



## ORIGINAL ARTICLE



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# Changes in the Vα7.2+ CD161++ MAIT cell compartment in early pregnancy are associated with preterm birth in HIV-positive women

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## Abstract

**Problem:** Human immunodeficiency virus (HIV) infection is associated with an increased risk of adverse pregnancy outcomes, including preterm birth (PTB), despite viral suppression with antiretroviral therapy. Mucosal-associated invariant T (MAIT) cells are an immune cell subset involved in antimicrobial immunity at mucosal surfaces. MAIT cells have been found at the maternal-foetal interface, and MAIT cells are typically depleted early in HIV infection. We aimed to investigate changes in MAIT cells in relation to maternal HIV/ART status and PTB.

**Method of Study:** We conducted flow cytometric analysis of peripheral blood samples from 47 HIV-positive (HIV+) and 45 HIV-negative (HIV-) pregnant women enrolled in a prospective pregnancy cohort study in Soweto, South Africa. Frequencies of Vα7.2+ CD161++ MAIT cells and proportions of CD4<sup>+</sup>, CD8<sup>+</sup> and double-negative MAIT cells were compared between women with and without HIV infection, and between women with and without PTB or spontaneous preterm labour (Sp-PTL).

**Results:** Although overall MAIT cell frequencies were the same between HIV+ and HIV- patients, HIV+ patients had a higher proportion of CD8<sup>+</sup> MAIT cells in the first two trimesters. Women with PTB and Sp-PTL also had a higher proportion of CD8<sup>+</sup> MAIT cells in the first trimester compared to women without these outcomes. The association between changes in MAIT cell subsets and PTB/Sp-PTL was present in both HIV+ and HIV- women, and an additive effect on MAIT cell subsets was seen in women with both HIV infection and PTB.

**Conclusions:** Interactions between HIV-related and pregnancy-related changes in MAIT cell subsets and distribution may lead to imbalances in peripheral MAIT cell subsets in early pregnancy. This may contribute to the increased risk of PTB in HIV+ patients by altering the overall functionality of the peripheral MAIT cell compartment.

## KEYWORDS

antiretroviral therapy, HIV, MAIT cells, mucosal-associated invariant T cells, preterm birth

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## 1 | INTRODUCTION

Evidence suggests that 5%-12% of all births worldwide occur prematurely.<sup>1</sup> Complications of preterm birth (PTB) are the primary cause of death among children under 5 years of age,<sup>2</sup> and lead to significant short-term and long-term morbidity.<sup>1</sup>

In 2014, UNAIDS reported that 91% of HIV-positive (HIV+) pregnant women resided in sub-Saharan Africa.<sup>3</sup> Antiretroviral therapy (ART)-naïve maternal HIV infection in this region is associated with an increased risk of PTB, small for gestational age newborns, low birth weight (LBW) and stillbirth.<sup>4</sup> Although ART improves maternal health and reduces the risk of mother-to-child transmission of HIV,<sup>5</sup> its use in pregnant women in low- and middle-income countries has been implicated in adverse pregnancy outcomes, including PTB.<sup>6</sup> Preconception ART initiation has been reported to be associated with worse perinatal outcomes, including PTB, than antenatal ART initiation.<sup>7</sup>

Both HIV infection and pregnancy induce significant immunological changes. HIV infection is characterized by chronic immune activation<sup>8</sup> and depletion of immune cell subsets<sup>9</sup>—most notably CD4<sup>+</sup> T cells—resulting in immunodeficiency. Placentation, pregnancy maintenance and the timely onset of labour depend on changes at the maternal-foetal interface involving various adaptive and innate immune cell subsets.<sup>10</sup>

Mucosal-associated invariant T (MAIT) cells are important for anti-bacterial immunity at mucosal interfaces.<sup>11</sup> They express a semi-invariant  $\alpha\beta$  T-cell receptor comprising an invariant TCR  $\alpha$  chain and a restricted range of V $\beta$  chains (V $\alpha$ 7.2J $\alpha$ 33 paired with V $\beta$ 2 or V $\beta$ 13).<sup>12</sup> They can be activated by vitamin B metabolites of bacteria and yeast presented on the MR1 protein,<sup>12</sup> or by innate cytokines.<sup>11</sup> MAIT cells display an intrinsic effector-memory phenotype and secrete pro-inflammatory cytokines and cytolytic products.<sup>11</sup>

Early MAIT cell depletion, irreversible with ART, occurs in HIV+ adults independently of markers of disease severity (eg CD4<sup>+</sup> T cell count or viral load).<sup>13-19</sup> Some suggest that MAIT cells are depleted due to excess activation and subsequent apoptosis<sup>13,15</sup>; alternative theories include the downregulation of the MAIT cell marker CD161<sup>15,17,20</sup> and PD-1-mediated inhibition of MAIT cell proliferation.<sup>17</sup> In HIV infection, MAIT cells have defective cytolytic function,<sup>21</sup> and reduced IFN- $\gamma$  and TNF- $\alpha$  expression in response to activating ligands.<sup>18</sup>

The role of MAIT cells in pregnancy is incompletely understood. They accumulate in the placental intervillous space in uncomplicated pregnancies<sup>22</sup> and are depleted in the peripheral circulation in early-onset pre-eclamptic patients compared with healthy controls.<sup>23</sup>

Given that maternal HIV infection and ART are associated with PTB, and MAIT cells are irreversibly depleted in adults with HIV infection, we investigated circulating MAIT cell frequencies in relation to maternal HIV/ART status and PTB.

## 2 | METHODS

### 2.1 | Patients

Blood samples were obtained from patients participating in a prospective cohort study at Chris Hani Baragwanath Academic Hospital, Soweto, South Africa.<sup>24</sup> Patient inclusion criteria were as follows: black South African, living in Soweto, aged 18 or above, spontaneous conception, singleton pregnancy. Exclusion criteria were as follows: multiple pregnancy, body mass index (BMI) >35 kg/m<sup>2</sup> or an intellectual or physical disability. All patients had a first trimester dating ultrasound scan. HIV testing was routinely offered to those not known to be HIV+. Patient data from medical records, antenatal cards and/or interviews were collected on around 200 items, including socioeconomic characteristics; and smoking, alcohol, drug, medical, gynaecological and obstetric history. HIV disease stage, ART initiation timing, use of ART and ART regimens were documented (with consent) for HIV+ women. Perinatal outcomes were recorded at delivery, with birthweight accurately measured by dedicated research midwives within 12 hours of birth.

### 2.2 | Exposure definitions

HIV+ women were categorized as preconception ART initiators if they had started ART before the date of their last menstrual period (LMP) and as antenatal ART initiators if they had started ART after the LMP date. Two patients in the antenatal ART initiation group had samples collected in the first trimester before the ART initiation date, the remaining patients after the ART initiation date.

### 2.3 | Outcome definitions

PTB was defined as any birth before 37 weeks' gestation, spontaneous preterm labour (Sp-PTL) as spontaneous onset of labour before 37 weeks' gestation, LBW newborns as newborns weighing under 2500g and small for gestational age (SGA) newborns as newborns under the 10th centile of the INTERGROWTH-21st Newborn Standard birth-weight-for-gestational-age/sex.<sup>25</sup>

### 2.4 | Sample collection and processing

Between 27 November 2013 and 20 October 2015, trained study nurses collected peripheral blood samples in each trimester from HIV+ and HIV- patients, and at delivery and 6 weeks postnatally from a subset of HIV+ patients. For each patient, samples were not necessarily available for all time points. Peripheral blood mononuclear cells (PBMCs) were isolated using standard density gradient centrifugation, frozen in a solution of 50% (v/v) FCS, 10% (v/v) DMSO and R10 media, and stored at -80°C. Samples were then shipped to Oxford on dry ice, where they were stored in liquid nitrogen.

## 2.5 | Flow cytometry

South African patient samples were chosen for analysis based on the pregnancy outcome, rather than to be representative of HIV+ or HIV- pregnant women. Healthy HIV-negative (HIV-) non-pregnant control PBMCs were isolated from leukocyte cones (NHS Blood Services, UK) and used to optimize flow staining; these samples are anonymized, so data on age and gender are not available. Frozen PBMCs were thawed in a water bath at 37°C. Each vial of thawed cells ( $\sim 2.5 \times 10^6$  cells) was added to 50  $\mu$ L of DNase I solution and suspended in warm R10 medium (37°C). To identify live cells, cells were stained with the Zombie Aqua Fixable Viability Kit (BioLegend) according to the manufacturer's protocol. Cells were then stained with the following fluorescent antibodies in the dark for 15 minutes: anti-CD3 (REA613), CD4 (OKT4), CD8 $\alpha$  (RPA-T8), TCR V $\alpha$ 7.2 (3C10) and CD161 (HP-3G10) (anti-CD3 antibodies from Miltenyi Biotec; other antibodies from BioLegend). MR1 5-OP-RU tetramers were obtained from the NIH Tetramer Core Facility at Emory University, Atlanta, GA, USA. After antibody staining, cells were washed in ice-cold staining buffer (PBS with 10% FBS) and fixed in 200  $\mu$ L of 2% paraformaldehyde. Fixed cells were resuspended in 300  $\mu$ L ice-cold staining buffer prior to processing in a LSR II flow cytometer (Becton Dickinson). Flow cytometric analysis, including compensation for spectral overlap, was done using FlowJo V10 software (FlowJo LLC).

## 2.6 | Statistical analysis

Patient characteristics at baseline were analysed for normality and compared using the Mann-Whitney U test or unpaired t test for continuous variables, and Fisher's exact test or chi-squared test for categorical variables. Global changes during pregnancy were assessed by comparing values at each trimester with those at every other trimester using the Kruskal-Wallis test. Mann-Whitney U tests were used to compare median cell frequencies/proportions between two groups. For paired samples, a Friedman test followed by a Dunn's multiple comparisons test was used.

## 2.7 | Ethical approval

Written informed consent was obtained from all study participants upon enrolment. Ethical approval was obtained from the University of Oxford Tropical Research Ethics Committee (OxTREC Number 1008-13) and the Human Research Ethics Committee (Medical) of the University of Witwatersrand, Johannesburg, South Africa (Numbers M130134 and M180791).

## 3 | RESULTS

### 3.1 | Patient characteristics

Characteristics of HIV+ and HIV- patients were largely comparable (Table 1). Maternal age was higher among HIV+ women

compared to HIV- women. Maternal age was not associated with overall MAIT cell levels, nor with frequencies of MAIT cell subsets in trimester 1, 2 or 3 except for a weak positive correlation seen between maternal age and CD8 $^+$  MAIT cell proportions in trimester 1 (Spearman coefficient = 0.295,  $P = .037$ ). However, when analysing HIV+ and HIV- patients separately, this correlation was not seen (data not shown). There was a significant difference in maternal education, but, importantly, there were no significant differences in parity, obstetric history, pre-pregnancy BMI, smoking status or alcohol intake.

### 3.2 | MAIT cell frequencies remain stable throughout pregnancy

In accordance with previous literature, we defined blood MAIT cells as T cells co-expressing the V $\alpha$ 7.2 TCR $\alpha$  chain and high levels of CD161<sup>26,27</sup>; the majority of V $\alpha$ 7.2+CD161++ cells stained with an MR1 tetramer loaded with the 5-OP-RU ligand (Figure 1A). Overall, for all patients, the median MAIT cell frequency is between 0.73% and 1.06% of CD3 $^+$  lymphocytes during pregnancy. There was no significant change in MAIT cell frequencies during pregnancy in all women (Figure 1B), nor within HIV+ (Figure 1C) or HIV- (Figure 1D) subgroups. In HIV+ women, for some of whom we also took samples at delivery and 6 weeks postnatally, there was no change in MAIT cell frequencies at these later time points (Figure 1C). No changes in MAIT cell frequencies were seen in the paired analysis including only patients who had samples for all time points (Figure S1). For comparison, levels of CD3 $^+$  T cells also remained stable throughout pregnancy in all women and in the postnatal period in HIV+ patients (Figure S2).

### 3.3 | Overall proportions of MAIT cell subsets remain stable throughout pregnancy, but show notable inter-patient variation within trimesters

There was remarkable variation in the proportions of MAIT cell subsets within each trimester (Figure 1). CD8 $^+$  MAIT cells form the highest proportion of MAIT cells during pregnancy, delivery and the early postnatal period, accounting for a median of between 57.0% and 79.3% of MAIT cells (range: 21.6%-92%) (Figure 1H-J). CD4- CD8- double-negative (DN) MAIT cells are the second largest MAIT cell subset, constituting a median of 15.9%-31.2% of MAIT cells (range: 3.6%-69.5%) (Figure 1K-M). Finally, CD4 $^+$  MAIT cells constitute a median of between 0.62% and 6.94% of the MAIT cell compartment (range: 0%-50.1%) (Figure 1E-G).

No significant changes in the proportions of MAIT cell subsets during pregnancy, at delivery or postnatally were found overall or in HIV+ women. However, in HIV- women, the proportion of CD8 $^+$  MAITs increased during pregnancy and was significantly higher in trimester 3 than in trimester 1 ( $P < .05$ , Figure 1J), with a reciprocal decrease in the proportion of DN MAITs ( $P < .01$ , Figure 1M);

**TABLE 1** Characteristics of HIV-positive (HIV+) and HIV-negative (HIV-) patients

	HIV+ patients	HIV- patients	Statistical comparison (P)
Number of patients	47	45	
Maternal age (median [IQR])	33 [28-37]	29 [26-33]	.036
Pre-pregnancy BMI (mean [SD])	27.7 [4.3]	26.2 [3.5]	.084
Number of previous pregnancies (median [IQR])	2 [1-3]	2 [1-3]	.902
History of adverse pregnancy outcomes (number [%])			
History of low birth weight (LBW)	4 [9]	11 [24]	.105
History of preterm birth (PTB)	10 [21]	11 [24]	.922
History of stillbirth (SB)	2 [4]	4 [9]	.655
History of neonatal death (NND)	2 [4]	1 [2]	.859
History of miscarriage (MC)	21 [45]	18 [40]	.355
At least 1 APO (LBW, PTB, SB, NND, MC)	26 [55]	28 [62]	.402
Unknown	7 [15]	7 [16]	
Smoking during pregnancy (number [%])			
Yes	5 [11]	2 [4]	.435
No	42 [89]	43 [96]	
Alcohol intake during pregnancy (number [%])			
Yes	8 [17]	4 [9]	.355
No	39 [83]	41 [91]	
Number of years of education (median [IQR])	12 [11-12]	12 [12-12]	.0056
ART initiation category (number [%])			
Preconception	17 [36]	N/A	
Antenatal	19 [40]	N/A	
Unknown	11 [23]	N/A	
Number of samples			
Trimester 1	25	25	
Trimester 2	37	32	
Trimester 3	14	16	
Weeks + days of gestation at sample collection (median [range])			
Trimester 1	12 + 4 [8 + 0 – 13 + 6]	12 + 0 [8 + 0 – 14 + 2]	.573
Trimester 2	26 + 2 [23 + 4 – 29 + 0]	26 + 0 [20 + 6 – 27 + 6]	.327
Trimester 3	35 + 3 [31 + 1 – 37 + 4]	35 + 4 [30 + 2 – 39 + 0]	.997
Pregnancy outcomes (number [%])			
Term birth (TB)	24 [51]	20 [44]	.525
Preterm birth (PTB)	23 [49]	25 [56]	
Spontaneous term labour (Sp-TL)	11 [23]	11 [24]	.649
Induced term labour	3 [6]	1 [2]	
Elective term Caesarean section	10 [21]	8 [18]	
Spontaneous preterm labour (Sp-PTL)	13 [28]	12 [27]	.711

(Continues)

**TABLE 1** (Continued)

	HIV+ patients	HIV- patients	Statistical comparison (P)
Induced preterm labour	2 [4]	4 [9]	
Elective preterm Caesarean section	8 [17]	9 [20]	
Small for gestational age (SGA)	19 [40]	11 [24]	.221

the proportion of CD4<sup>+</sup> MAITs in HIV- women showed a decreasing trend (Figure 1G). Paired analysis including only patients with samples for all time points showed a similar pattern, although among HIV- women the increase in the proportion of CD8<sup>+</sup> MAITs was not significant, whereas the decreases in the proportions of CD4<sup>+</sup> and DN MAITs were significant (Figure S1). There were no changes in frequencies of CD4<sup>+</sup>, CD8<sup>+</sup> or DN CD3<sup>+</sup> T cells during pregnancy among all women and the HIV+ and HIV- subgroups (Figure S2).

### 3.4 | HIV-positive women have a higher proportion of CD8<sup>+</sup> MAIT cells in early pregnancy compared to HIV-negative women

There are no significant differences in overall MAIT cell frequencies between HIV+ and HIV- women in trimester 1, 2 or 3 (Figure 2A). However, HIV+ women have a higher proportion of CD8<sup>+</sup> and lower proportion of CD4<sup>+</sup> MAIT cells in trimesters 1 and 2 than HIV- women (Figure 2B,C). The proportion of DN MAIT cells is also lower in HIV+ women than HIV- women in trimester 1 (Figure 2D). Overall, there is a shift towards CD8<sup>+</sup> MAIT cells in early pregnancy in HIV infection.

### 3.5 | Timing of antiretroviral therapy initiation in HIV+ women affects MAIT cell subset proportions in early pregnancy compared to HIV- women

During pregnancy, there are no differences in overall MAIT cell frequencies or in the proportions of individual MAIT cell subsets between HIV+ women who initiated ART preconception or antenatally (Figure 3A). However, in trimester 1, HIV+ women on preconception ART have a higher CD8<sup>+</sup> and lower CD4<sup>+</sup> MAIT cell proportion compared to HIV- women, but antenatal ART initiators have similar CD8<sup>+</sup> and CD4<sup>+</sup> MAIT cell proportions to HIV- women (Figure 3B,C). In trimester 2, both antenatal and preconception ART initiators have a higher CD8<sup>+</sup> and lower CD4<sup>+</sup> MAIT cell proportion than HIV- women.

### 3.6 | Women with preterm birth have a higher proportion of CD8<sup>+</sup> MAIT cells in early pregnancy than women with term birth

There are no differences in total MAIT cell frequencies between women with PTB and term birth (TB) in trimester 1, 2 or 3 overall, or within HIV+

and HIV- subgroups (Figure 4A,E,I). However, in trimester 1, there is a higher proportion of CD8<sup>+</sup> MAIT cells in PTB than in TB women overall and within HIV+ and HIV- subgroups (Figure 4C,G,K), and a lower proportion of CD4<sup>+</sup> MAIT cells in PTB women overall (Figure 4B). Therefore, within HIV+ women, who already have higher CD8<sup>+</sup> MAIT proportions in early pregnancy compared to HIV- women, there is a shift towards even higher CD8<sup>+</sup> MAIT cell proportions in those with PTB.

### 3.7 | Women with spontaneous preterm labour have a higher proportion of CD8<sup>+</sup> MAIT cells compared to spontaneous term labour (Sp-TL)

As Sp-PTL leads to the majority of PTBs,<sup>28,29</sup> we analysed differences between women with Sp-PTL and those with spontaneous term labour (Sp-TL). As seen with PTB, those with Sp-PTL had a higher proportion of CD8<sup>+</sup> MAIT cells in the first trimester overall and within the HIV- subgroup (Figure 5C,K); conversely, the CD4<sup>+</sup> MAIT cell proportion was lower in those with Sp-PTL than in those with Sp-TL overall and among HIV- women (Figure 5B,J).

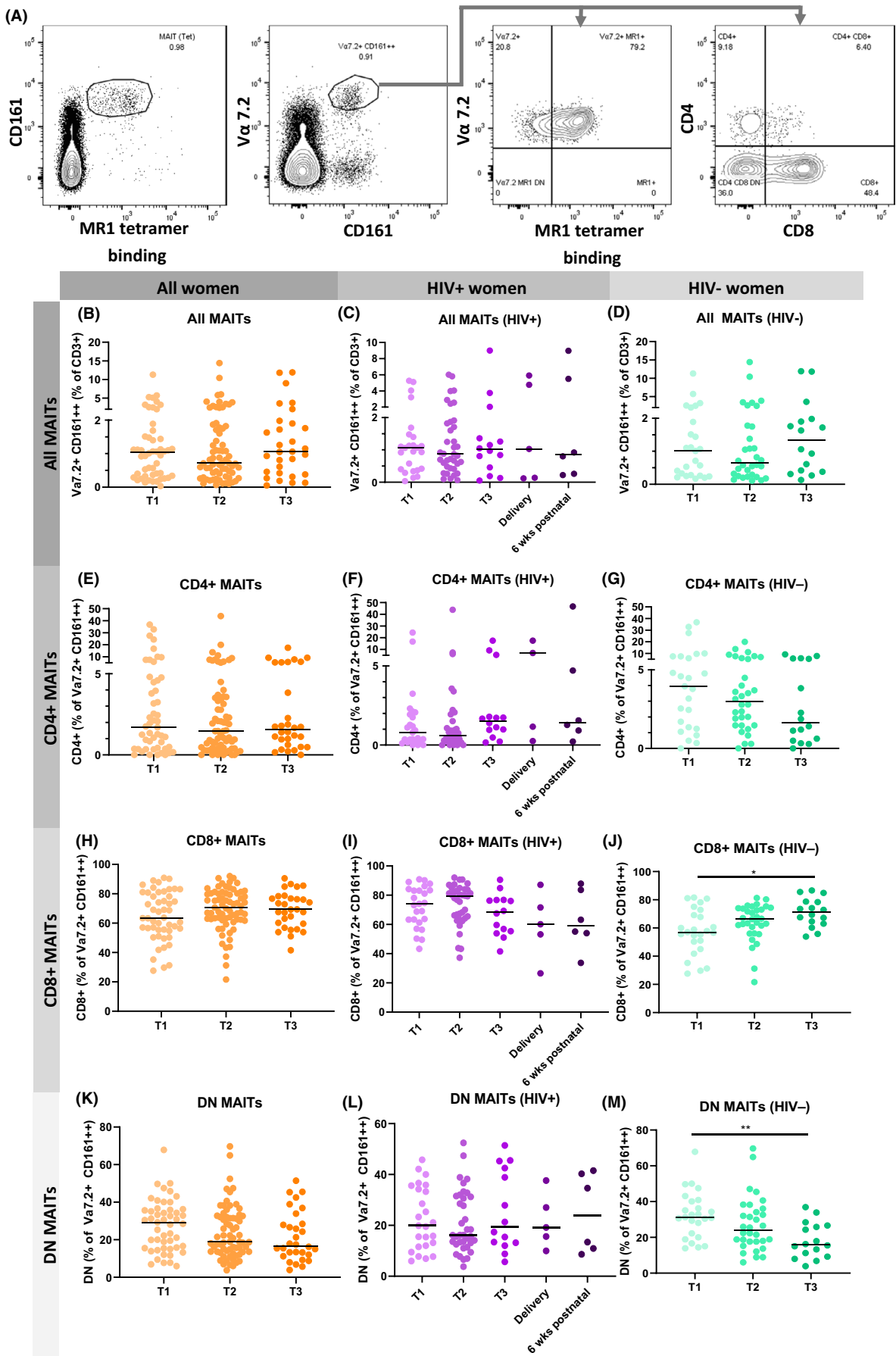
### 3.8 | The MAIT cell compartment characteristics of women with preterm birth (PTB) and spontaneous preterm labour are specific and do not extend to other adverse pregnancy outcomes

To investigate whether the differences in MAIT cell subsets between those with and without PTB were specific, we analysed MAIT cells in women with small for gestational age (SGA) infants and women with appropriate for or large for gestational age infants (AGA/LGA).

There were no differences in overall MAIT cell frequencies, nor in the proportions of MAIT cell subsets, between SGA and AGA/LGA women in any trimester (Figure S3A,D,G,J,M). This was also the case within HIV+ and HIV- subgroups (Figure S3B,C,E,F,H,I,K,L,N,O).

## 4 | DISCUSSION

In early pregnancy, the CD8<sup>+</sup> MAIT cell proportion is higher (and the CD4<sup>+</sup> MAIT cell proportion lower) in HIV+ compared to HIV- women, in those with PTB compared to those with term birth and in those with Sp-PTL compared to those with Sp-TL. There is an additive effect on MAIT cell subsets in women with both HIV infection





**FIGURE 1** MAIT cells throughout pregnancy. (A) Gating strategy identifying MR1 tetramer-binding CD161<sup>++</sup> Vα 7.2<sup>+</sup> MAIT cells among live CD3<sup>+</sup> lymphocytes. CD4<sup>+</sup>, CD8<sup>+</sup> and CD4<sup>+</sup> CD8<sup>+</sup> double-negative (DN) subsets are shown. The sample used for this staining was from a healthy HIV-negative non-pregnant control. (B-D) MAIT cell frequencies as percentages of CD3<sup>+</sup> lymphocytes in trimester 1 (T1), trimester 2 (T2) and trimester 3 (T3) of pregnancy in all women (B) and HIV-negative (HIV-) women (D); MAIT cell frequencies as percentages of CD3<sup>+</sup> lymphocytes in T1, T2, T3, at delivery and at 6 weeks postnatal in HIV-positive (HIV+) women (C). (E-M) MAIT cell subsets throughout pregnancy as percentages of total MAIT cells in all women (E) (H) (K), in HIV+ women (F)(I)(L), and HIV- women (G)(J)(M). Horizontal bars among data points on graphs indicate median values. A Kruskal-Wallis test followed by a Dunn's multiple comparisons test was used to compare values at each time point with values at every other time point. Results of statistical tests are indicated by horizontal bars above the graphs. \**P* < .05, \*\**P* < .01

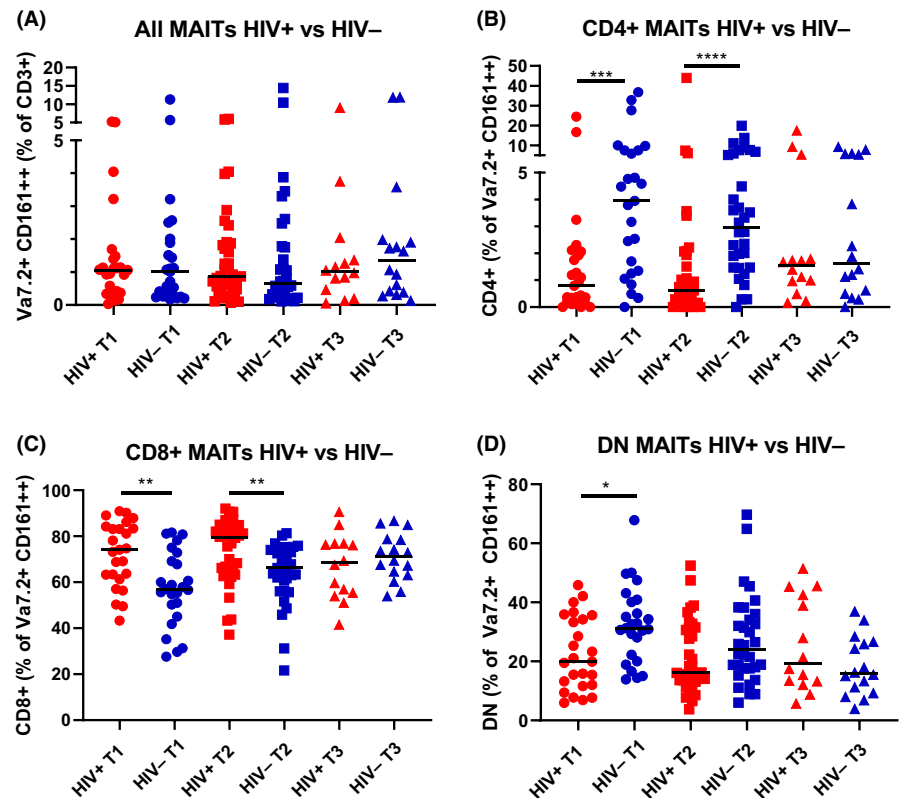
and PTB. No differences were seen between SGA and non-SGA pregnancies. HIV infection is therefore associated with changes in the MAIT cell compartment which are also present in women with PTB and Sp-PTL, but not in women with SGA. Therefore, changes in the MAIT cell compartment may provide a mechanistic link between maternal HIV infection and PTB.

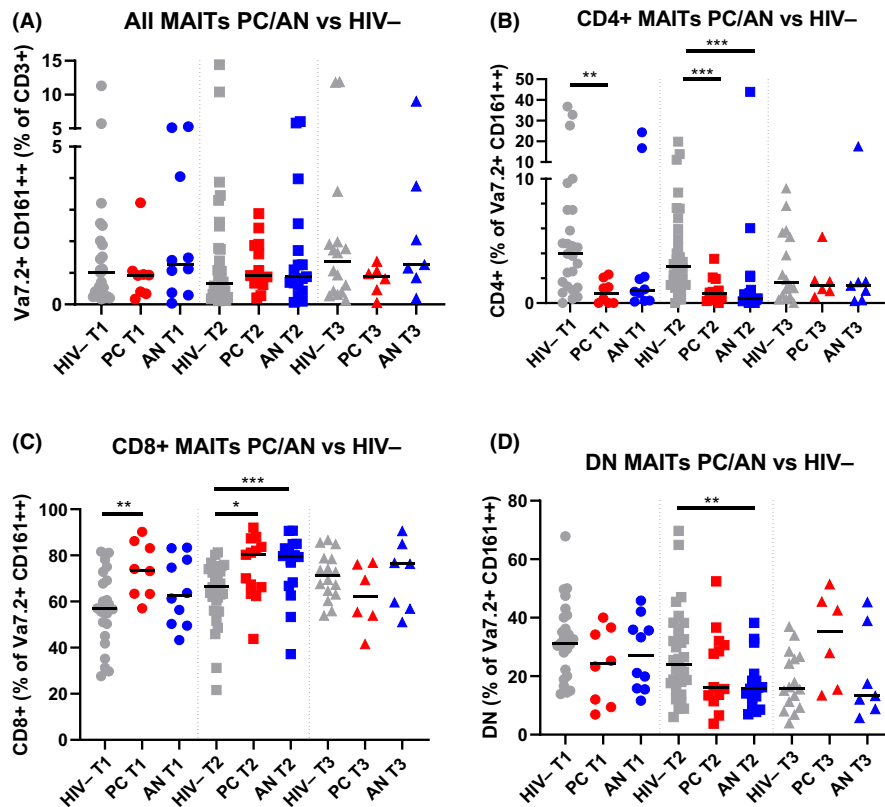
We demonstrate no difference in overall MAIT cell frequencies in peripheral blood between HIV+ and HIV- patients during pregnancy, in contrast to previous findings in non-pregnant populations.<sup>13-19</sup> Given that peripheral MAIT cell frequencies in our study (median between 0.73% and 1.06% of CD3<sup>+</sup> lymphocytes) are lower than those reported for non-pregnant populations, it is possible that reductions in overall MAIT cell levels during pregnancy have resulted in an equalization of MAIT levels between HIV+ and HIV- pregnant women. Differences in MAIT cell frequencies between HIV+ and HIV- women may still be present in other body compartments on account of MAIT cell redistribution in pregnancy, such as recruitment to intervillous blood spaces by placenta-derived chemokines. Furthermore, a notable number of patients have a higher proportion

of CD4<sup>+</sup> MAIT cells (up to 50.1%) and a lower proportion of CD8<sup>+</sup> MAIT cells (as low as 21.6%) than previously described in healthy subjects<sup>26,27,30</sup> and in those with chronic inflammation or HIV infection.<sup>18,31</sup> These differences may be due to pregnancy-specific changes in MAIT cells. MAIT cell frequencies in HIV+ patients remained unchanged until 6 weeks postnatally, although we had very few samples at this time point and it may be too early for pregnancy-specific changes to reverse. We did not have postnatal samples for HIV- patients, or any preconception samples. However, patients with autoimmune diseases commonly experience flares in the later postnatal period, implying that systemic immune changes occur later postnatally.<sup>32</sup> Levels of some cytokines may take up to 3–4 months after delivery to normalize,<sup>33</sup> whereas certain immune cell types appear to normalize within 6 weeks.<sup>34</sup> The importance of early exposure to microbiota for MAIT cell development has been recently shown<sup>35</sup> and may explain some of the variation in MAIT cell frequencies in our study population.

Downregulation of the MAIT cell marker CD161 may be a potential mechanism for the reduction in MAIT cell frequencies in HIV

**FIGURE 2** MAIT cells in HIV-positive (HIV+) vs HIV-negative (HIV-) women. T1 = trimester 1, T2 = Trimester 2, T3 = Trimester 3. (A) Total MAIT cell frequencies as percentages of CD3<sup>+</sup> lymphocytes. (B-D) CD4<sup>+</sup>, CD8<sup>+</sup> and CD4-CD8- double-negative (DN) MAIT cell subsets as percentages of total MAIT cells. Horizontal bars among data points on graphs indicate median values. Values for HIV+ and HIV- women were compared within each trimester using a Mann-Whitney *U* test. Results of statistical tests are indicated by horizontal bars above the graphs. \**P* < .05, \*\**P* < .01, \*\*\**P* < .001, \*\*\*\**P* < .0001





**FIGURE 3** MAIT cells in HIV-positive (HIV+) women according to timing of initiation of antiretroviral therapy (ART) and HIV-negative (HIV-) women. AN: antenatal ART initiation. PC: preconception ART initiation. T1 = trimester 1, T2 = trimester 2, T3 = trimester 3. (A) MAIT cells as a percentage of CD3<sup>+</sup> lymphocytes. CD4<sup>+</sup> (B), CD8<sup>+</sup> (C) and CD4<sup>-</sup> CD8<sup>-</sup> double-negative (DN) (D) MAIT cells as percentages of total MAIT cells. Horizontal bars among data points on graphs indicate median values. Values were compared within each trimester between AN or PC HIV+ women and HIV- women, and between AN and PC HIV+ women using Mann-Whitney U tests. Results of statistical tests are indicated by horizontal bars above the graphs. \* P < .05, \*\* P < .01, \*\*\* P < .001

infection.<sup>15,17,20</sup> This change may not occur or may be reversed in pregnancy, resulting in equivalent MAIT cell frequencies in HIV+ and HIV- pregnant women. Lectin-like transcript-1 (LLT1), the ligand for CD161,<sup>36</sup> is expressed by human trophoblast cells in the placenta.<sup>37</sup> LLT1 downregulates CD161 expression and inhibits the cytotoxicity and IFN- $\gamma$  secretion of uterine NK cells.<sup>36</sup> MAIT cells may be maintaining CD161 expression during pregnancy to allow their cytotoxic, pro-inflammatory phenotype to be moderated at the maternal-foetal interface by the CD161-LLT1 interaction. Alternatively, placental LLT1 may cause CD161 downregulation in HIV- women in pregnancy, leading to measurement of MAIT levels equivalent to HIV+ women.

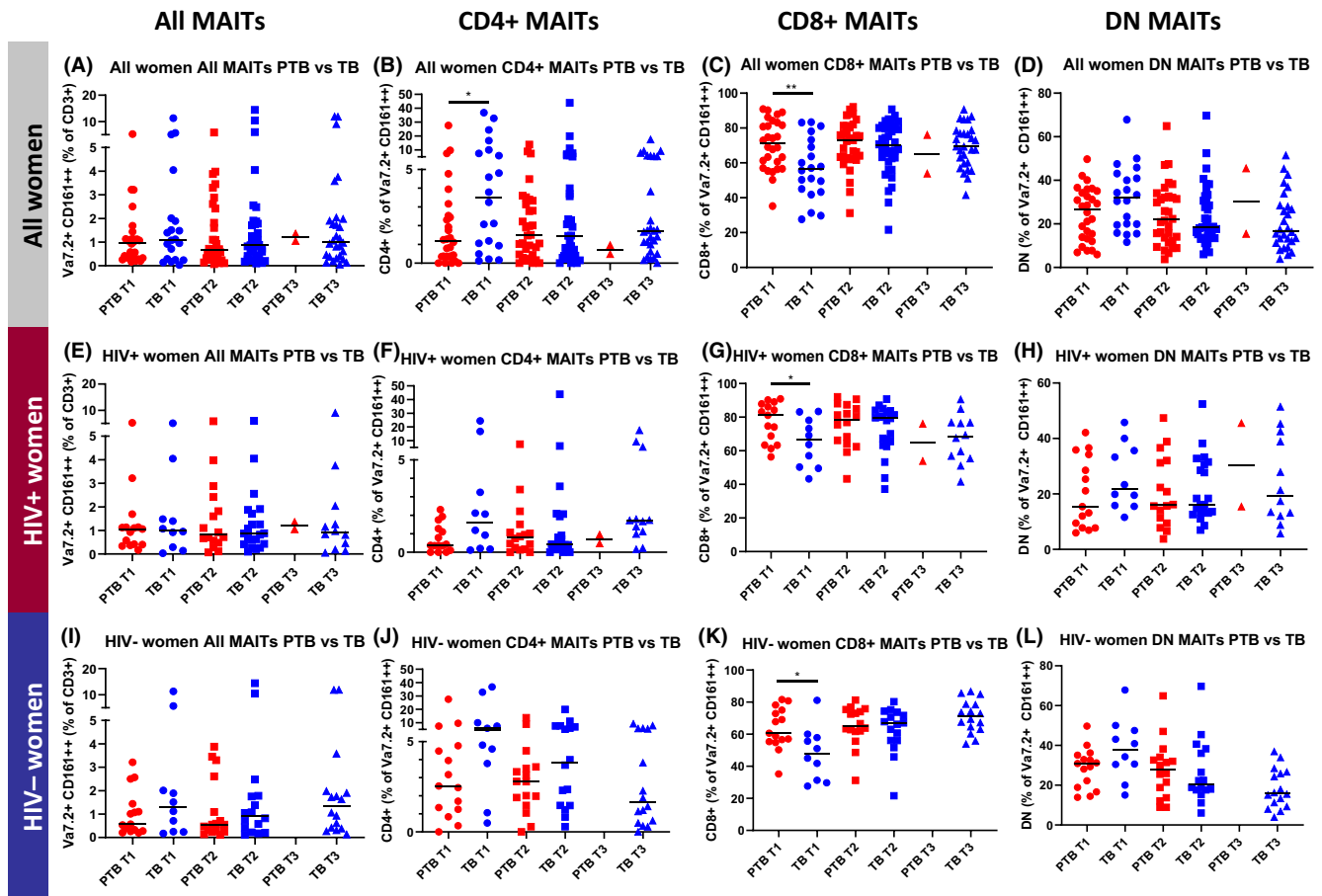
Changes in proportions of immune cell subtypes can have dramatic consequences. In HIV infection, the ratio of CD4<sup>+</sup> to CD8<sup>+</sup> T cells has been used as a marker of poor outcomes, including lack of CD4<sup>+</sup> T-cell recovery with ART,<sup>38</sup> neurocognitive disorders,<sup>39</sup> lung cancer,<sup>39</sup> chronic pulmonary conditions<sup>39</sup> and suboptimal responses to hepatitis B and yellow fever vaccination.<sup>39</sup> HIV+ patients whose CD4:CD8 T-cell ratios remain abnormally low following ART have increased morbidity and mortality.<sup>40,41</sup> Even in HIV- individuals, an inverted CD4:CD8 T-cell ratio is associated with ageing, and increased mortality above age 60.<sup>42</sup>

Considering CD8<sup>+</sup>, DN and CD4<sup>+</sup> MAIT cell subsets have distinct phenotypic features,<sup>27,43</sup> altered CD8:DN:CD4 MAIT cell ratios may alter the functionality of the MAIT cell compartment (Figure S4). This analysis has been hampered by the very low frequencies of CD4<sup>+</sup> MAIT cells typically found in blood, which means that even low levels of contamination of the relevant FACS gate

can impact on the apparent cellular phenotype. Studies with tetramers have provided some clarity here, although the differences between the subsets have not yet been fully clarified at a fundamental level (eg through transcriptional studies). CD4<sup>+</sup> Va7.2+ CD161<sup>++</sup> cells have been described to have lower expression of the transcription factor Eomes compared to bona fide CD8<sup>+</sup> MAIT cells, suggesting that they may have low cytotoxicity,<sup>27</sup> although this needs further verification. The differences between CD8<sup>+</sup> and DN MAIT cells appear more subtle: Dias et al show CD8<sup>+</sup> MAIT cells have greater Eomes and T-bet expression than DN MAIT cells,<sup>44</sup> whereas Kurioka et al show that expression of these transcription factors is comparable between the two subsets.<sup>27</sup> Functionally, Kurioka et al show no difference in the frequency of CD8<sup>+</sup> or DN MAIT cells expressing IFN- $\gamma$  in response to *E coli* stimulation, but Dias et al report that CD8<sup>+</sup> MAIT cells express more IFN- $\gamma$ . With regard to cytolytic potential, although both studies show similar levels of Granzyme A expression, Dias et al show greater expression of Granzyme B and perforin in CD8<sup>+</sup> MAIT cells, whereas Kurioka et al show similar expression of Granzyme B, K and perforin in CD8<sup>+</sup> and DN MAIT cells.<sup>27,44</sup>

The imbalance of cytotoxic CD8<sup>+</sup> MAIT cells to DN and CD4<sup>+</sup> MAIT cells with reduced cytotoxicity in early pregnancy may be contributing to increased risk of Sp-PTL in HIV infection. In humans, some studies show an association of maternal serum levels of IFN- $\gamma$  and TNF- $\alpha$  with PTB,<sup>45-47</sup> whereas others demonstrate no significant associations.<sup>48</sup> IFN- $\gamma$  and TNF- $\alpha$  have also been associated with embryo toxicity, pregnancy loss and PTB in non-human primates<sup>49</sup> and mouse models.<sup>50-53</sup>





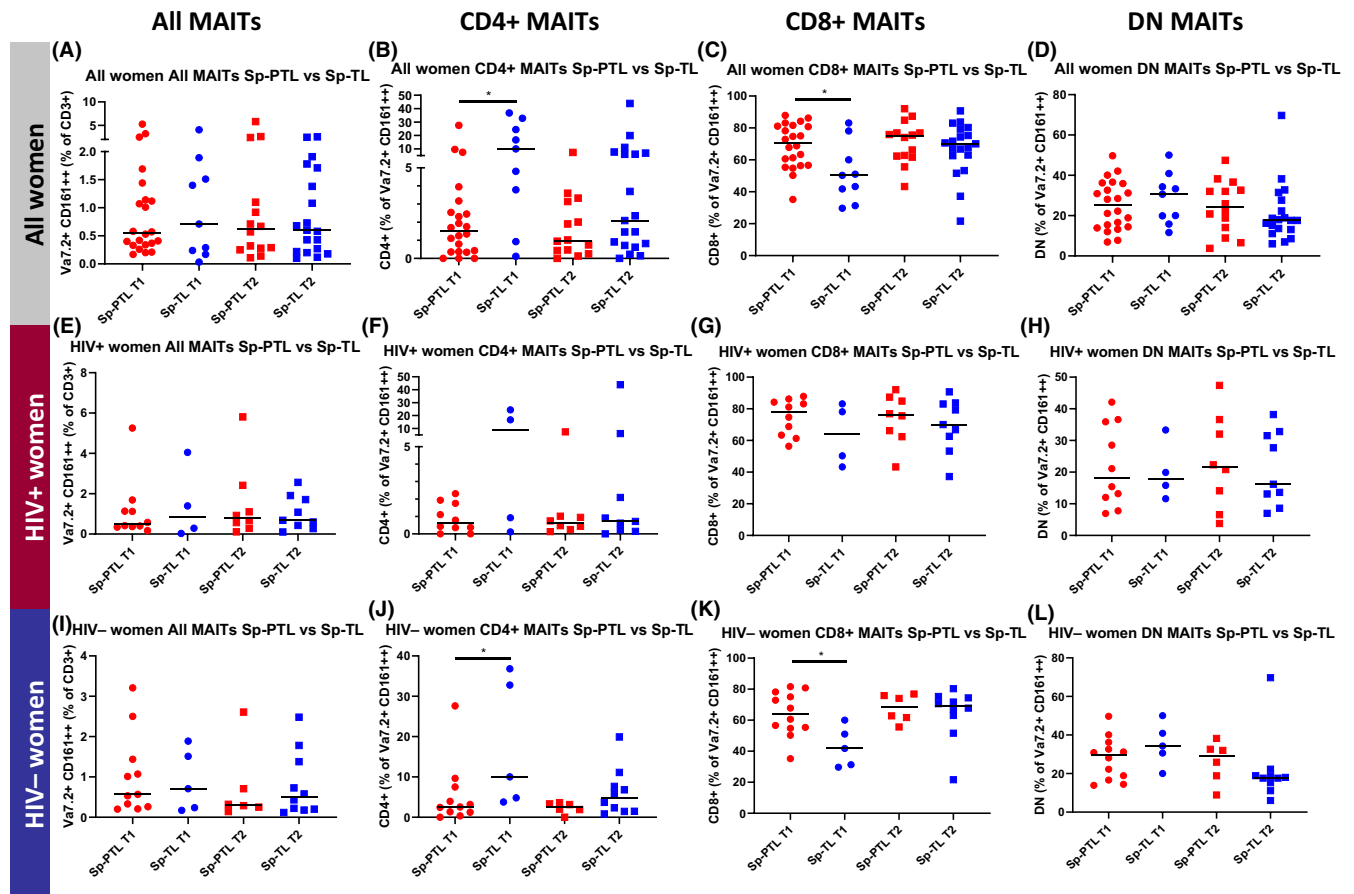
**FIGURE 4** MAIT cells in women with pregnancies resulting in preterm birth (PTB) and term birth (TB). T1 = trimester 1, T2 = trimester 2, T3 = trimester 3. (A) (E) (I) Total MAIT cell frequencies as percentages of CD3<sup>+</sup> lymphocytes. CD4<sup>+</sup> (B) (F) (J), CD8<sup>+</sup> (C) (G) (K) and CD4-CD8-double-negative (DN) (D) (H) (L) MAIT cell subsets as percentages of total MAIT cells. Horizontal bars among data points on graphs indicate median values. Values for PTB and TB women were compared within each trimester using a Mann-Whitney U test. Results of statistical tests are indicated by horizontal bars above the graphs. \*  $P < .05$ , \*\*  $P < .01$

As changes in the MAIT cell compartment in trimester 1 have been most consistently associated with HIV infection, PTB and Sp-PTL, it is notable that HIV+ women who started ART preconception, but not those that started ART antenatally, had a higher CD8<sup>+</sup> MAIT cell level than HIV- women in the first trimester. This correlates with the finding from a systematic review and meta-analysis that preconception ART initiation is associated with a higher risk of preterm and very preterm delivery compared to antenatal ART initiation; this association was greater in studies done in low- and middle-income countries.<sup>7</sup> It may be that preconception ART initiators have lived with HIV infection for longer and have more deranged MAIT cell immunology. Alternatively, pre-existing immune reconstitution due to ART in combination with the immunological changes during pregnancy in HIV+ women may have altered the MAIT cell compartment. Studies assessing MAIT cell function following ART focus on in-vitro cytokine production<sup>13-15</sup> and suggest a partial restoration of function, but the effect of ART on overall MAIT cell antimicrobial activity has not been evaluated.

Our study does not investigate MAIT cells at the maternal-foetal interface. MAIT cells from the female genital tract appear to be phenotypically different, with a highly cytotoxic phenotype,<sup>54</sup> producing more IL-17 and IL-22 and less IFN- $\gamma$  and TNF- $\alpha$  than

circulating MAIT cells.<sup>55</sup> Considering intrauterine infection has been implicated in a large proportion of PTB cases,<sup>1</sup> and that maternal immune cells are recruited from intervillous blood in intrauterine infection,<sup>56</sup> deranged placental MAIT cell immunology may be significantly contributing to an increased risk of PTB in HIV+ women. Further exploration of the relationship between circulating MAIT cells and MAIT cells at the maternal-foetal interface in HIV+ women is warranted.

In summary, we found that pregnant women have lower proportions of CD8<sup>+</sup> MAIT cells and relatively higher proportions of CD4<sup>+</sup> MAIT cells than previously described in healthy non-pregnant subjects, which may indicate a pregnancy adaptation. We found no difference in overall MAIT cell frequencies between HIV+ and HIV- women during pregnancy, but HIV+ women have a higher CD8<sup>+</sup> MAIT cell proportion in early pregnancy compared to HIV- women. Moreover, PTB is also associated with a higher CD8<sup>+</sup> MAIT cell proportion in early pregnancy. Together, these data suggest that changes in MAIT cell subsets may provide a mechanistic link between maternal HIV infection and PTB. Further characterization of these mechanisms may lead to the development of preventative and therapeutic interventions to reduce the global burden of PTB. Finally, MAIT characteristics



**FIGURE 5** MAIT cells in women with pregnancies resulting in spontaneous preterm labour (Sp-PTL) and spontaneous term labour (Sp-TL). T1 = trimester 1, T2 = trimester 2, T3 = trimester 3. (A) (E) (I) Total MAIT cell frequencies as percentages of CD3<sup>+</sup> lymphocytes. CD4<sup>+</sup> (B) (F) (J), CD8<sup>+</sup> (C) (G) (K) and CD4-CD8- double-negative (DN) (D) (H) (L) MAIT cell subsets as percentages of total MAIT cells. No values were available for Sp-PTL women in trimester 3, so third trimester analysis was not performed. Horizontal bars among data points on graphs indicate median values. Values for Sp-PTL and Sp-TL women were compared within each trimester using a Mann-Whitney U test. Results of statistical tests are indicated by horizontal bars above the graphs. \**P* < .05

in early pregnancy may serve as predictive biomarkers for women at risk of delivering preterm.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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