@westerncape.gov.za).
3. The reference number above should be quoted in all future correspondence.

Yours sincerely

DR A HAWKRIDGE
DIRECTOR: HEALTH IMPACT ASSESSMENT
DATE: 13/10/2015.
CC P OLCKERS **DIRECTOR: KLIPFONTEIN/ MITCHELLS PLAIN**

3. INFORMED CONSENT FORMS

3A PIMS Informed Consent Form 1

PIMS: Informed Consent Form #1
English Version 1.0, March 2015

PIMS INFORMED CONSENT 1
GROUP 1 PREGNANCY CONSENT

WHAT IS THE PURPOSE OF THIS STUDY?
We are from the University of Cape Town and University of Southampton, UK. You are being asked to take part in a study that is being conducted at the Gugulethu Midwife Obstetric Unit (MOU). The purpose of this study is to understand how to improve health care services for women during their pregnancy.

You are being asked to take part in this study because you are a pregnant woman and you are getting your pregnancy care here at the Gugulethu MOU. The purpose of this consent is to give you information to help you decide if you want to take part in this study.

WHAT DO I HAVE TO DO IF I AGREE TO TAKE PART?
If you agree to take part, you will do the following at today's visit:

- Answer a short questionnaire about yourself and your health
- If it appears that you are still early in your pregnancy, we would like you to have an ultrasound scan to see how far along you are in pregnancy
- Based on this information, we may invite you to participate in further research

Review of medical records
As part of this study, we will also be looking at and taking information from your antenatal, obstetric, medical, and laboratory records. From these records, we are interested in learning about the pregnancy care you received as well as information about your delivery. If you are HIV-positive, we also want to learn about the HIV care and treatment that you received during your pregnancy and after you delivered. All data that we review and abstract is confidential and no participant names are recorded on study documents.

Contact for future study
After the completion of this visit, it is possible that we will contact you again at your next clinic visit to take part in additional studies like this one. At that time, you would be asked to review and sign another consent form. You can choose to not take part in these additional visits if you are asked.

WHAT ARE THE POTENTIAL RISKS?
If you decide to participate, you may feel uncomfortable about some of the personal questions you are asked about your health or your pregnancy. You may refuse to answer any question that you do not want to answer. There is some risk in sharing personal and medical information. We will be careful to keep all your information as private as possible.

WHAT ARE THE POTENTIAL BENEFITS?
There is no direct benefit to you if you take part in this study but if we identify any health care problem during the course of the study, we will make sure you are referred to the appropriate health care services. The information gained in this study may help to improve services for pregnant women in Cape Town, the Western Cape Province, and across South Africa.

WHAT ARE THE ALTERNATIVES TO TAKING PART?
The alternative to taking part in this study is to continue with your usual health care. If you decide not to participate in this study, your usual health care will not be changed in any way.

WHAT ABOUT CONFIDENTIALITY?
If you agree to take part, all information collected during the study will be kept strictly confidential. Your name will not be written on the study forms and will not be used in connection with any information or lab specimens that are collected as part of the study.

All study materials will be stored in locked filing cabinets. Only study staff and personnel involved in routine audits will have access to these materials. All staff involved in data collection and management will get specific training in confidentiality.

Even with these procedures in place, if the study staff learns that you are at risk of hurting yourself or someone else or of possible child abuse and/or neglect, study staff will tell the proper authorities.

WILL I BE GIVEN ANYTHING FOR TAKING PART?
No, there is no compensation for taking part in the study today.

ARE THERE ANY COSTS?
There is no cost for being in this study.

CAN I LEAVE THE STUDY?
You have the right to decide not to not take part in the study, to refuse to answer any questions, or to withdraw from the study at any time without any penalty. It will have no effect on the care that you receive at the Gugulethu MOU or any other health facility.

DO YOU HAVE ANY QUESTIONS?
If there is anything that is unclear or if you need further information, please ask us and we will provide it.
Do you have any questions?

FOR ADDITIONAL INFORMATION:
If you have any questions or have any problems while taking part in this research study, you should contact:

Prof Landon Myer
School of Public Health and Family Medicine
Faculty of Health Sciences
University of Cape Town

Tel: 021 406 6661
Email: Landon.Myer@uct.ac.za

Prof Marie Louise Newell
Human Development and Health Unit
Faculties of Medicine & Social and Human Sciences
University of Southampton, UK
Tel: +44 (023) 8059 3901
Email: m.newell@soton.ac.uk

If you have any questions about your rights as a research participant, you may contact the following member of the ethics committee:

Prof Marc Blockman, Chair
Human Research Ethics Committee
Faculty of Health Sciences, University of Cape Town
Tel: 021 406 6338

Dr Angela Fenwick, Chair
Southampton Faculty of Medicine Ethics Committee
Building 85, Life Sciences, Highfield Campus
Southampton SO17 1BJ, United Kingdom
Tel: +44 2381 208692

SIGNATURE PAGE

For participant to complete (please tick):

☐ I have read the information in this document (or it has been read to me). I have been offered a copy of this consent form. I was encouraged and given time to ask questions and all my questions about the study and my participation in it have been answered. I freely consent to be in this research study and agree to participate and know that I may withdraw at any time. My being in the study is voluntary. I understand that whether or not I participate will not affect my health care services received today, or at any time in the future.

☐ I agree that the study team can access my medical records at this hospital or another hospital if necessary for this study. My information will be kept confidential.

Participant Name (Please print)	Participant Signature	Date/Time

Interviewer Name (Please print)	Interviewer Signature	Date/Time

If this consent form is read to the participant because the participant is unable to read the form or if the participant must use a thumbprint to sign his/her name, an impartial witness not affiliated with the research or investigator must be present for the consent and sign the following statement:

I confirm that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the participant. The participant freely consented to be in the research study.

Witness Name (Please print)	Witness Signature	Date/Time

Thank you

3B PIMS Informed Consent Form 2

PIMS INFORMED CONSENT 2
GROUP 2 PREGNANCY AND DELIVERY CONSENT

WHAT IS THE PURPOSE OF THIS STUDY?
We are from the University of Cape Town and University of Southampton, UK. You are being asked to take part in a study that is being conducted at the Gugulethu Midwife Obstetric Unit {MOU). The purpose of this study is to understand how to improve health care services for HIV-positive women during their pregnancy and after they deliver the baby.

We know that it is important for their own health as well as the health of their baby, that HIV-positive women receive the HIV care and treatment that they need both during and after delivery. Information learned in this study will help us to improve HIV services for pregnant women.

You are being asked to take part in this study because you are a pregnant woman with known HIV infection who is taking HIV drugs (antiretroviral therapy) and you took part in the first part of this study. The purpose of this consent form is to give you information to help you decide if you want to take part in the next part of this study.

WHAT DO I HAVE TO DO IF I AGREE TO TAKE PART?
If you agree to take part, you will come in for up to 6 study visits. These visits will take place today while you are in the clinic, and then every 2 months until you deliver, within one week of delivering your baby and again when your baby is about 10 weeks and 6 months old. All of these study visits are separate from the usual clinic visits that you will have for your pregnancy and HIV care. Study visits can be timed so that they take place on the same days that you come in for your usual pregnancy and/or HIV care. The first visit today will take about 60 minutes and each of the subsequent visits will take about 30 minutes.

At the visits that are conducted *while you are pregnant*, you will do the following:
- Answer questions about your pregnancy and HIV-related health care and use of HIV drugs.
- Have your blood pressure, weight and height measured
- Have 6 tubes {SO mis) of blood drawn from your arm each time

One-week after delivery
One week after you give birth to your baby, you will come to the clinic for a visit that will include the following:
- Answer questions about your pregnancy and HIV-related health care and use of HIV drugs.
- Have your blood pressure, weight and height measured
- At this visit, we will ask you additional questions about your delivery, your baby's health, infant feeding and health care.
- Have 6 tubes {SO mis) of blood drawn from your arm.
- Have your baby's weight and height measured.

At the visits that are conducted *after your baby is born*, you will do the following:
- Answer questions about your recent health and HIV-related health care and use of HIV drugs.
- Answer questions about your baby's health, feeding practices and infant health care.
- Have your blood pressure, weight and height measured
- Have 6 tubes {SO mis) of blood drawn from your arm.
- Have your baby's weight and height measured

NOTE: The blood that is drawn today will be stored and used to check on your immune system (the part of your body that fights infections like HIV) at a later time. Results from these tests will not be available to you, the clinic, or the study

staff. If the health care providers at the clinic need to check your blood, they will take a separate blood specimen. When it is stored, your blood and test results will not have your name or any other way of identifying you attached to it.

Delivery specimens
As part of being in this study, we would like to take blood and pieces from your baby's placenta ('afterbirth'). Often the placenta is thrown away after delivery, because your baby does not need it anymore. We would like to take about 10 mls of blood (1 tube) of blood from the placenta, and then take some pieces of the placenta itself, because these can help us understand the health of babies while they are still in the uterus, before they are born. Taking these specimens does not hurt you or your baby in any way. If you would prefer that we do not take specimens from the placenta, this is OK, and you can still be in the study.

Please initial below to indicate whether or not you give permission for us to take specimens from the placenta after your baby is born. You may still remain in the study, no matter which you choose.

______ (initial) I agree to have specimens taken from the placenta as part of this research.

______ (initial) I do **NOT** agree to have specimens taken from the placenta as part of this research.

Review of medical records
As part of this study, we will also be looking at and taking information from your antenatal, obstetric, medical, and laboratory records. We will also be looking at and taking information from the health care records of your baby after she or he is born. From these records, we are interested in learning about the pregnancy care you received as well as information about your delivery and your baby's health. All data that we review and abstract is confidential and no participant names are recorded on study documents.

Follow-up of missed visits
You will be asked to provide contact information so that we may get in touch with you during the study. Study staff will talk with you about the best way to contact you. In the event that you miss one of the scheduled study visits, a member of the study staff will contact you in order to find another day and time to complete your visit. If you repeatedly miss study visits or the staff is unable to contact you using the information that you provide, it may be necessary to visit you at home in order to reschedule the missed study visit.

Contact for future study
After the completion of the visit one week after delivery, we will speak to you again to make sure you are happy to be involved in further research.

WHAT ARE THE POTENTIAL RISKS?
You may feel uncomfortable about some of the personal questions you are asked. You may refuse to answer any question that you do not want to answer. There is some risk in sharing personal and medical information. We will be careful to keep all your information as private as possible.

Drawing blood for the study is the same procedure that is normally done as part of routine medical care and presents a slight risk of discomfort. Experienced staff will draw blood under sterile conditions and will make every effort to protect you against these risks.

WHAT ARE THE POTENTIAL BENEFITS?
There is no direct benefit to you if you take part in this study. The information gained in this study may help to improve ART services for HIV-infected pregnant women and their babies in Cape Town, the Western Cape Province, and across South Africa.

WHAT ARE THE ALTERNATIVES TO TAKING PART?
The alternative to taking part in this study is to continue with your usual health care. If you decide not to participate in this study, your usual health care will not be changed in any way.

WHAT ABOUT CONFIDENTIALITY?
If you agree to take part, all information collected during the study will be kept strictly confidential. Your name will not be written on the study forms and will not be used in connection with any information or lab specimens that are collected as part of the study.

All study materials will be stored in locked filing cabinets. Only study staff and personnel involved in routine audits will have access to these materials. All staff involved in data collection and management will get specific training in confidentiality.

Even with these procedures in place if the study staff learns that you are at risk of hurting yourself or someone else or of possible child abuse and/or neglect, study staff will tell the proper authorities.

WILL I BE GIVEN ANYTHING FOR TAKING PART?
At the end of each visit, you will be given a R80 grocery voucher, R20 for transport and food and drink while you are at the visit.

ARE THERE ANY COSTS?
There is no cost for being in this study.

CAN I LEAVE THE STUDY?
You have the right to decide not to not take part in the study, to refuse to answer any questions, or to withdraw from the study at any time without any penalty. It will have no effect on the care that you receive at the Gugulethu MOU or any other health facility.

FUTURE USE OF SPECIMENS:
If you agree, some of the blood drawn at study visits or the placenta specimens (if we have your permission to collect these) may be used for future HIV-related research. At this time, we cannot provide details of when this testing may be conducted, or exactly what tests we would like to do. However, additional testing will not be done using these stored samples without the approval of the appropriate research ethics committees involved in this research.

If you agree to let us keep your stored samples for future research, they may be kept in a locked freezer for up to 5 years. If we do use your samples in the future, your name or other identifiers will not be included with this information (as with the rest of the information we collect for this study).

Please initial below to indicate whether or not you give permission for your specimens to be used for future research. You may still remain in the study, no matter which you choose.

______ (initial) I agree to have my samples (blood and/or placenta) stored for future research.

______ (initial) I do **NOT** agree to the storage of my samples (blood and/or placenta) stored for future research.

DO YOU HAVE ANY QUESTIONS?

If there is anything that is unclear or if you need further information, please ask us and we will provide it.

Do you have any questions?

FOR ADDITIONAL INFORMATION:

If you have any questions or have any problems while taking part in this research study, you should contact:

Prof Landon Myer
School of Public Health and Family Medicine
Faculty of Health Sciences
University of Cape Town
Tel: 021 406 6661
Email: Landon.Myer@uct.ac.za

Prof Marie Louise Newell
Academic Unit of Human Development and Health
Faculties of Medicine & Social and Human Sciences
University of Southampton, UK
Tel: +44 (023) 8059 3901
Email: m.newell@soton.ac.uk

If you have any questions about your rights as a research participant, you may contact the following member of the ethics committee:

Prof Marc Blockman
Chair, Human Research Ethics Committee
Faculty of Health Sciences, University of Cape Town
Tel: 021 406 6338

Dr Angela Fenwick, Chair
Southampton Faculty of Medicine Ethics Committee
Building 85, Life Sciences, Highfield Campus
Southampton SO17 1BJ, United Kingdom
Tel: +44 2381 208692

SIGNATURE PAGE

For participant to complete (please tick):

☐ I have read the information in this document (or it has been read to me). I have been offered a copy of this consent form. I was encouraged and given time to ask questions and all my questions about the study and my participation in it have been answered. I freely consent to be in this research study and agree to participate and know that I may withdraw at any time. My being in the study is voluntary. I understand that whether or not I participate will not affect my health care services received today, or at any time in the future.

☐ I agree that the study team can access my medical records at this hospital or another hospital if necessary for this study. My information will be kept confidential.

☐ I agree to provide contact information for myself which will be kept confidential by the study team.

☐ I agree to be called on my telephone during the course of the study

---

Participant Name (Please print) Participant Signature Date/Time

---

Interviewer Name (Please print) Interviewer Signature Date/Time

If this consent form is read to the participant because the participant is unable to read the form or if the participant must use a thumbprint to sign his/her name, an impartial witness not affiliated with the research or investigator must be present for the consent and sign the following statement:

I confirm that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the participant. The participant freely consented to be in the research study.

Witness Name (Please print) Witness Signature Date/Time

Thank you

4. SUPPLEMENTARY DATA

4A Tables and figures

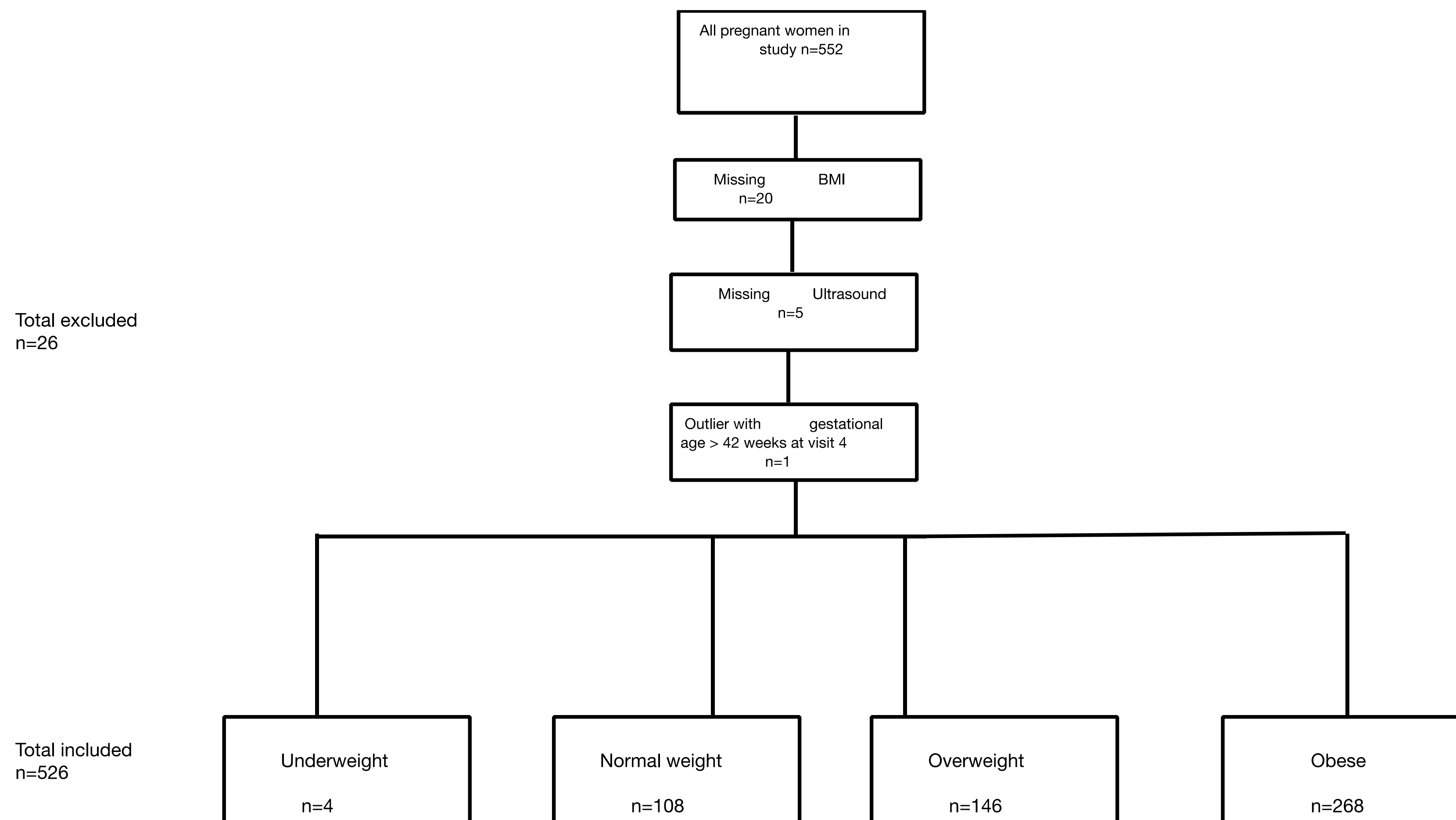

Figure 1. Handling of data

Table 1. Pairwise comparison using T-test with pooled standard deviation (SD) by BMI category

		BMI category	
Immune marker		Normal	Overweight
CRP (ng/mL)	Overweight	0.29	-
	Obese	**0.03**	**<0.01**
IP10 (pg/mL)	Overweight	0.65	-
	Obese	0.35	0.40
SAA (ng/mL)	Overweight	0.26	
	Obese	**<0.01**	0.04

Abbreviations: BMI, body mass index; CRP, C-reactive protein; SAA, Serum amyloid A;IP-10,interferon-gamma inducible protein-10; ng/mL, nanograms per millilitre; pg/mL, picograms per millilitre. P<0.05=statistically significant.

Table 2. Median (IQR) plasma concentration of immune markers by antiretroviral treatment (ART) initiation status

Immune marker by Visit	Total (N)	Initiated in current pregnancy (N)	Initiated preconception (N)	P
Visit 1	**526**	**278**	**248**	
Gestational age (GA), weeks Median (IQR)	14 (11 – 18)	14 (11 – 18)	14 (11 – 17)	0.29
CRP (ng/mL)	12412.78 (7547.28 – 15158.60)	11646.94 (7112.06 – 14991.56)	12841.04 (7716.59 – 15168.60)	0.18
IP-10 (pg/mL)	160.76 (146.02 – 259.35)	226.97 (147.72 – 353.29)	121.05 (86.72 – 171.33)	**<0.001**
SAA (ng/mL)	8.93 (4.79 – 16.33)	9.29 (4.84 – 17.38)	8.16 (4.67 – 15.37)	**0.04**
Visit 2*	**223**	**223**		
Gestational age (GA), weeks Median (IQR)	17.14 (14 – 21)	17.14 (14 – 21)		
CRP	12779.89 (8018.67 – 16586.46)	12779.89 (8018.67 – 16586.46)		
IP-10 (pg/mL)	197.84 (139.13 – 274.96)	197.84 (139.13 – 274.96)		
SAA (ng/mL)	8.78 (5.32 – 15.12)	8.78 (5.32 – 15.12)		
Visit 3	**440**	**232**	**208**	
Gestational age (GA), weeks Median (IQR)	27.14 (27.14 – 28.04)	27.14 (27.29 – 28.14)	27.57 (27.14 – 28)	**0.004**
CRP ng/mL)	12336.16 (8093.75 – 15302.67)	12252.19 (7983.61 – 15196.68)	12779.89 (8166.22 – 15277.87)	0.67
IP-10 (pg/mL)	148.65 (105.50 – 214.12)	165.23 (122.14 – 247.65)	135.56 (99.29 – 182.17)	**<0.001**
SAA (ng/mL)	10.01 (5.10 – 16.66)	10.76 (5.69 – 18.34)	9.23 (4.87 – 16.21)	0.27
Visit 4	**415**	**217**	**198**	
Gestational age (GA), weeks Median (IQR)	33.71 (33.14 – 34.14)	33.86 (33.29 – 34.14)	33.57 (33.14 – 34)	**0.003**
CRP (ng/mL)	12252.19 (7808.32 – 14278.48)	12090.30 (7477.11 – 14245.81)	12335.46 (8491.51 – 14703.41)	0.45
IP-10 (pg/mL)	164.51 (127.43 – 229.90)	179.88 (133.71 – 251.92)	150.61 (116.16 – 201.20)	**0.004**
SAA (ng/mL)	9.97 (5.78 – 16.70)	10.24 (6.08 – 17.89)	9.25 (5.36 – 15.66)	0.55

Abbreviations: CI, confidence interval; CRP, c-reactive protein; SAA, serum amyloid A; IP-10, interferon-gamma inducible protein-10.

*Visit 2 comprised of those who initiated ART in pregnancy; N, number.

P-value <0.05 -statistically significant.

Table 3. Associations between immune markers and covariates for the unadjusted mixed model

	CRP (ng/mL)			**IP-10 (pg/mL)**			**SAA (ng/mL)**		
Predictors	*β*	*CI*	*P*	*β*	*CI*	*P*	*β*	*CI*	
BMI category									
Normal (ref)	–	–	–	–	–	–	–	–	
Overweight	113.41	-725.63 – 952.46	0.79	-3.36	-23.28 – 16.55	0.74	-1.49	-4.35 – 1.36	0.31
Obese	1058.45	120.49 – 1996.40	**0.03**	-11.22	-32.16 – 9.72	0.29	-3.66	-6.39 – -0.92	**<0.01**

Abbreviations: BMI, body mass index; CRP, c-reactive protein; SAA, serum amyloid A; IP-10, interferon-gamma inducible protein-10; ng/mL, nanograms per millilitre; pg/mL, picograms per millilitre; CI, confidence interval, *β*, beta coefficient; ref, reference.
P-value < 0.05=statistically significant.

4B Immune marker assays

Quality control

Samples with confirmed detectable levels of all three cytokines were then chosen to be included as inter-plate controls (samples with large enough volume to be included on all plates run) and intra- plate controls (samples duplicated on the same plate) to respectively assess variation between runs and variation within run. Duplicated intra-plate controls were allocated to different columns on the 96-well plate to allow for an assessment of variation due to pipetting skills or machine dependent variations during the plate washing and reading. Coefficient of variation and Spearman rank correlation coefficients were generated for each of the cytokines to assess intra-plate variation.

Biological principle of assay

The assay used for determination is based on the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human CRP (R&D systems), SAA (Novus Biologicals, NBP2-68119), or IP-10 (R&D systems) had been pre-coated onto their specific microplate. Standards and samples are pipetted into the designated wells and any of the assayed cytokine present is bound by the immobilised antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for the cytokine is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of cytokine bound in the initial step. The colour development is stopped, and the intensity of the colour is measured. Sample assay for CRP required a 100-fold dilution. This was achieved by using 10µL of sample + 990µL of Calibrator Diluent RD5P (diluted 1:5).

4C Post-hoc analysis between BMI groups

The Shapiro-Wilks test showed that the continuous variables were not normally distributed, ($p<0.05$). Using the non-parametric Kruskal-Wallis test, the null hypothesis was rejected and at least one BMI category differs in CRP concentration ($p<0.001$). The Dunn post-hoc test revealed that for CRP, normal and obese, and obese and overweight BMI categories differ significantly ($p<0.001$). No statistically significant difference in CRP concentration was observed between normal and overweight BMI categories ($p>0.05$). The Dunn post-hoc test also revealed that for SAA, normal and obese, and obese and overweight BMI categories differ significantly ($p<0.001$). No statistically significant difference in SAA concentration was

observed between normal and overweight BMI categories ($p>0.05$). No statistically significant difference in IP-10 concentration was observed between BMI categories ($p>0.05$) in this cohort.

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- Huynh TV, Young RA, Davis RW. DNA Cloning. In: Glover DM. DNA Cloning - A Practical Approach. Vol 1. Oxford, UK: IRL Press; **1988**: 49-78.
- Public Health Service Task Force. Recommendations for the use of antiretroviral drugs in pregnant HIV-1 infected women for maternal health and interventions to reduce perinatal HIV-1 transmission in the United States. Available at: http://www.aidsinfo.nih.org. Accessed 24 April 2002.
- Lyon DJ, Cheng AFB, Norrby SR. Mechanisms of cefotaxime resistance in blood culture isolates of Enterobacter high prevalence of extended-spectrum β-lactamases [abstract C43]. In: Program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy (San Francisco). Washington, DC: American Society for Microbiology, 1995: 47.
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