Title: Blood cytokine, chemokine and growth factor profiling in a cohort of pregnant women from tropical countries

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ABSTRACT

The immune status of women changes during and after pregnancy, differs between blood compartments at delivery and is affected by environmental factors particularly in tropical areas endemic for multiple infections. We quantified the plasma concentration of a set of thirty-one T_H1, T_H2, T_H17 and regulatory cytokines, pro-inflammatory and anti-inflammatory cytokines and chemokines, and growth factors (altogether biomarkers), in a cohort of 540 pregnant women from five malaria-endemic tropical countries. Samples were collected at recruitment (first antenatal visit), delivery (periphery, cord and placenta) and postpartum, allowing a longitudinal analysis. We found the lowest concentration of biomarkers at recruitment and the highest at postpartum, with few exceptions. Among them, IL-6, HGF and TGF-β had the highest levels at delivery, and even higher concentrations in the placenta compared to peripheral blood. Placental concentrations were generally higher than peripheral, except for eotaxin that was lower. We also compared plasma biomarker concentration between the tropical cohort and a control group from Spain at delivery, presenting overall higher biomarker levels the tropical cohort, particularly pro-inflammatory cytokines and growth factors. Only IL-6 presented lower levels in the tropical group. Moreover, a principal component analysis of biomarker concentrations at delivery showed that women from Spain grouped more homogenously, and that IL-6 and IL-8 clustered together in the tropical cohort but not in the Spanish one. Plasma cytokine concentrations correlated with *Plasmodium* antibody levels at postpartum but not during pregnancy. This basal profiling of immune mediators over gestation and in different compartments at delivery is important to subsequently understand response to infections and clinical outcomes in mothers and infants in tropical areas.

Keywords: Pregnancy; Tropical country; Placenta; Cord; Malaria; Cytokine; Chemokine; Growth factor

1. Introduction

The immune system adapts during pregnancy to tolerate the semiallogenic fetus while protecting both the mother and fetus from infections. From a clinical point of view, this immune alteration manifests as worsening or mitigation of certain autoimmune diseases during gestation (1,2) and increased incidence and/or severity of some infectious diseases (reviewed in 3). The T helper (T_H)2/T_H1 cytokine paradigm, according to which successful pregnancies depend on a switch to a T_H2 response, was accepted for a long time (4). However, controlled pro-inflammatory cytokine responses during gestation are necessary during embryo implantation, placentation, labor and defense against infections (5). As the status of the immune system evolves during pregnancy, some studies have investigated longitudinal changes in cytokines over different trimesters or compared to postpartum (6–8), showing in general a decrease in levels over pregnancy with some exceptions and conflicting results. Other studies focused on the distinct patterns present in the peripheral, placental and cord compartments at delivery (9,10).

Although chemokines and growth factors are essential mediators in the development and communication of immune cells, they have been less well studied in pregnancy. With few exceptions (6,8), prior works have only analyzed limited sets of biomarkers, while a wider breath of responses needs to be simultaneously studied to unravel the complex relationships between cytokine networks.

Immunity is influenced by hereditability and the environment (11), including exposure to infectious diseases. We have previously described how both pregnancy and malaria can have distinct effects on B and T cells, as well as *Plasmodium*-specific cytokine responses in endemic populations, and how these alterations may impact pregnancy

89 outcomes (12–14). Moreover, recurrent infections by *Plasmodium falciparum* and/or
90 *P. vivax* in malaria-endemic areas cause chronic activation and alteration of the
91 immune system (13,15–17).

92 As part of a series of studies profiling humoral and cellular immune responses in
93 pregnancy in a diverse cohort of pregnant women from five countries where malaria
94 and other infectious diseases are endemic, we set out to assess plasma biomarkers in
95 peripheral, cord and placental blood samples collected during pregnancy, at delivery
96 and after the puerperium. The heterogeneity of the cohort in relation to malaria
97 endemicity was addressed with available antibody data reflecting exposure to *P.*
98 *falciparum* and *P. vivax* antigens. We selected a comprehensive set of T_H1, T_H2, T_H17
99 and regulatory cytokines, pro-inflammatory and anti-inflammatory cytokines and
100 chemokines, and growth factors, to cover the main immune functions attributed to
101 these biomarkers, towards understanding the basal conditions of these women
102 cohorts, during and after pregnancy. This information is essential to subsequently
103 analyze the effect of infections with significant impact on pregnancy such as malaria,
104 on immunity, and the impact of infection and immune responses on pregnancy
105 outcomes.

106

2. Materials and methods

2.1. Study design and population

This study was performed in the context of a health facility-based observational study of pregnant women aimed to determine the burden of *P. vivax* infection in pregnancy, conducted between 2008 and 2012 in five countries of different *P. vivax* and *P. falciparum* endemicity: Brazil (BR), Colombia (CO), Guatemala (GT), India (IN) and Papua New Guinea (PNG). Approximately 2,000 women per site were recruited at the first antenatal visit (which in some cases occurred late in pregnancy, including the third trimester), and followed up until delivery. A random subgroup of 10% constituted the immunology cohort that was invited to return to the clinic at postpartum (at least 10 weeks after delivery). A venous blood sample was collected at recruitment, delivery and postpartum for immunological assessment. Samples collected at recruitment and postpartum were obtained in the morning, as visits were scheduled at that time. Plasmas obtained at delivery were sampled just after the baby was born.

In addition, total cord blood was collected at delivery and placental blood was obtained in CO and PNG from small incisions on the maternal facing sides. *P. vivax* and *P. falciparum* parasitemia were assessed in blood smears.

For this particular study, 50 recruitment samples per country were randomly selected and analyzed with their paired delivery and postpartum samples. When paired recruitment/delivery/postpartum samples available were less than 50, additional random samples were included to achieve N=50. Because postpartum follow-up was low in some countries, it was not always possible to achieve N=50. In addition, total samples available in IN were fewer than 50 for the three time points, as plasma vials had been freeze-thawed several times which is known to be deleterious for cytokine

concentration. Therefore only samples with a second untouched vial available were measured. A total of 61 paired periphery-cord plasmas and 101 paired periphery-placenta plasmas were analyzed. For the delivery analysis, peripheral plasma samples from pregnant women who were unexposed to malaria recruited in Barcelona-Spain (BCN) were also included (n=16) (naïve control group).

The protocol was approved by the national and/or local ethics committees of each recruiting site, the CDC IRB (USA), and the Hospital Clinic Ethics Review Committee (Barcelona, Spain, registry 2007/3978). Written informed consent was obtained from all study participants.

2.2. Isolation of plasma

Five to 10 mL of venous, cord and placental blood were collected aseptically in heparinized tubes. Plasma was separated from the cellular fraction by centrifugation at 600 x g for 10 min at room temperature (RT), aliquoted and stored at -80ºC. To minimize inter-site variability, samples from BR, CO, GT and PNG were shipped to the Barcelona Institute for Global Health in dry ice for measurement of cytokines, chemokines and growth factors (henceforth referred to as biomarkers). Samples from IN were analyzed at ICGEB, Delhi (India).

2.3. Multiplex bead array assay

Plasmas were thawed at 4ºC overnight and biomarkers analyzed with a multiplex suspension detection system, the *Cytokine Magnetic 30-Plex Panel* (Invitrogen, Madrid, Spain) that allows detection of the following biomarkers: epidermal growth factor (EGF), Eotaxin-1/CCL11, fibroblast growth factor (FGF), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), hepatocyte growth factor (HGF), interferon (IFN)-α, IFN-γ, interleukin (IL)-1RA,

IL-1β, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8/CXCL8, IL-10, IL-12(p40/p70), IL-13, IL-15, IL-17, IFN-γ induced protein (IP-10/CXCL10), monocyte chemoattractant protein (MCP-1/CCL2), monokine induced by IFN-γ (MIG/CXCL9), macrophage inflammatory protein (MIP)-1α/CCL3, MIP-1β/CCL4, regulated on activation, normal T cell expressed and secreted (RANTES/CCL5), tumor necrosis factor (TNF), and vascular endothelial growth factor (VEGF). Fifty µL of the plasma samples were tested in single replicates (dilution 1:2, recommended by the vendor) in 96-well flat-bottom plates. Each plate contained serial dilutions (1:3) in duplicates of a standard sample of known concentration of each analyte provided by the manufacturer, as well as two blank controls and a control in duplicate of medium concentration prepared from a reference sample for quality control purposes. Upper and lower values of the standard curves for each analyte are displayed in Supplementary table 1.

The assays were carried out according to the manufacturer's instructions. Beads were acquired on the BioPlex100 system (Bio-Rad, Hercules, CA) and concentrations calculated using the Bioplex software. When values were out of range (OOR) according to the software, the following were assigned: for OOR values under the curves, a value three-times lower than the lowest standard concentration was assigned (as standard dilutions were 1:3), and for OOR values above the curve, a value three-times higher than the highest standard concentration was assigned. Moreover, the software extrapolated values below and above the lower and higher concentrations, respectively, of the standard curves when they fitted into the curves and were not OOR. These values were kept as they were extrapolated by the software, with the exception of values below three-times the lowest standard concentration and above three-times the highest standard concentration, for which those respective values were assigned.

179 In addition, the cytokine transforming growth factor (TGF)-β1 was analyzed in all
180 plasmas except those from IN, with a DuoSet ELISA kit (R&D Systems). Following the
181 vendor's recommendations, latent TGF-β1 was activated to its immunoreactive form
182 with HCl and neutralized with NaOH/HEPES. A 40-fold plasma dilution was used.

183 **2.4. Quantification of IgG antibodies**

184 Measurement of plasma IgG antibodies at each time point was performed by an in-
185 house multiplex suspension array assay using the Luminex™ technology. Antigens
186 used corresponded to different *P. falciparum* and *P. vivax* stages (sporozoite,
187 merozoite and proteins expressed on the erythrocyte surface) and included: PfMSP-
188 1_{19} (18), PfAMA-1 (19), PfEBA175 (PfF2) (20), DBL3X, DBL5ε, DBL6ε (21), Pv200L
189 (PvMSP$1_{121–416}$) (22), PvMSP-1_{19} (23), PvCSP-N, PvCSP-C, PvCSP-R (24), full-length
190 PvCSP, full-length PvMSP-5 (25), PvDBP (RII) (26), PvLP1, PvLP 2 (27), and VIR
191 proteins (14). Magnetics beads (xMAP technology) were covalently coupled with the
192 antigens. Beads were mixed in a single batch and ~1000 beads per analyte were
193 incubated with each plasma sample (dilution 1:100) in duplicates, and subsequently
194 with anti-human IgG-biotin (Sigma-Aldrich), followed by streptavidin-conjugated R-PE
195 (Fluka, Madrid, Spain). Beads were analyzed on the BioPlex100 system (Bio-Rad,
196 Hercules, CA), and results were expressed as median fluorescence intensity.

197 **2.5. Statistical methods**

198 To compare biomarker concentrations between recruitment, delivery and postpartum,
199 a two-way crossed-effects model was estimated, with the subject effect being crossed
200 with the site effects. For this objective, pairwise statistical significance was interpreted
201 based on 95% confidence intervals, considering significant when the interval did not
202 include 0. To compare the biomarker concentrations between paired periphery and

placental plasma samples or paired periphery and cord plasma samples, a Wilcoxon matched-pairs signed rank test was performed. P-values were corrected for multiple comparisons with the Benjamini-Hochberg test. To compare biomarker concentrations among sites at delivery, a Kruskal-Wallis test was performed followed by Dunn's multiple comparisons test of BCN *vs.* each other country. Additionally, a principal component analysis (PCA) was performed to asses how a) cytokines and b) study subjects analyzed at delivery clustered, excluding TGF-β as this cytokine was analyzed by a differetn technique (ELISA) and was not measured in IN. To analyze the association between biomarker concentrations and anti-malarial antibody levels, the Spearman's correlation test was used and the result classified based on the rho coefficient as: very weak (0-|0.19|), weak (|0.2|-|0.39|), moderate (|0.4|-|0.59|), strong (|0.6|-|0.79|) and very strong (|0.8|-|1|). Only results with p-value <0.05 (after adjustment for multiple comparisons, Benjamini-Hochberg test) and rho value >0.4 were considered 'biologically relevant'.

Overall, significance was defined at $p<0.05$. Analyses and graphs were performed using Stata/SE 10.1 (College Station, TX, USA) and GraphPad Prism (La Jolla, CA, USA).

3. Results

3.1. Study population

A total of 797 plasma samples collected in 546 women from the five tropical countries at different compartments and timepoints were analyzed. The number of samples included in the analysis by site and time point are provided in Supplementary table 2. Addititonally, 16 peripheral plasma samples collected from women in BCN were

analyzed. Characteristics of the cohort are shown in Supplementary table 3. Prevalence of *P. vivax* and *P. falciparum* infection at recruitment was 1.7% and 2.5%, respectively and at delivery 0.97% and 0.48% respectively (data not shown).

3.2. Effect of pregnancy and labor on plasma biomarker concentrations

To assess any specific effect of pregnancy and/or labor on blood biomarkers, we compared concentrations measured in plasma at recruitment, delivery and postpartum in the tropical cohort. Overall, recruitment samples presented the lowest concentration and postpartum samples the highest, with the exception of IL-1β, IL-6, TGF-β, IL-2, FGF and HGF, for which the highest concentration was found at delivery (Table 1).

		Recruitment	Delivery		Postpartum		p-value
		Expected change	Expected change	95% CI	Expected change	95% CI	
Pro-inflammatory	**TNF**	0	0.15	0.01; 0.29	0.27	0.11; 0.43	0.0026
	IL-1β	0	0.31	0.17; 0.45	0.25	0.10; 0.41	< 0.0001
	IL-6	0	1.47	1.26; 1.68	0.62	0.40; 0.85	< 0.0001
Anti-inflammatory	**IL-10**	0	-0.02	-0.14; 0.11	0.02	-0.12; 0.16	0.8900
	TGF-β	0	0.38	0.28; 0.48	0.22	0.11; 0.32	< 0.0001
	IL-1RA	0	0.38	0.21; 0.54	0.61	0.43; 0.79	< 0.0001
	IFN-α	0	0.11	0.01; 0.21	0.27	0.16; 0.37	< 0.0001
Chemokines	**IL-8**	0	1.13	0.78; 1.48	1.29	0.92; 1.67	< 0.0001
	MIP-1α	0	0.22	0.07; 0.37	0.5	0.33; 0.66	< 0.0001
	MIP-1β	0	0.37	0.16; 0.58	0.86	0.62; 1.09	< 0.0001
	MCP1	0	0.34	0.19; 0.48	0.93	0.77; 1.08	< 0.0001
	IP10	0	0.1	-0.00; 0.20	0.07	-0.04; 0.18	0.1398
	EOTAXIN	0	0.14	0.03; 0.25	0.91	0.79; 1.02	< 0.0001
	RANTES	0	0.13	0.02; 0.23	0.11	-0.00; 0.23	0.0455
	MIG	0	-0.09	-0.25; 0.08	0.58	0.40; 0.76	< 0.0001
T_H1	**IFN-γ**	0	0.01	-0.06; 0.08	0.18	0.11; 0.26	< 0.0001
	IL-12	0	0.01	-0.06; 0.08	0.29	0.22; 0.37	< 0.0001
	IL-2	0	0.4	0.23; 0.56	0.25	0.07; 0.44	< 0.0001
	IL-15	0	0.35	0.21; 0.49	0.35	0.20; 0.51	< 0.0001
	IL-2R	0	0.04	-0.07; 0.15	0.31	0.18; 0.43	< 0.0001
T_H2	**IL-4**	0	0.06	0.00; 0.12	0.11	0.04; 0.18	0.0039
	IL-5	0	0.09	-0.04; 0.22	0.3	0.15; 0.44	0.0002
	IL-13	0	0.2	0.07; 0.33	0.52	0.38; 0.67	< 0.0001
T_H17	**IL-17**	0	0.05	-0.04; 0.14	0.25	0.15; 0.35	< 0.0001
Growth factors	**EGF**	0	0.2	0.06; 0.34	0.56	0.40; 0.71	< 0.0001
	FGF	0	0.51	0.32; 0.69	0.45	0.24; 0.65	< 0.0001
	HGF	0	0.8	0.60; 1.00	0.16	-0.06; 0.38	< 0.0001
	VEGF	0	0.4	0.28; 0.52	0.68	0.55; 0.81	< 0.0001
	G-CSF	0	-0.01	-0.08; 0.07	0.16	0.08; 0.24	< 0.0001
	GM-CSF	0	0.03	-0.14; 0.20	0.15	-0.03; 0.34	0.2496
	IL-7	0	0.29	0.12; 0.47	0.7	0.51; 0.89	< 0.0001

Table 1. Pregnancy and labor effect on biomarker concentration.

Two-way crossed-effect model, with the subject effect being crossed with the site effect. Only samples from the tropical countries were analized in the model, N=618. CI: confidence interval. Expected change: change in mean concentration measured in pg/mL.

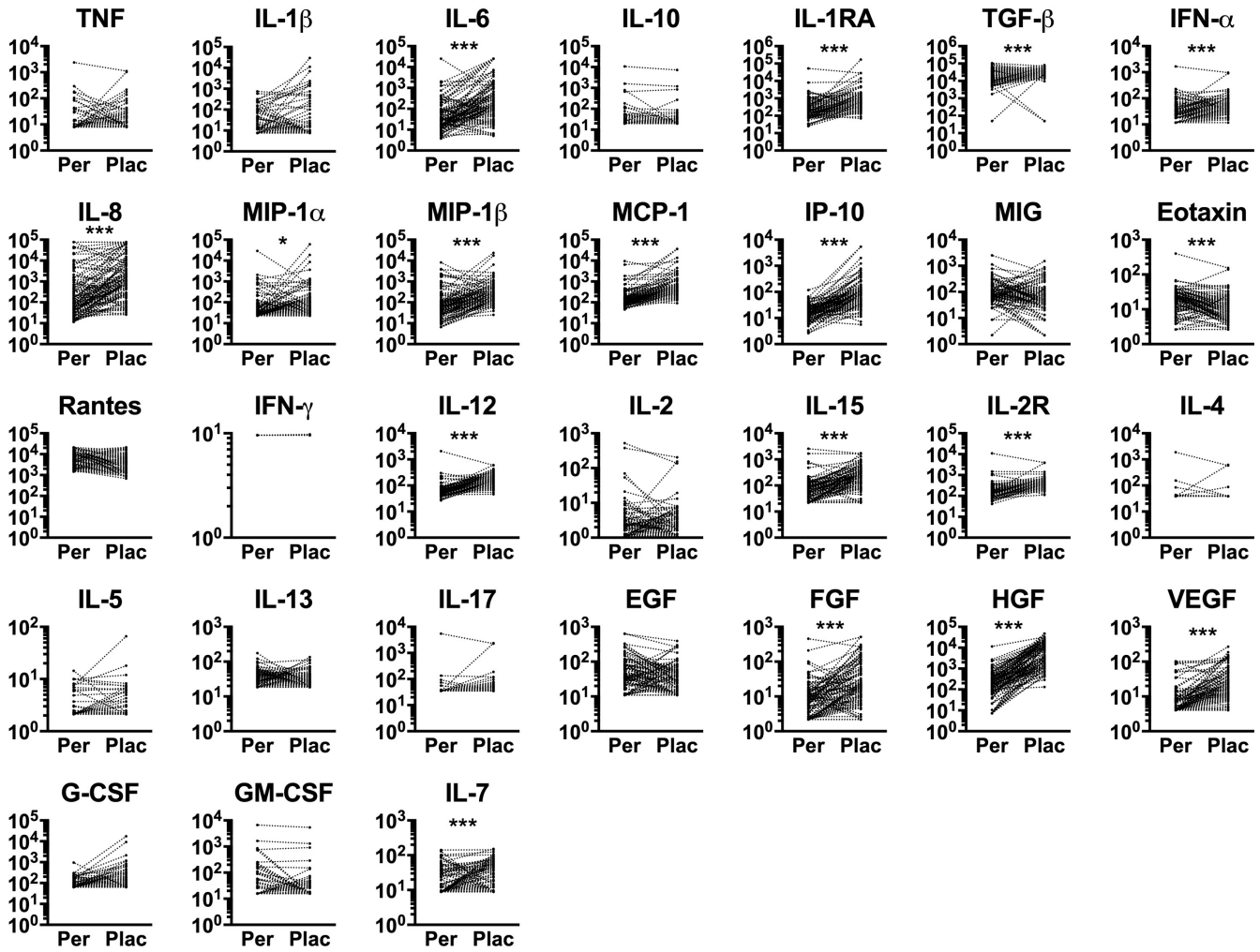
As concentrations at delivery were significantly higher than at recruitment for many biomarkers, we wondered whether there was a gradual increase throughout pregnancy or rather a labor-specific up-regulation or release of biomarkers. At recruitment, the correlation between the biomarker concentrations and women's gestational age (women were enrolled throughout the whole pregnancy) was low in all cases (Spearman's rho<|0.33| in all cases, Supplementary table 4).

3.3. Comparison of biomarker concentrations in placental, cord and peripheral blood at delivery

Next, we assessed whether biomarker concentrations differed between blood compartments at delivery in the tropical cohort. We evaluated separately periphery *vs.* placenta and periphery *vs.* cord blood due to the low number of paired samples.

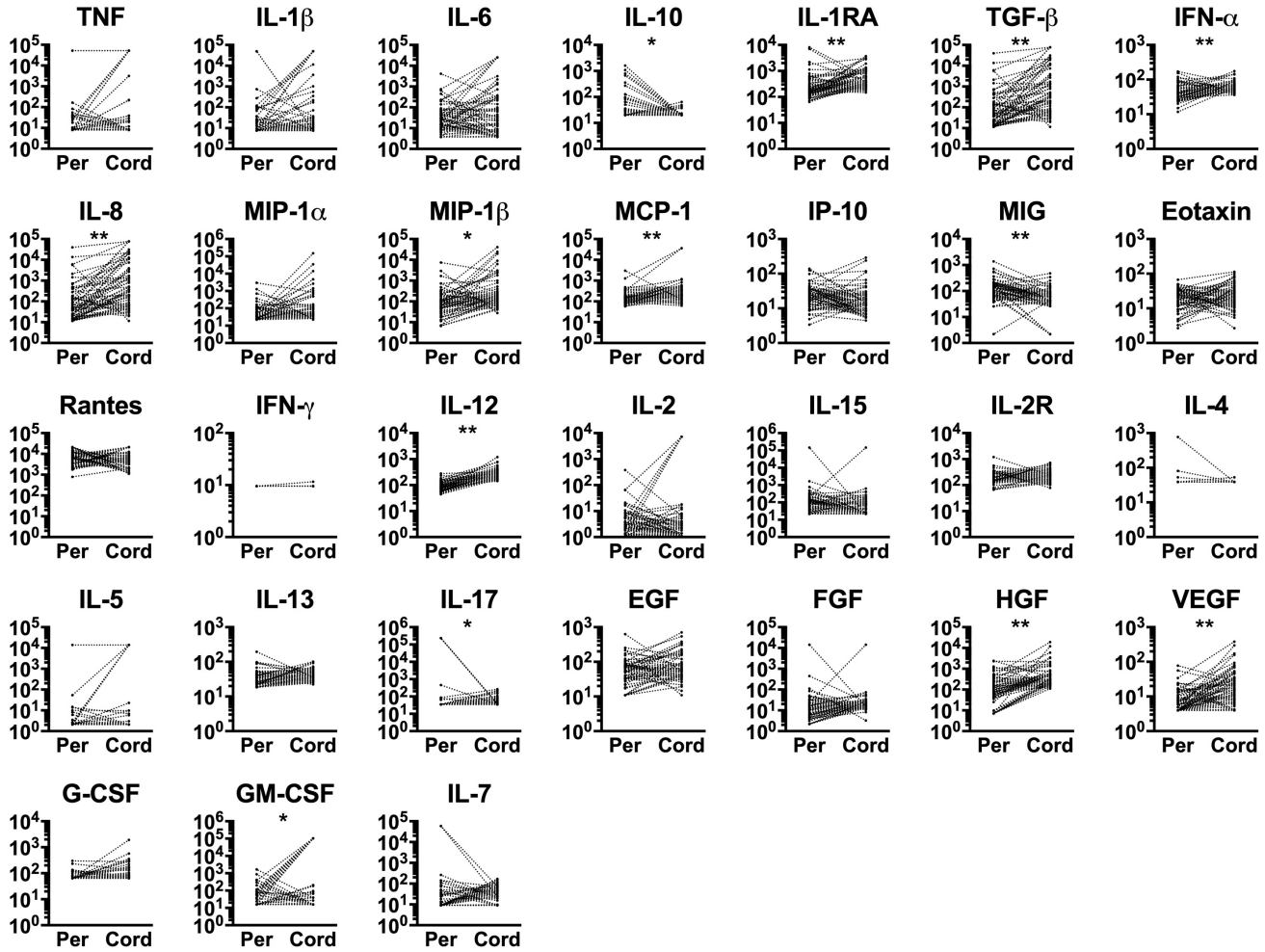
For the biomarkers with significant differences between placenta and periphery, concentrations were always higher in placental than peripheral blood: pro-inflammatory biomarkers IL-6, IL-8, MIP-1α, MIP-1β, MCP-1, IP-10; anti-inflammatory cytokines TGF-β, IL-1RA, IFN-α; T_H1-related cytokines IL-12, IL-15, IL-2R; and growth factors VEGF, FGF, HGF, IL-7 (Fig. 1). This variation was very pronounced for HGF, with a 25 fold-change (Fig. 1). There was only one exception, eotaxin, whose concentration was significantly lower in placenta compared to peripheral plasma (Fig. 1).

Biomarker concentrations were also higher in cord than peripheral plasma for the following markers: pro-inflammatory chemokines IL-8, MIP-1β, MCP-1; anti-inflammatory cytokines TGF-β, IL-1RA, IFN-α; T_H-related cytokines IL-12 and IL-17; and growth factors VEGF, HGF and GM-CSF (Fig. 2). The exceptions were IL-10 and MIG, which showed lower concentrations in cord than in peripheral plasma (Fig. 2).



286

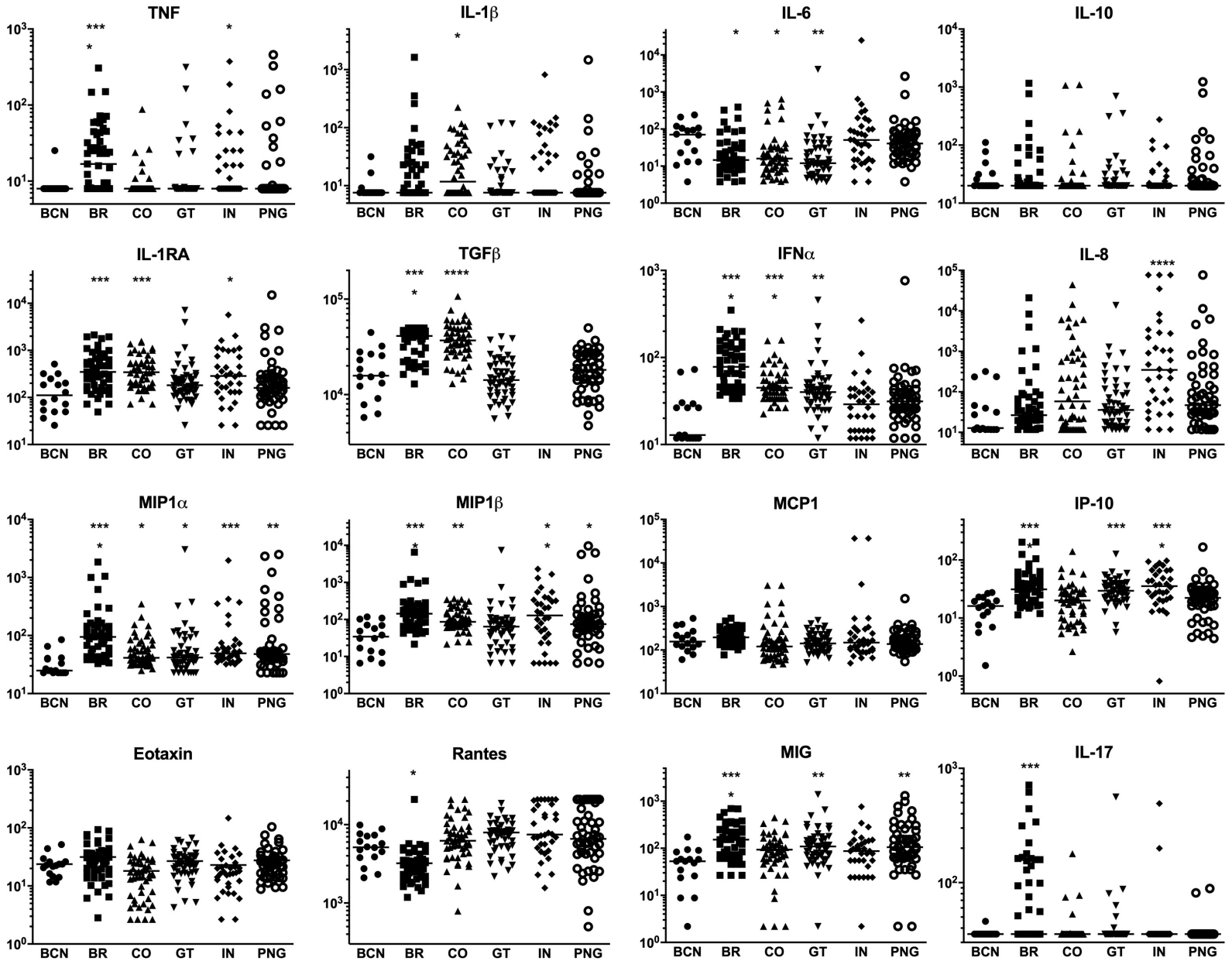
287 **Figure 1.** Peripheral and placental plasma biomarker concentrations. Graphs depict biomarker concentrations in paired peripheral
288 (Per) and placental (Pla) plasmas obtained at delivery. Concentrations for all biomarkers are expressed in pg/mL. N= 101, samples
289 belong to women from Colombia or Papua New Guinea only. P-value corresponds to the Wilcoxon signed-rank test corrected for
290 multiple comparisons using the Benjamini-Hochberg test. *p<0.05, **p<0.01, ***p<0.001.



291

292 **Figure 2.** Peripheral and cord plasma biomarker concentrations. Graphs depict biomarker concentrations in paired peripheral (Per)
293 and cord (Cord) plasmas obtained at delivery. Concentrations for all biomarkers are expressed in pg/mL. N= 61, samples belong to
294 the five tropical study countries. P-value corresponds to the Wilcoxon signed-rank test corrected for multiple comparisons using the
295 Benjamini-Hochberg test. *p<0.05, **p<0.01, ***p<0.001.

3A


TNF
IL-1β
IL-6
IL-10
IL-1RA
TGFβ
IFNα
IL-8
MIP1α
MIP1β
MCP1
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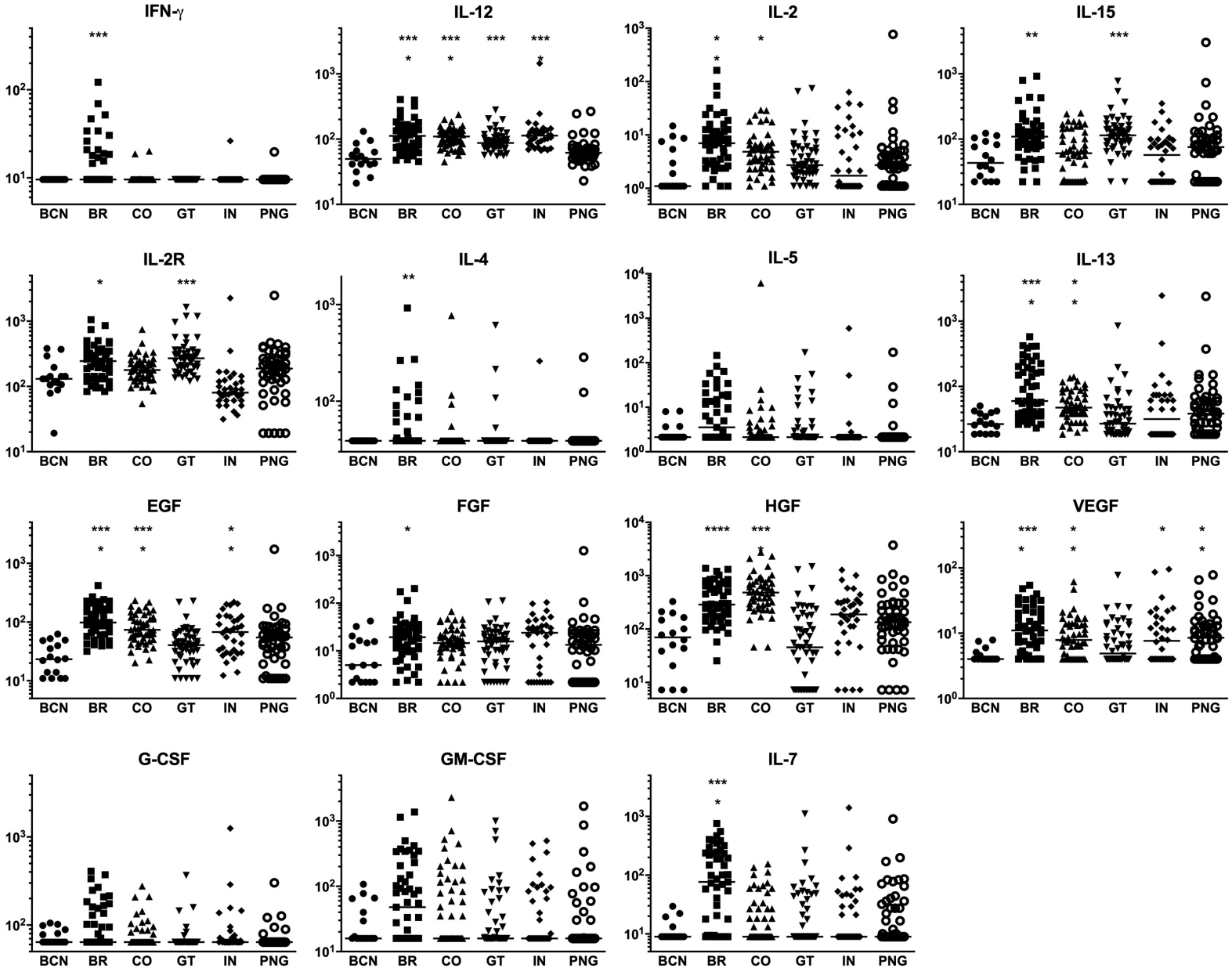
Eotaxin
Rantes
MIG
IL-17
BCN
BR
CO
GT
IN
PNG

3B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IL-12
IL-2
IL-15
IL-2R
IL-4
IL-5
IL-13
EGF
FGF
HGF
VEGF
G-CSF
GM-CSF
IL-7
BCN
BR
CO
GT
IN
PNG

Figure 3. Biomarker concentrations at delivery by country.
Graphs depict biomarker concentrations in peripheral plasma at delivery in individual countries. BCN: Barcelona control group, N=16; BR: Brazil, N=49; CO: Colombia, N=50; GT: Guatemala, N=50; IN: India, N=34; PNG: Papua New Guinea, N=50. P-value corresponds to a Kruskal-Wallis test followed by Dunn's multiple comparisons test of BCN *versus* each other country. *p<0.05, **p<0.01, ***p<0.001.

3.4. Plasma biomarker concentrations at delivery vary between tropical countries and the control group

Because the tropical cohort has been largely exposed to infectious diseases that have a chronic impact on the immune system, we analyzed differences on peripheral plasma biomarker concentrations measured at delivery between countries, including samples from BCN (controls). All biomarkers showed statistically significant differences between countries (Kruskal-Wallis test, $p<0.05$) except IL-10. When we compared biomarker concentrations between the control BCN group and each other country individually, we observed differences for most analytes, with women from tropical countries generally having higher levels than the control group. That was the case for the pro-inflammatory biomarkers TNF, IL-1β, IL-8, MIP-1α, MIP-1β, IP-10 and MIG, although the difference did not reach statistical significance for every country (Fig. 3A). Interestingly, the pro-inflammatory cytokine IL-6 showed lower concentrations in all countries compared to BCN (only statistically significant for BR, CO and GT), and RANTES concentrations were also lower in BR compared to BCN (Figure 3A). Other chemokines like MCP-1 or eotaxin did not show evident differences among countries (Fig. 3A). Regarding anti-inflammatory cytokines, women from BR and CO had consistently higher concentrations of TGF-β, IL1-RA and IFN-α than those from BCN (Fig. 3A).

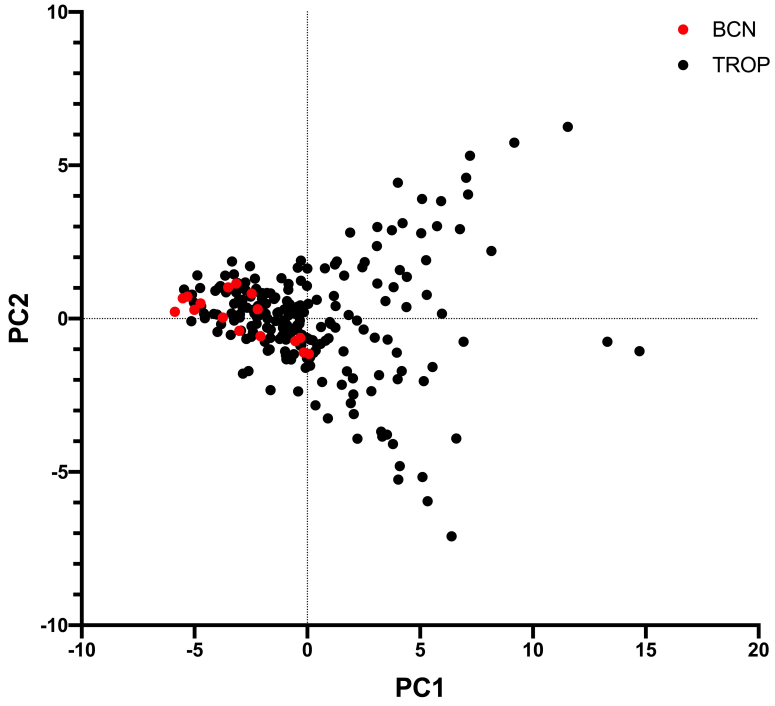
The T_H-related cytokines IFN-γ, IL-4 and IL-17 presented low plasma levels in general, with only few women having concentrations above the limit of detection, mostly from BR (Fig. 3A and 3B). In addition, T_H1-related IL-12, IL-2, IL-2R and IL-15 had increased plasma concentrations in women from tropical countries compared to BCN, although differences were not significant for every country. Furthermore, T_H2-

related IL-5 did not show statistically significant differences among countries and IL-13 presented increased levels in women from BR and CO than BCN (Fig. 3B).

Additionally, women from tropical countries had increased concentrations of the growth factors EGF, FGF, HGF and VEGF compared to women from BCN, which was less evident in women from GT. Other growth factors such as G-CSF, GM-CSF and IL-7 showed overall lower concentrations in women from BCN, but only for Brazilian women the difference was significant (Fig. 3B).

We also performed a PCA with biomarkers measured at delivery. Six principal components (PC) resulted in a Eigenvalue>1 (overall kmo=0.8954); however two of them contributed mostly to variation, PC1 with 39% and PC2 with 11% (data not shown). Therefore only these two PC were further taken into account. When study subjects were displayed according to their predicted scores for PC1 and PC2, women from BCN grouped more homogenously (Fig 4A). Then, PCA analyses were performed separately for women from BCN and the tropical cohort, in order to detect if cytokines clustered differently in both groups. PC1 and PC2 eigenvectors for each biomarker were displayed, showing that while IL-6 and IL-8 clustered together in the tropical cohort, they occupied opposite PC2-directions in the Spanish women.

4A


BCN
TROP
PC2
PC1
10
5
0
-5
-10
-10
-5
0
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TROP
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IL-1β
IL-13
TNF
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MIG
MIP-1α
Rantes
VEGF
IL-1RA
FGF
IL-12
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EGF
IL-1RA
IL-2R
G-CSF
HGF
HGF
IP-10
IP-10
EGF
Rantes
IL-15
MIP-1α
IL-17
TNF
IL-2
IL-15
MCP-1
IL-10
IL-10
Eotaxin
IL-12
IL-2R
GM-CSF
IFN-α
MIG
IL-7
IL-13
Eotaxin
IL-6
IL-4
IL-5
G-CSF
IL-5
VEGF
IL-2
IL-1β
FGF
IFN-γ
IL-17

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Figure 4. A principal component (PC) analysis of all biomarkers except TGF-β analyzed in peripheral plasmas collected at delivery from the tropical cohort (TROP, N=233) and Barcelona control cohort (BCN, N=16) was performed. A) Predicted PC1 and PC2 score values for each study subject are displayed. B) A loadingplot with PC1 and PC2 eigenvector values for each biomarker in the tropical and the control group is displayed.

3.5. Plasma cytokine concentrations correlate with *Plasmodium* antibody levels at postpartum but not during pregnancy

To test whether differences in biomarker concentrations between BCN and the five tropical countries were related to malaria exposure, we assessed the correlation between the biomarker concentrations and plasma levels of IgG against selected *Plasmodium* antigens measured at the same bleeding. Anti-*Plasmodium* IgG levels are frequently used as surrogate of malaria exposure. Correlations were analyzed separately at recruitment, delivery and postpartum.

Overall, correlations at recruitment and delivery were weak or very weak. At recruitment, only anti-PvCSP-N antibodies presented a biologically relevant positive correlation with TGF-β, MIP-1β and HGF concentrations (Supplementary table 5). At delivery, anti-PvMSP5 IgG levels correlated negatively with TGF-β levels (Supplementary table 6).

Postpartum, however, multiple potentially biologically relevant correlations were found ($p<0.05$ and Spearman's rho>0.4). TNF was the pro-inflammatory cytokine that showed better correlations, with moderate and strong negative associations for *P. vivax* as well as *P. falciparum* antibodies (Table 2). In contrast, IL-8 presented a relevant positive correlation with several antibodies. In the anti-inflammatory cytokine group, IFN-α levels correlated negatively with antibodies to merozoite antigens from

both species and anti-PfDBL6ε antibodies, while IL-10, TGF-β and IL-1RA did not show any biologically relevant correlations with antibody levels (Table 2).

There were consistently negative moderate to strong correlations between several T_H-related cytokine concentrations (especially IFN-γ, IL-12, IL-4, IL-5, IL-13 and IL-17) and antibodies against both *P. falciparum* and *P. vivax* antigens (Table 2).

Finally, the correlation pattern between antibodies and growth factors was very similar to the T_H-related cytokines, with consistently negative moderate correlations between several (remarkably G-CSF, GM-CSF and IL-7) and antibodies against merozoite antigens of both species and PfDBL6ε (Table 2).

386 Table 2. Correlations between antibody levels and biomarker concentrations postpartum.

	TNF	IL1B	IL-6	IL-10	TGF-β	IL-1RA	IFN-α	IL-8	MIP-1α	MIP-1β	MCP1	IP10	EOTAXIN	RANTES	MIG	IFN-γ	IL-12	IL-2	IL-15	IL-2R	IL-4	IL-5	IL-13	IL-17	EGF	FGF	HGF	VEGF	G-CSF	GM-CSF	IL-7
PvCSP-N	0,16	0,02	0,15	0,02	0,28	0,11	0,08	**0,38**	0,20	0,24	0,05	-0,05	-0,08	-0,23	0,09	0,18	-0,12	-0,06	-0,09	-0,07	0,12	0,11	0,20	0,15	0,28	-0,05	0,23	0,28	0,13	0,04	0,19
PvCSP-C	0,07	0,09	0,14	-0,04	0,28	0,13	0,12	**0,37**	0,08	0,20	0,14	0,01	-0,10	-0,19	-0,04	0,06	-0,13	-0,07	-0,01	-0,01	0,04	0,07	0,12	0,06	0,20	-0,01	0,17	0,16	0,02	0,04	0,09
PvCSP-R	0,03	0,18	0,18	-0,03	0,24	0,17	0,16	**0,34**	0,09	0,25	0,23	0,10	-0,06	-0,06	-0,14	0,05	-0,06	-0,02	0,03	0,01	-0,01	0,03	0,12	0,04	0,17	0,02	0,17	0,17	-0,01	0,05	0,10
PvCSP	-0,28	-0,05	-0,03	-0,09	-0,07	-0,12	-0,16	0,07	-0,16	-0,10	0,07	0,08	0,02	0,09	-0,11	-0,25	-0,18	-0,12	0,00	-0,13	-0,26	-0,25	-0,18	-0,25	-0,30	-0,18	-0,15	-0,16	-0,27	-0,12	-0,21
PvDBP	**-0,48**	0,05	-0,07	-0,22	-0,13	-0,28	**-0,35**	0,18	-0,27	-0,09	0,17	**0,39**	0,01	0,18	-0,14	**-0,39**	-0,32	-0,25	0,01	-0,32	**-0,38**	**-0,37**	-0,28	**-0,40**	-0,27	-0,17	-0,32	-0,20	**-0,40**	**-0,35**	**-0,38**
PvMSP1$_{19}$	**-0,46**	0,09	0,03	-0,21	-0,18	-0,24	-0,31	0,17	-0,24	-0,08	0,17	0,28	-0,05	0,18	-0,16	**-0,41**	**-0,33**	-0,24	0,03	-0,26	**-0,39**	**-0,35**	-0,31	**-0,38**	-0,30	-0,14	-0,31	-0,16	**-0,45**	**-0,34**	**-0,38**
Pv200L	**-0,37**	0,00	-0,08	-0,24	-0,29	-0,31	**-0,44**	0,26	-0,21	-0,07	0,05	-0,03	-0,03	0,04	-0,05	**-0,48**	**-0,53**	-0,23	-0,14	-0,23	**-0,41**	**-0,43**	**-0,42**	**-0,42**	**-0,35**	-0,18	**-0,34**	-0,16	**-0,45**	**-0,43**	**-0,47**
PvMSP1-N	-0,28	-0,03	-0,08	-0,16	-0,07	-0,22	-0,30	0,27	-0,11	0,05	0,11	0,10	0,09	-0,02	-0,03	-0,28	**-0,40**	-0,15	-0,03	-0,15	-0,25	-0,25	-0,27	-0,21	-0,18	-0,07	-0,25	-0,13	-0,32	-0,22	-0,31
PvMSP5	**-0,42**	0,04	-0,06	-0,26	-0,28	-0,30	**-0,41**	0,23	-0,20	-0,05	0,16	0,19	0,16	0,16	-0,05	**-0,53**	**-0,47**	-0,18	0,06	-0,24	**-0,47**	**-0,47**	**-0,43**	**-0,44**	**-0,40**	-0,19	-0,32	-0,22	**-0,55**	**-0,43**	**-0,50**
VIR25	0,12	-0,09	0,04	-0,01	0,11	0,10	0,08	0,03	0,12	0,05	0,10	0,05	0,07	-0,10	0,15	0,05	0,02	0,02	0,06	0,11	0,05	0,02	-0,02	0,11	0,04	0,10	0,08	0,10	0,09	0,00	0,03
VIR5	0,04	-0,08	0,04	-0,06	0,16	0,01	0,07	0,15	0,13	0,12	0,05	-0,19	0,09	-0,10	0,11	-0,02	-0,15	-0,03	-0,05	0,08	0,04	0,02	0,03	0,04	0,04	0,06	0,02	0,06	-0,01	-0,03	0,03
LP1	-0,01	0,08	0,17	-0,07	0,24	0,07	-0,02	**0,42**	0,10	0,23	0,15	0,08	-0,16	-0,09	-0,05	0,03	-0,18	-0,14	-0,05	-0,05	0,00	-0,06	0,10	0,02	0,16	-0,06	0,11	0,16	0,01	-0,02	0,03
LP2	-0,32	0,01	-0,08	-0,25	-0,04	-0,21	-0,32	**0,41**	-0,11	0,10	0,09	0,11	0,04	-0,06	-0,03	-0,31	**-0,48**	-0,25	-0,06	-0,28	-0,30	**-0,37**	-0,29	-0,28	-0,14	-0,15	-0,21	-0,03	**-0,36**	**-0,40**	-0,29
PfMSP1$_{19}$	**-0,45**	-0,01	-0,09	-0,16	**-0,35**	**-0,35**	**-0,50**	0,30	-0,25	-0,05	0,10	0,10	0,03	0,07	-0,04	**-0,53**	**-0,59**	-0,22	-0,04	-0,31	**-0,47**	**-0,47**	**-0,47**	**-0,48**	**-0,36**	-0,26	**-0,35**	-0,19	**-0,49**	**-0,40**	**-0,49**
PfAMA	**-0,50**	-0,02	-0,09	-0,18	-0,31	**-0,37**	**-0,58**	**0,36**	-0,24	-0,03	0,10	0,12	0,05	0,08	0,01	**-0,59**	**-0,65**	-0,25	-0,01	**-0,33**	**-0,52**	**-0,61**	**-0,52**	**-0,52**	**-0,42**	-0,25	**-0,40**	-0,17	**-0,56**	**-0,43**	**-0,53**
PfEBA175	**-0,39**	0,01	-0,11	-0,17	-0,30	**-0,33**	**-0,55**	0,27	-0,20	0,02	0,02	0,09	0,05	0,12	0,00	**-0,54**	**-0,56**	-0,27	-0,11	**-0,36**	**-0,44**	**-0,60**	**-0,51**	**-0,43**	-0,30	-0,22	**-0,33**	-0,15	**-0,47**	**-0,39**	**-0,47**
PfDBL3x	-0,15	0,07	0,06	-0,13	0,02	-0,06	-0,23	**0,38**	0,02	0,13	0,06	0,00	-0,09	-0,09	-0,05	-0,20	**-0,41**	-0,22	-0,16	-0,26	-0,18	-0,29	-0,17	-0,16	-0,02	-0,15	-0,01	0,09	-0,15	-0,16	-0,16
PfDBL5ε	-0,30	0,12	0,03	-0,14	0,02	-0,13	-0,29	**0,35**	-0,10	0,15	0,15	0,13	0,02	0,02	-0,11	-0,29	-0,31	-0,15	-0,02	-0,29	-0,30	-0,31	-0,15	-0,28	-0,09	-0,06	-0,11	0,11	-0,31	-0,22	-0,20
PfDBL6ε	**-0,47**	0,02	-0,08	-0,23	-0,15	-0,29	**-0,42**	0,24	-0,23	-0,01	0,08	0,01	-0,04	0,13	-0,19	**-0,41**	**-0,45**	-0,29	-0,14	-0,32	**-0,41**	**-0,41**	-0,30	**-0,45**	-0,30	-0,26	-0,31	-0,13	**-0,45**	**-0,33**	**-0,40**
	Pro-inflammatory			Anti-inflammatory				Chemokines								T_H1					T_H2			T_H17	Growth factors						

387 Spearman's correlation coefficient (rho, range 0-|1|) is displayed in the cells. N=147. Grey-color scale (used for quick data comprehension) range from dark
388 grey (Spearman's rho value=|0.65|) to white (Spearman's rho value=0). Bold indicates p<0.05 after multiple testing correction with the Benjamini-Hochberg
389 method. Bold and squared indicates rho>0.4 AND p<0.05.

4. Discussion

Here we present a descriptive analysis of plasma cytokine, chemokine and growth factor concentrations during and after pregnancy in tropical areas. Although women were recruited in malaria-endemic countries, the prevalence of *Plasmodium* infection was very low, especially at delivery, thus the study cohort was malaria-exposed but not infected.

Pregnant women were recruited at the first antenatal visit, regardless of gestational age. We could not perform a full longitudinal analysis of pregnancy (excluding delivery) but we were able to study the correlation of biomarker concentrations and gestational age, finding limited evidences of association, in contrast to a recent study showing in general higher biomarker levels in the second compared to the first trimester of pregnancy (8). Nevertheless, we observed that biomarker concentrations postpartum were higher than during pregnancy, and at delivery higher than at enrolment. The exceptions were the pro-inflammatory IL-1β and IL-6; the anti-inflammatory TGF-β; and IL-2, FGF and HGF, for which the highest concentrations were found at delivery, not at postpartum. Three of these biomarkers (IL-6, TGF-β and HGF) presented even higher concentrations in placental samples, suggesting an important role of these biomarkers in labor. Previous research in uncomplicated pregnancies had shown that IL-1, IL-6 and TNF (but not IL-10) were significantly up-regulated in sera of women in labor compared to women not in labor at term (10). Moreover, one recent study demonstrated that IL-8 and IL-6 produced during uncomplicated labor are actually of fetal origin, with the placenta having a prominent role in clearing them (especially IL-6) from fetal circulation (9). Thus, at present it is well accepted that parturition presents as a localized and physiological inflammatory process that favors the contraction of the uterus, the delivery of the baby and detachment of the placenta (29), explaining the

higher concentrations of proinflammatory cytokines observed at delivery. The TGF-β concentration and role have been assessed mainly in placenta/decidua at early pregnancy and in cord blood at delivery. Two studies showed higher levels of this anti-inflammatory cytokine in cord blood after spontaneous vaginal delivery compared to elective caesarean section (with no labor) in term pregnancies (30,31). Thus, while in early pregnancy TGF-β is known to inhibit trophoblast invasion of uterine arteries (32), which may be associated with intrauterine growth retardation and preeclampsia, during labor TGF-β may counteract the excessive inflammation in the placenta/fetal interface (30,31). HGF has a role in cell proliferation, migration, and morphogenesis in different tissues and its elevated levels in placenta are well recognized (33).

Our results show overall higher biomarker concentrations in both placenta (maternal side) and cord blood than in periphery. This suggests that either they are produced by the mother and traverse the placenta getting accumulated in the fetal circulation, or they are in fact produced by the fetus (9). However, a few exceptions were found in our study. First, MIG presented lower levels in cord and placenta. This chemokine is silenced in the murine decidua (34) and elevated MIG levels have been associated with poor outcomes in malaria-infected pregnant women (35,36) and in pregnant women with autoimmune thyroiditis (37). This might be the reason for presenting lower levels in the placenta and cord, compartments that are more related to the fetus. Second, eotaxin concentration was remarkably lower in placental than in peripheral plasma. The eotaxin receptor CCR3 is present in the placenta and the interaction with eotaxin-2/CCL24 *in vitro* favors decidualization (38) that is essential in early pregnancy. Thus, a reduced concentration of competitor eotaxin/CCL11 in the placenta seems logical in the context of uncomplicated pregnancies. Unfortunately, we could not measure eotaxin-2/CCL24 concentration in our samples, but microarray assays in

440 villous and extravillous trophoblast as well as decidual cells in early pregnancy have
441 shown that mRNA expression of eotaxin-2 is at least two-times higher than expression
442 of eotaxin (39). More studies about the role of eotaxin in pregnancy are necessary.
443 Finally IL-10 was decreased in cord blood but not in placental plasma compared to
444 periphery. This result contrasts partially with one recent publication showing that labor
445 increases cord but decreases placental IL-10 concentrations compared to the same
446 tissues after elective cesarean section with no labor (9). A study on IL-10 expression
447 throughout pregnancy showed that just before and during labor placental IL-10
448 expression is down-regulated (compared to early pregnancy) but PBMCs hold the
449 capacity of secreting IL-10 (7), supporting our finding of increased IL-10 levels in
450 periphery.

451 The effect of pregnancy and labor on plasma biomarker concentrations in women from
452 tropical countries followed a similar pattern to what has been described in the literature
453 for uncomplicated pregnancies. However, when we specifically compared plasma
454 biomarker concentrations at delivery between our cohort and the control group from
455 BCN, women from all tropical countries showed consistently higher plasma
456 concentrations of most biomarkers than pregnant women from BCN, with one clear
457 exception: IL-6. This suggests a general over-activation of the women's immune
458 system during labor in these countries, at least compared to the control group.
459 Moreover, in the PCA analysis women from BCN seemed to group more
460 homogenously than women from tropical countries, maybe as a result of this general
461 activation of the immune system in the tropical cohort that is not equally presented in
462 every woman.

463 At postpartum, many biomarker concentrations correlated well with malaria antibody
464 levels, suggesting that chronic malaria-exposure may have an impact on the circulating

cytokine profile, although it might just be a confounder of exposure to other pathogens or socio-demographic variables. However, at enrolment and delivery the correlation was poor or non-existent. The reason why pregnant women in tropical countries present a more activated status of the immune system at delivery, and whether this also happens throughout pregnancy, remain unclear, but these results should be taken into consideration when analyzing the immune impact of infections during pregnancy in tropical countries.

As mentioned, despite general immune activation, women from tropical countries had lower IL-6 concentration than women from BCN, in contrast with all other biomarkers. Of note, in our PCA analysis at delivery, IL-6 clustered with IL-8 in the tropical cohort but segregated in an opposite PC2-direction in the control group. IL-6 is produced physiologically during labor (9) and can drive prostaglandin synthesis (40), inducing subsequently myometrial contractility. In early pregnancy, IL-6 may promote trophoblast invasion of spiral uterine arteries (at least *in vitro)* (41), a critical step for the establishment of maternal blood flow towards the placenta whose failure leads to miscarriage or pregnancy disorders such as intrauterine growth restriction (42). Thus, although elevated levels have been associated with negative outcomes during pregnancy including preterm delivery (43,44), gestational diabetes mellitus (45) or recurrent spontaneous abortion (46), IL-6 seems to also have a physiological and important role in pregnancy and parturition. Further studies are necessary to unravel if IL-6 “abnormal” levels at delivery in women from malaria-endemic areas have an impact on delivery outcomes.

Our study presents some limitations. Some important clinical variables of the cohort were not recorded, such as smoking status or gestational diabetes. And although recorded, the *blood pressure* variable had multiple missing values. Moreover, other

infectious diseases were not analyzed except for syphilis screening. Finally, comparison of biomarker concentrations between uncomplicated pregnancies (from BCN) and pregnancies from tropical countries could only be done at delivery, as first trimester and postpartum samples were not collected in BCN.

In summary, profiling of cytokine, chemokine and growth factor plasma concentrations during pregnancy in women from tropical countries showed a similar pattern to what has been described for uncomplicated pregnancies although with exacerbated or increased biomarker levels. The exception may be IL-6, an important pro-inflammatory cytokine during pregnancy and labor, which showed a decreased concentration in our cohort of women from tropical settings.

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Declaration of interest

The authors declare no conflicts of interest.

Authorship contribution statement

Carlota Dobaño: Conceptualization, Funding acquisition, Investigation, Visualization, Writing - original draft, Writing - review & editing.

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Aina Casellas: Data curation, Formal analysis, Methodology, Writing - review & editing.

Pilar Requena: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing - original draft, Writing - review & editing.

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Figure legends

Figure 1. Peripheral and placental plasma biomarker concentrations.

Graphs depict biomarker concentration in paired peripheral (Per) and placental (Pla) plasmas obtained at delivery. Concentrations for all biomarkers are expressed in pg/mL. N= 101, samples belong to women from Colombia or Papua New Guinea only. P-value corresponds to the Wilcoxon signed-rank test. *p<0.05, **p<0.01, ***p<0.001.

Figure 2. Peripheral and cord plasma biomarker concentrations.

Graphs depict biomarker concentration in paired peripheral (Per) and cord (Cord) plasmas obtained at delivery. Concentrations for all biomarkers are expressed in pg/mL. N= 61, samples belong to the five tropical study countries. P-value corresponds to the Wilcoxon signed-rank test. *p<0.05, **p<0.01, ***p<0.001.

Figure 3. Biomarker concentrations at delivery by country.

Graphs depict biomarker concentration in peripheral plasma at delivery in individual countries. BCN: Barcelona control group, N=16; BR: Brazil, N=49; CO: Colombia, N=50; GT: Guatemala, N=50; IN: India, N=34; PNG: Papua New Guinea, N=50. P-value corresponds to a Kruskal-Wallis test followed by Dunn's multiple comparisons test of BCN *vs* each other country. *p<0.05, **p<0.01, ***p<0.001.

Figure 4. A principal component (PC) analysis of all biomarkers except TGF-β analyzed in peripheral plasmas collected at delivery from the tropical cohort (TROP, N=233) and Barcelona control cohort (BCN, N=16) was performed. A) Predicted PC1 and PC2 score values for each study subject are displayed. B) A loadingplot with PC1

567 and PC2 eigenvector values for each biomarker in the tropical and the control group is
568 displayed.
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Table S1. Upper and lower values of the biomarker standard curves

Biomarker	Std 1 (upper) pg/mL	Std 7 (lower) pg/mL
TNF	8700	12
IL1B	8300	11
IL-6	4150	6
IL-10	21900	30
TGF-β	2500	39
IL-1RA	28000	38
IFN-α	12900	18
IL-8	12800	18
MIP-1α	25000	34
MIP-1β	7200	10
MCP1	6100	8
IP10	900	1
EOTAXIN	2900	4
RANTES	3500	5
MIG	2400	3
IFN-γ	10500	14
IL-12	6500	9
IL-2	1200	1.6
IL-15	24300	33
IL-2R	21000	29
IL-4	42600	58
IL-5	2330	3
IL-13	20300	28
IL-17	38800	53
EGF	12000	16
FGF	2400	3
HGF	7900	11
VEGF	4400	6
G-CSF	70000	96
GM-CSF	17350	24
IL-7	9800	13

Table S2. Sample size by time point and country

	Recruitment	Delivery-periphery	Delivery-placenta	Delivery-cord	Post-partum
Brazil	50	49	X	2	43
Colombia	50	50	31	13	20
Guatemala	50	50	X	21	50
India	35	34	X	X	11
PNG	50	50	74	25	49

PNG: Papua New Guinea

Table S3. Baseline characteristics

		BCN	BR	CO	GT	IN	PNG	p-value
Age (years)[a,b]		30.4 (4.4) [16]	23.4 (5.7) [114]	22.0 (5.6) [114]	25.3 (7.9) [114]	23.3 (3.3) [66]	25.9 (6.0) [132]	<0.0001[c]
GA recruitment [a]		N/R	23 (9) [111]	21 (9) [114]	27 (8) [112]	25 (7) [66]	24 (5) [138]	<0.0001[c]
Parity[d]	0	6 (37)	31 (28)	34 (30)	41 (36)	30 (45)	56 (41)	0.001[e]
	1-3	7 (44)	59 (52)	56 (49)	34 (30)	32 (49)	51 (37)	
	+4	3 (19)	23 (20)	24 (21)	39 (34)	4 (6)	30 (22)	
BMI [a,b]		26.0 (3.5) [16]	25.8 (4.4) [111]	23.3 (3.4) [113]	26.2 (3.7) [114]	22.9 (4.3) [66]	24.1 (3.5) [128]	<0.0001[c]
GA delivery[a]		39.8 (1.0) [16]	39.1(1.6) [70]	37.9(2.3) [72]	39.6(2.1) [85]	36.4(1.5) [47]	37.8(3.5) [106]	<0.0001[c]
Birth weigth[a]		3337 (490) [16]	3192 (494) [72]	3216 (406) [79]	3183 (527) [87]	2970 (452) [47]	2874 (512) [118]	<0.0001[c]
Delivery mode	V	12 (75)	59 (74)	64 (81)	62 (69)	40 (82)	119 (100)	<0.0001[f]
	C	4 (25)	21 (26)	15(19)	28 (31)	9 (18)	0 (0)	
Syphilis screening	POS	0 (0)	0 (0)	6 (8)	N/A	0 (0)	4 (5)	0.101[f]
	NEG	16 (100)	70 (100)	64 (92)	N/A	16 (100)	81 (95)	

BCN: Barcelona (Spain); BR: Brazil; CO: Colombia; GT: Guatemala; IN: India; PNG: Papua New Guinea. GA: gestational age (weeks). BMI: body mass index (kg/m^2). V: vaginal. C: cesarean section. POS: positive. NEG: negative. N/R: not relevant, samples available collected at delivery. N/A: not available. [a] Arithmetic Mean (standard deviation) [N]. [b] At recruitment. [c] One-way ANOVA. [d] N (percentage). [e] Chi-squared test.[f] Fisher's exact test.

Table S4. Correlation of gestational age at recruitment and biomarker concentration.

	rho	q-value
TNF	-0.044	0.860
IL-1β	0.0122	0.860
IL-6	0.0728	0.860
IL-10	0.0485	0.860
IL-1RA	-0.2582	0.018
TGF-β	-0.1421	0.860
IFN-α	-0.0491	0.860
IL-8	-0.0448	0.860
MIP-1α	-0.0873	0.860
MIP-1β	-0.162	0.500
MCP-1	-0.0884	0.860
IP-10	0.2179	0.042
MIG	0.0263	0.860
EOTAXIN	-0.0826	0.860
RANTES	0.0481	0.860
IFN-γ	-0.1217	0.860
IL-12	-0.2171	0.042
IL-2	-0.0241	0.860
IL-15	0.1306	0.860
IL-2R	-0.0469	0.860
IL-4	-0.0734	0.860
IL-5	-0.0414	0.860
IL-13	-0.0826	0.860
IL-17	-0.1363	0.860
EGF	-0.3215	0.003
FGF	-0.0303	0.860
HGF	-0.1596	0.530
VEGF	-0.0129	0.860
G-CSF	-0.1109	0.860
GM-CSF	-0.069	0.860
IL-7	-0.0592	0.860

Rho: Spearman's coefficient. q-value: p-value adjusted for multiple comparisons (Hochberg-Benjamini)

Table S5. Correlations between antibody levels and biomarker concentrations at recruitment.

	TNF	IL1B	IL-6	IL-10	TGF-β	IL-1RA	IFN-α	IL-8	MIP-1α	MIP-1β	MCP1	IP10	EOTAXIN	RANTES	MIG	IFN-γ	IL-12	IL-2	IL-15	IL-2R	IL-4	IL-5	IL-13	IL-17	EGF	FGF	HGF	VEGF	G-CSF	GM-CSF	IL-7
PvCSP-N	0,11	0,12	0,20	0,16	**0,40**	**0,28**	0,26	0,27	0,25	**0,41**	0,03	-0,06	-0,13	-0,04	0,01	0,06	0,19	0,22	0,07	0,08	0,11	0,08	0,12	0,11	**0,29**	0,16	**0,46**	**0,29**	-0,05	0,08	0,12
PvCSP-C	0,06	0,11	0,10	0,09	0,20	0,20	0,26	0,21	0,19	**0,29**	0,05	0,08	-0,05	-0,11	0,07	0,03	0,15	0,22	0,13	0,15	0,03	0,07	0,03	0,06	0,21	0,19	**0,30**	0,14	-0,01	0,01	0,08
PvCSP-R	0,06	0,08	0,16	0,12	0,27	0,20	0,26	0,10	0,17	0,23	0,07	0,00	-0,07	-0,16	0,00	0,07	0,22	0,24	0,11	0,16	0,06	0,13	0,11	0,10	0,24	0,22	**0,30**	0,20	0,02	0,09	0,15
PvCSP	-0,10	-0,07	-0,04	-0,04	**-0,31**	-0,24	-0,15	0,01	-0,15	-0,17	-0,03	0,05	0,14	-0,06	0,00	-0,09	-0,26	-0,03	0,05	0,12	-0,02	-0,10	-0,25	-0,07	**-0,28**	-0,05	**-0,31**	-0,12	-0,13	-0,17	-0,19
PvDBP	-0,19	0,04	0,05	-0,02	-0,07	-0,02	0,00	0,18	-0,05	0,08	-0,11	0,05	-0,08	0,08	-0,01	-0,15	0,06	0,13	0,08	0,09	-0,10	-0,13	-0,19	-0,13	-0,04	-0,04	0,03	0,00	**-0,32**	-0,15	-0,11
$PvMSP1_{19}$	-0,16	0,00	0,07	0,04	-0,17	-0,05	-0,01	0,14	-0,11	0,06	-0,08	0,07	0,05	0,17	0,03	-0,15	0,00	0,12	0,12	0,18	-0,08	-0,15	-0,23	-0,13	-0,12	-0,01	-0,07	0,07	**-0,32**	-0,11	-0,16
Pv200L	-0,13	0,07	0,10	0,09	-0,21	-0,06	0,00	0,20	-0,04	0,09	-0,02	0,06	0,07	0,05	0,06	-0,17	-0,07	0,07	0,10	0,08	-0,05	-0,19	-0,23	-0,07	-0,14	-0,01	-0,05	0,08	-0,26	-0,12	-0,21
PvMSP1-N	-0,04	0,03	-0,05	0,10	-0,27	-0,06	0,03	0,09	-0,01	0,07	-0,01	0,09	0,14	0,12	0,11	-0,02	-0,13	0,07	0,11	0,10	-0,06	-0,07	-0,11	0,02	-0,18	-0,03	-0,19	-0,03	-0,20	-0,05	-0,15
PvMSP5	-0,08	0,04	-0,04	-0,09	**-0,37**	-0,13	-0,09	0,07	-0,12	-0,03	0,01	0,06	0,20	0,12	0,08	-0,20	-0,16	0,01	0,09	0,09	-0,16	-0,17	-0,26	-0,15	**-0,29**	-0,09	-0,25	-0,06	-0,26	-0,19	-0,24
VIR25	0,12	-0,14	0,04	0,09	0,04	-0,05	0,08	-0,05	-0,02	0,02	0,02	-0,07	0,09	-0,08	-0,01	0,17	0,02	-0,02	-0,07	0,03	0,11	0,16	0,06	0,15	0,02	-0,01	-0,01	0,05	0,02	0,07	0,05
VIR5	0,01	-0,05	-0,01	0,01	-0,18	-0,10	-0,06	0,01	0,02	0,03	-0,02	-0,02	0,12	-0,03	0,04	0,10	-0,17	-0,01	-0,01	-0,01	0,04	0,07	-0,06	0,05	-0,17	-0,02	-0,14	-0,07	0,00	0,00	-0,08
LP1	0,06	0,12	0,14	0,12	0,22	0,17	0,20	0,24	0,14	0,27	0,02	0,07	0,00	-0,04	0,08	0,05	0,13	0,17	0,11	0,19	0,08	0,06	0,05	0,11	0,19	0,15	**0,28**	0,21	-0,06	0,05	0,09
LP2	-0,13	0,02	0,05	-0,01	0,02	0,03	0,06	**0,28**	0,00	0,21	-0,08	0,01	-0,02	0,03	0,01	-0,10	-0,02	0,12	0,09	0,06	-0,03	-0,10	-0,10	-0,06	0,03	-0,02	0,10	0,08	**-0,28**	-0,15	-0,12
$PfMSP1_{19}$	-0,15	0,03	0,05	-0,01	-0,26	-0,05	-0,05	0,19	-0,06	0,11	-0,08	0,11	0,07	0,17	0,09	-0,19	-0,04	0,07	0,14	0,19	-0,07	-0,16	-0,25	-0,09	-0,14	-0,07	-0,06	0,00	**-0,28**	-0,23	-0,23
PfAMA	-0,15	-0,05	0,03	-0,01	**-0,29**	-0,12	-0,12	0,20	-0,11	0,03	-0,11	0,10	0,08	0,19	0,11	-0,16	-0,08	-0,03	0,04	0,10	-0,06	-0,22	**-0,31**	-0,09	-0,23	-0,16	-0,11	-0,03	-0,27	-0,21	**-0,30**
PfEBA175	-0,14	-0,04	0,00	-0,02	-0,19	-0,07	-0,09	0,21	-0,11	0,10	-0,14	0,08	0,01	0,19	0,04	-0,11	-0,07	-0,05	0,01	0,09	-0,08	-0,17	-0,25	-0,03	-0,12	-0,16	-0,04	0,02	-0,23	-0,18	-0,21
PfDBL3x	-0,06	0,01	0,09	0,04	0,17	0,09	0,11	0,23	0,07	0,23	-0,08	-0,09	-0,09	-0,06	0,00	-0,02	0,06	0,10	-0,03	0,07	0,02	-0,04	-0,06	0,01	0,09	0,00	0,24	0,11	-0,15	-0,07	-0,01
PfDBL5ε	-0,17	0,01	0,08	-0,02	0,04	0,00	0,04	0,21	-0,04	0,12	-0,11	-0,03	-0,02	0,06	-0,01	-0,10	0,02	0,10	0,02	0,10	-0,04	-0,09	-0,11	-0,08	-0,02	0,00	0,13	0,09	-0,23	-0,16	-0,07
PfDBL6ε	-0,13	-0,02	0,07	0,00	-0,03	-0,01	-0,04	0,19	0,02	0,12	-0,07	0,00	-0,05	0,06	-0,05	-0,12	0,02	0,06	0,03	0,11	0,00	-0,07	-0,13	-0,08	-0,03	-0,04	0,03	0,05	-0,19	-0,18	-0,09

Spearman's correlation coefficient (rho, range 0-1) is shown (N=213) in a grey-color scale ranging from dark grey (Spearman's rho value=0.65) to white (Spearman's rho value=0). Bold numbers indicate p<0.05 after multiple correction adjustment by the Benjamin-Hochberg method; bold numbers and margins on a cell indicate rho>0.4 AND p<0.05.

Table S6. Correlations between antibody levels and biomarker concentrations at delivery.

	TNF	IL1B	IL-6	IL-10	TGF-β	IL-1RA	IFN-α	IL-8	MIP-1α	MIP-1β	MCP1	IP10	EOTAXIN	RANTES	MIG	IFN-γ	IL-12	IL-2	IL-15	IL-2R	IL-4	IL-5	IL-13	IL-17	EGF	FGF	HGF	VEGF	G-CSF	GM-CSF	IL-7
PvCSP-N	0,05	0,09	0,07	0,15	0,24	0,14	0,12	0,02	0,11	0,15	0,08	0,01	-0,07	0,01	0,11	0,00	-0,02	0,12	0,00	0,09	0,02	-0,04	0,23	0,01	0,16	0,09	**0,30**	0,23	-0,02	0,10	0,05
PvCSP-C	0,03	0,16	0,07	0,11	0,07	0,14	0,09	0,02	0,10	0,12	0,05	-0,01	-0,03	0,15	0,18	0,03	0,08	0,19	0,06	0,13	-0,02	0,06	0,19	0,02	0,10	0,17	0,24	0,18	-0,03	0,09	0,10
PvCSP-R	0,00	0,13	0,00	0,05	0,11	0,14	0,11	0,06	0,05	0,09	0,02	-0,07	-0,14	0,14	0,06	-0,05	0,10	0,17	0,00	0,14	-0,07	0,01	0,16	-0,03	0,08	0,14	0,23	0,13	-0,05	0,05	0,06
PvCSP	-0,17	-0,11	-0,01	0,01	-0,21	-0,17	-0,16	-0,09	-0,12	-0,10	-0,06	0,03	-0,05	0,05	-0,09	-0,13	**-0,33**	-0,07	0,03	-0,05	-0,04	-0,19	-0,25	-0,17	-0,12	-0,11	-0,20	-0,09	-0,18	-0,13	-0,27
PvDBP	-0,01	0,09	0,01	0,03	-0,12	0,08	0,09	-0,07	0,00	0,01	-0,01	0,00	0,03	0,05	0,18	-0,03	0,03	0,10	0,11	0,23	0,04	0,00	0,09	0,10	-0,05	0,06	0,13	0,09	0,04	0,04	-0,01
$PvMSP1_{19}$	-0,05	0,06	0,00	0,09	-0,18	0,01	0,00	-0,13	-0,06	-0,07	-0,07	0,05	-0,04	0,16	0,16	-0,07	-0,07	0,04	0,12	0,24	0,02	-0,01	-0,03	0,06	-0,12	0,02	-0,02	-0,01	-0,06	-0,01	-0,13
Pv200L	-0,12	0,02	0,10	0,04	**-0,34**	-0,06	-0,04	-0,08	-0,06	-0,07	-0,05	-0,10	-0,04	0,18	0,09	-0,22	-0,16	-0,04	0,00	0,16	-0,09	-0,12	-0,10	-0,03	-0,21	-0,01	-0,05	0,01	-0,08	-0,03	-0,19
PvMSP1-N	-0,03	-0,01	-0,07	0,10	-0,32	-0,10	-0,03	-0,17	-0,03	-0,07	-0,04	0,00	0,06	0,09	0,09	-0,08	-0,19	-0,03	0,07	0,07	0,03	-0,08	-0,17	0,05	-0,12	-0,03	-0,14	-0,05	-0,14	-0,03	-0,11
PvMSP5	-0,05	-0,04	0,08	0,07	**-0,42**	-0,09	-0,07	-0,14	-0,05	-0,10	0,01	0,02	0,05	0,05	0,11	-0,18	-0,23	-0,08	0,12	0,13	-0,05	-0,07	-0,20	-0,06	**-0,28**	-0,08	-0,15	-0,06	-0,11	-0,10	-0,25
VIR25	-0,13	-0,14	-0,11	-0,08	0,11	-0,15	-0,01	-0,11	-0,10	-0,09	-0,02	0,00	0,01	-0,03	0,04	-0,06	-0,15	-0,01	-0,02	-0,10	0,04	-0,04	-0,05	-0,09	-0,02	-0,11	-0,14	-0,06	-0,17	-0,13	-0,07
VIR5	-0,11	-0,10	0,00	0,05	-0,23	-0,19	-0,15	-0,09	-0,08	-0,05	-0,07	-0,03	0,01	0,14	0,05	-0,13	-0,21	-0,03	-0,04	-0,11	-0,09	-0,05	-0,08	-0,13	-0,20	-0,07	-0,20	-0,06	-0,13	-0,07	-0,07
LP1	0,08	0,13	-0,01	0,07	0,01	0,12	0,11	-0,05	0,06	0,11	0,03	-0,04	-0,03	0,09	0,16	-0,01	0,03	0,15	0,06	0,17	0,03	0,02	0,17	0,07	0,05	0,10	0,20	0,16	-0,01	0,08	0,05
LP2	-0,06	0,07	0,07	0,04	-0,04	0,06	0,03	0,01	0,02	0,06	0,02	-0,07	0,03	0,13	0,17	-0,03	-0,08	0,09	0,05	0,14	-0,01	-0,05	0,12	0,02	-0,04	0,06	0,17	0,14	-0,03	-0,02	0,02
$PfMSP1_{19}$	-0,13	-0,11	0,00	-0,04	**-0,34**	-0,12	-0,13	-0,11	-0,15	-0,11	-0,14	-0,14	-0,11	0,22	0,06	-0,14	-0,24	-0,09	-0,04	0,13	-0,03	-0,11	-0,08	-0,11	-0,26	-0,13	-0,13	-0,13	-0,14	-0,07	-0,16
PfAMA	-0,14	-0,09	0,04	0,01	-0,28	-0,17	-0,16	-0,08	-0,10	-0,12	-0,10	-0,12	0,03	0,14	0,12	-0,11	**-0,29**	-0,10	0,00	0,11	-0,05	-0,14	-0,13	-0,07	-0,25	-0,15	-0,12	-0,09	-0,15	-0,08	-0,19
PfEBA175	-0,11	-0,06	0,11	-0,02	-0,18	-0,16	-0,16	-0,03	-0,09	-0,08	-0,11	-0,02	-0,11	0,14	0,04	-0,14	-0,27	-0,07	-0,04	0,02	-0,10	-0,21	-0,08	-0,11	-0,21	-0,07	-0,08	0,01	-0,11	-0,09	-0,19
PfDBL3x	-0,02	0,07	0,09	-0,01	0,10	0,04	0,01	0,02	-0,01	0,05	0,02	-0,05	-0,04	0,00	0,12	-0,05	-0,12	0,06	-0,05	0,00	-0,05	-0,11	0,11	-0,05	0,03	0,05	0,18	0,11	-0,08	-0,06	-0,02
PfDBL5ε	-0,13	0,01	-0,02	0,00	-0,04	0,01	-0,03	-0,01	-0,02	0,04	-0,02	-0,05	-0,02	0,12	0,07	-0,18	-0,16	0,00	-0,02	0,08	-0,11	-0,10	0,06	-0,11	-0,04	0,00	0,08	0,05	-0,13	-0,07	-0,06
PfDBL6ε	-0,14	-0,01	0,12	-0,03	-0,13	-0,09	-0,13	0,03	-0,08	-0,01	-0,12	0,07	-0,06	0,18	0,04	-0,19	-0,17	-0,09	0,04	0,07	-0,11	-0,10	-0,04	-0,13	-0,14	-0,02	-0,04	0,04	-0,11	-0,09	-0,14

Spearman's correlation coefficient (rho, range 0-1) is shown (N=213) in a grey-color scale ranging from dark grey (Spearman's rho value=0.65) to white (Spearman's rho value=0). Bold numbers indicate p<0.05 after multiple correction adjustment by the Benjamin-Hochberg method; bold numbers and margins on a cell indicate rho>0.4 AND p<0.05.