

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Dobaño, C;Bardají, A;Kochar, S;Kochar, SK;Padilla, N;López, M;Unger, HW;Ome-Kaius, M;Castellanos, ME;Arévalo-Herrera, M;Hans, D;Martínez-Espinosa, FE;Bôtto-Menezes, C;Malheiros, A;Desai, M;Casellas, A;Chitnis, CE;Rogerson, S;Mueller, I;Menéndez, C;Requena, P

Title:

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

Date:

2020-01-01

Citation:

Dobaño, C., Bardají, A., Kochar, S., Kochar, S. K., Padilla, N., López, M., Unger, H. W., Ome-Kaius, M., Castellanos, M. E., Arévalo-Herrera, M., Hans, D., Martínez-Espinosa, F. E., Bôtto-Menezes, C., Malheiros, A., Desai, M., Casellas, A., Chitnis, C. E., Rogerson, S., Mueller, I. ,... Requena, P. (2020). Blood cytokine, chemokine and growth factor profiling in a cohort of pregnant women from tropical countries. *Cytokine*, 125, <https://doi.org/10.1016/j.cyto.2019.154818>.

Persistent Link:

<https://hdl.handle.net/11343/258767>

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

3 Carlota Dobaño^{a,*}, Azucena Bardají^a, Swati Kochar^b, Sanjay K. Kochar^b, Norma Padilla^c, Marta
4 López^d, Holger W. Unger^e, Maria Ome-Kaius^e, Maria Eugenia Castellanos^c, Myriam Arévalo-
5 Herrera^f, Dhiraj Hans^g, Flor E. Martínez-Espinosa^{h,i}, Camila Bôtto-Menezes^{h,j}, Adriana
6 Malheiros^k, Meghna Desai^l, Aina Casellas^a, Chetan E. Chitnis^{g,m}, Stephen Rogersonⁿ, Ivo
7 Mueller^o, Clara Menéndez^a, Pilar Requena^{a,p,*}

8 ^aISGlobal, Hospital Clínic - Universitat de Barcelona, Carrer del Rosselló, 132, 08036
9 Barcelona, Spain.

10 ^bMedical College, PBM Hospital, Bikaner, Rajasthan 334001, India.

11 ^cCentro de Estudios en Salud, Universidad del Valle de Guatemala, 18 Avenida 11-95
12 Guatemala, 01015, Guatemala.

13 ^dDepartment of Maternal-Fetal Medicine, Hospital Clínic-IDIBAPS, CIBER-ER, Carrer del
14 Rosselló, 149, 08036 Barcelona, Spain.

15 ^ePapua New Guinea Institute of Medical Research, P.O.Box 378. Madang, 511, Papua New
16 Guinea.

17 ^fCaucaseco Scientific Research Center, Km 6 Vía Cali-Puerto Tejada, Cali, Colombia.

18 ^gInternational Center for Genetic Engineering and Biotechnology, Jawaharlal Nehru University,
19 Aruna Asaf Ali Marg, New Delhi, Delhi 110067, India.

20 ^hFundação de Medicina Tropical Dr. Heitor Vieira Dourado, Av. Pedro Teixeira, s/n - Dom
21 Pedro, Manaus - AM, 69040-000, Brazil.

22 ⁱInstituto Leônidas e Maria Deane, Rua Teresina, 476 – Adrianópolis, Manaus, 69.057-070,
23 Brazil.

24 ^jUniversidade do Estado do Amazonas, 69850-000, R. Bloco Um e Três, 4-40 - Platô do Piquiá,
25 Boca do Acre - AM, 69850-000, Brazil.

26 ^kInstituto de Ciências Biológicas, Universidade Federal do Amazonas, Av. Jauary Marinho -
27 Setor Sul - Coroado, Manaus - AM, Brazil.

28 ^lCenters for Disease Control and Prevention, Division of Parasitic Diseases and Malaria,
29 Malaria Branch, 1600 Clifton Rd, Atlanta, GA 30333, USA.

30 ^mMalaria Parasite Biology and Vaccines Unit, Institut Pasteur, 25-28 Rue du Dr Roux, 75015,
31 Paris, France.

32 ⁿUniversity of Melbourne, Parkville VIC 3010, Australia.

33 ^oWalter and Eliza Hall Institute, 1G, Royal Parade, Parkville VIC 3052, Australia.

34 ^pDepartamento de Medicina Preventiva y Salud Pública, Universidad de Granada, Facultad de
35 Farmacia, Campus de Cartuja, 18071, Granada, Spain.

36 * **Correspondence:** pilar.requena.mendez@gmail.com, carlota.dobano@isglobal.org

37 **ABSTRACT**

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

61

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

64

65 **1. Introduction**

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

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

85 Immunity is influenced by heritability and the environment (11), including exposure
86 to infectious diseases. We have previously described how both pregnancy and malaria
87 can have distinct effects on B and T cells, as well as *Plasmodium*-specific cytokine
88 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

107 **2. Materials and methods**

108 **2.1. Study design and population**

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

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

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

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

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

140 **2.2. Isolation of plasma**

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

148 **2.3. Multiplex bead array assay**

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

155 IL-1 β , IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8/CXCL8, IL-10, IL-12(p40/p70), IL-13, IL-
156 15, IL-17, IFN- γ induced protein (IP-10/CXCL10), monocyte chemoattractant protein
157 (MCP-1/CCL2), monokine induced by IFN- γ (MIG/CXCL9), macrophage inflammatory
158 protein (MIP)-1 α /CCL3, MIP-1 β /CCL4, regulated on activation, normal T cell
159 expressed and secreted (RANTES/CCL5), tumor necrosis factor (TNF), and vascular
160 endothelial growth factor (VEGF). Fifty μ L of the plasma samples were tested in single
161 replicates (dilution 1:2, recommended by the vendor) in 96-well flat-bottom plates.
162 Each plate contained serial dilutions (1:3) in duplicates of a standard sample of known
163 concentration of each analyte provided by the manufacturer, as well as two blank
164 controls and a control in duplicate of medium concentration prepared from a reference
165 sample for quality control purposes. Upper and lower values of the standard curves for
166 each analyte are displayed in Supplementary table 1.

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

179 In addition, the cytokine transforming growth factor (TGF)- β 1 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- β 1 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-
188 1₁₉ (18), PfAMA-1 (19), PfEBA175 (PfF2) (20), DBL3X, DBL5 ϵ , DBL6 ϵ (21), Pv200L
189 (PvMSP1₁₂₁₋₄₁₆) (22), PvMSP-1₁₉ (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

203 placental plasma samples or paired periphery and cord plasma samples, a Wilcoxon
204 matched-pairs signed rank test was performed. P-values were corrected for multiple
205 comparisons with the Benjamini-Hochberg test. To compare biomarker concentrations
206 among sites at delivery, a Kruskal-Wallis test was performed followed by Dunn's
207 multiple comparisons test of BCN vs. each other country. Additionally, a principal
208 component analysis (PCA) was performed to assess how a) cytokines and b) study
209 subjects analyzed at delivery clustered, excluding TGF- β as this cytokine was analyzed
210 by a different technique (ELISA) and was not measured in IN. To analyze the
211 association between biomarker concentrations and anti-malarial antibody levels, the
212 Spearman's correlation test was used and the result classified based on the rho
213 coefficient as: very weak (0-|0.19|), weak (|0.2|-|0.39|), moderate (|0.4|-|0.59|), strong
214 (|0.6|-|0.79|) and very strong (|0.8|-|1|). Only results with p-value <0.05 (after
215 adjustment for multiple comparisons, Benjamini-Hochberg test) and rho value >0.4
216 were considered 'biologically relevant'.

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

220

221 **3. Results**

222 **3.1. Study population**

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

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

230 **3.2. Effect of pregnancy and labor on plasma biomarker concentrations**

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

236

237

238

239

240

241

242

243

244

245

246

247

248

249

250

251

252

253

254

		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

255

256

257 Table 1. Pregnancy and labor effect on biomarker concentration.

258

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

262

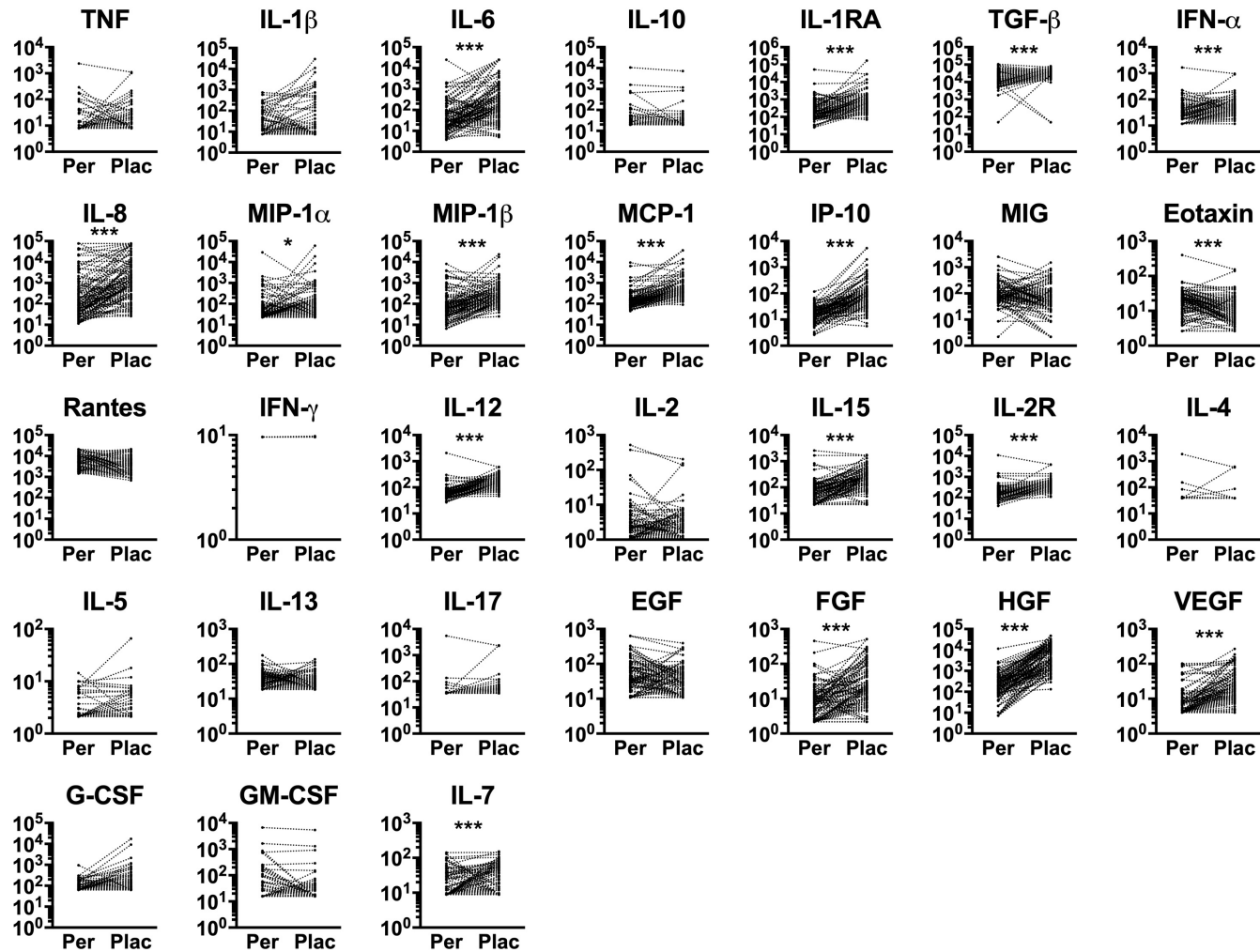
263 As concentrations at delivery were significantly higher than at recruitment for many
264 biomarkers, we wondered whether there was a gradual increase throughout pregnancy
265 or rather a labor-specific up-regulation or release of biomarkers. At recruitment, the
266 correlation between the biomarker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

269 **3.3. Comparison of biomarker concentrations in placental, cord and peripheral** 270 **blood at delivery**

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

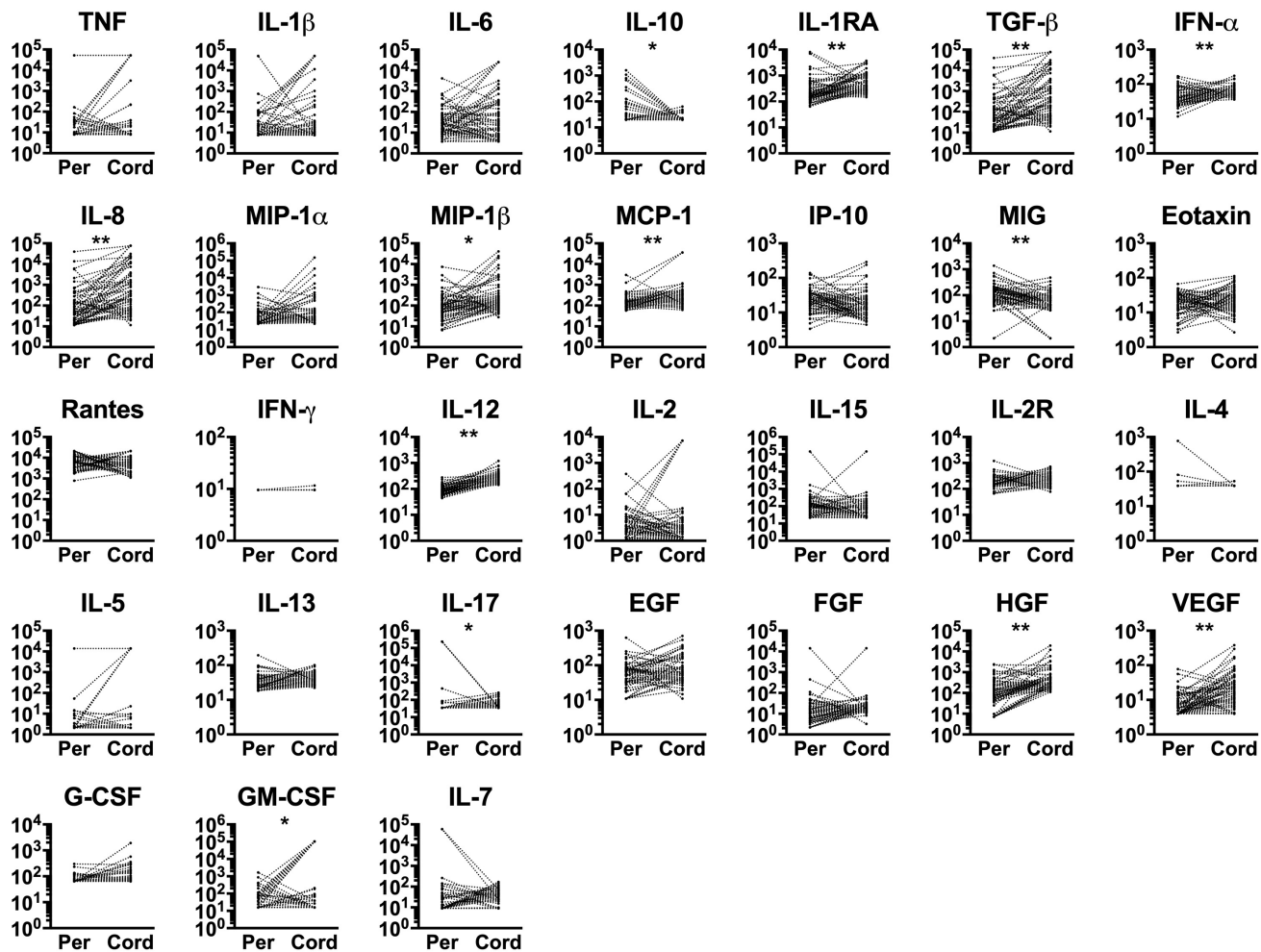
274 For the biomarkers with significant differences between placenta and periphery,
275 concentrations were always higher in placental than peripheral blood: pro-inflammatory
276 biomarkers IL-6, IL-8, MIP-1 α , MIP-1 β , MCP-1, IP-10; anti-inflammatory cytokines
277 TGF- β , IL-1RA, IFN- α ; T_H1-related cytokines IL-12, IL-15, IL-2R; and growth factors
278 VEGF, FGF, HGF, IL-7 (Fig. 1). This variation was very pronounced for HGF, with a
279 25 fold-change (Fig. 1). There was only one exception, eotaxin, whose concentration
280 was significantly lower in placenta compared to peripheral plasma (Fig. 1).

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



286

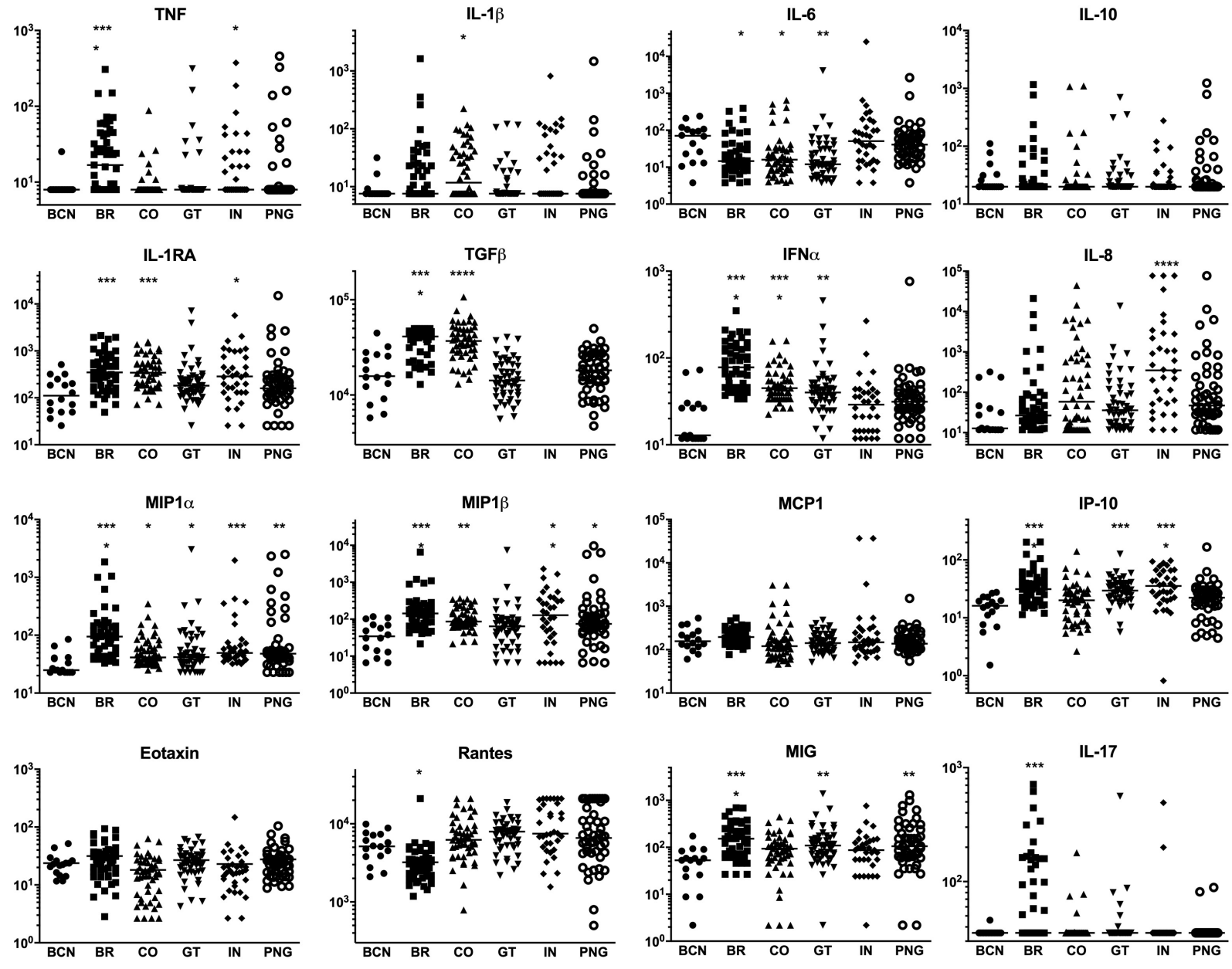
287 **Figure 1.** Peripheral and placental plasma biomarker concentrations. Graphs depict biomarker concentrations in paired peripheral
 288 (Per) and placental (Pla) plasmas obtained at delivery. Concentrations for all biomarkers are expressed in pg/mL. N= 101, samples
 289 belong to women from Colombia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



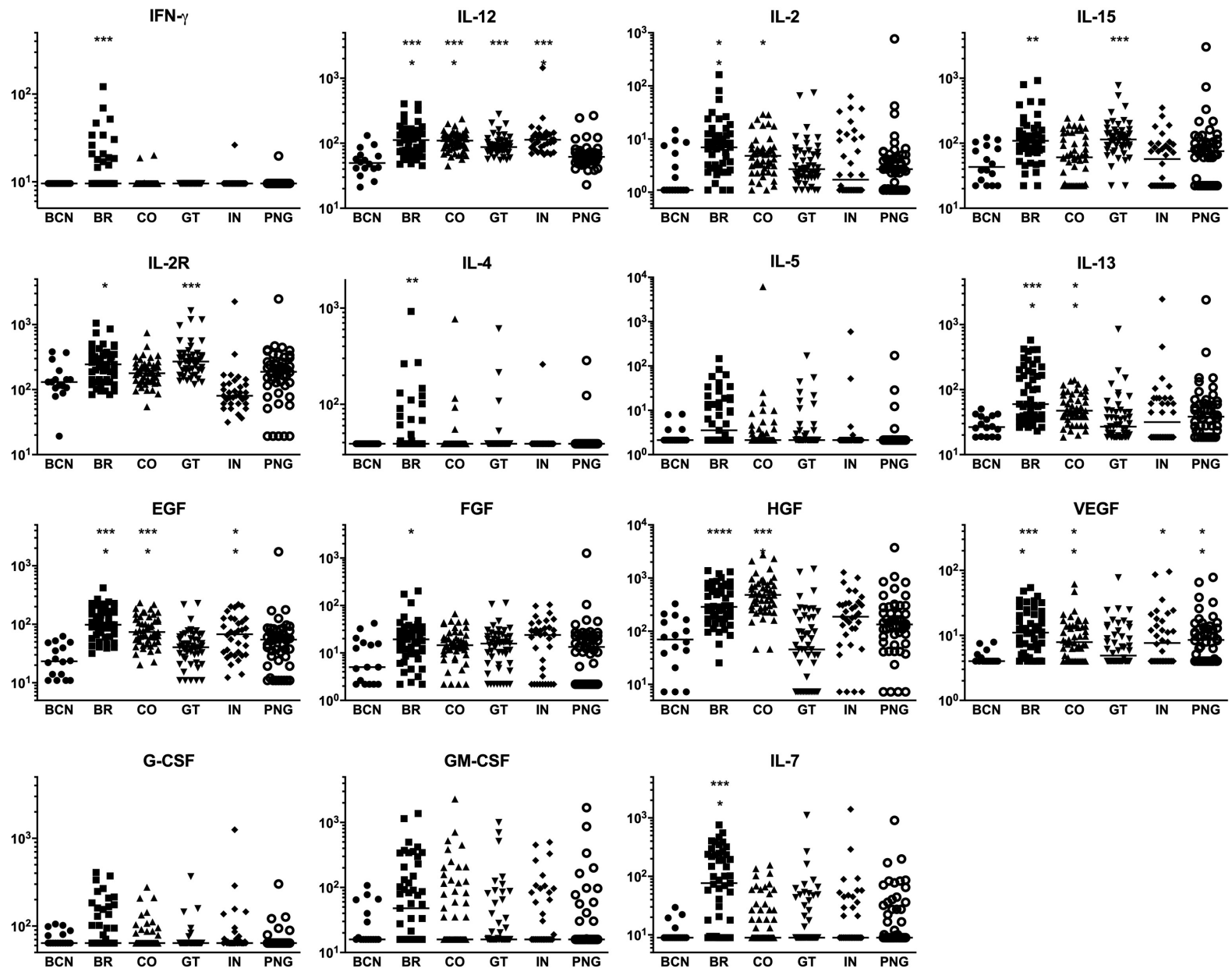
291

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



3B



298

299 **Figure 3.** Biomarker concentrations at delivery by country.

300 Graphs depict biomarker concentrations in peripheral plasma at delivery in individual countries. BCN: Barcelona control group, N=16;
301 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
302 to a Kruskal-Wallis test followed by Dunn's multiple comparisons test of BCN *versus* each other country. *p<0.05, **p<0.01,
303 ***p<0.001.

304 **3.4. Plasma biomarker concentrations at delivery vary between tropical**
305 **countries and the control group**

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

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

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

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

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

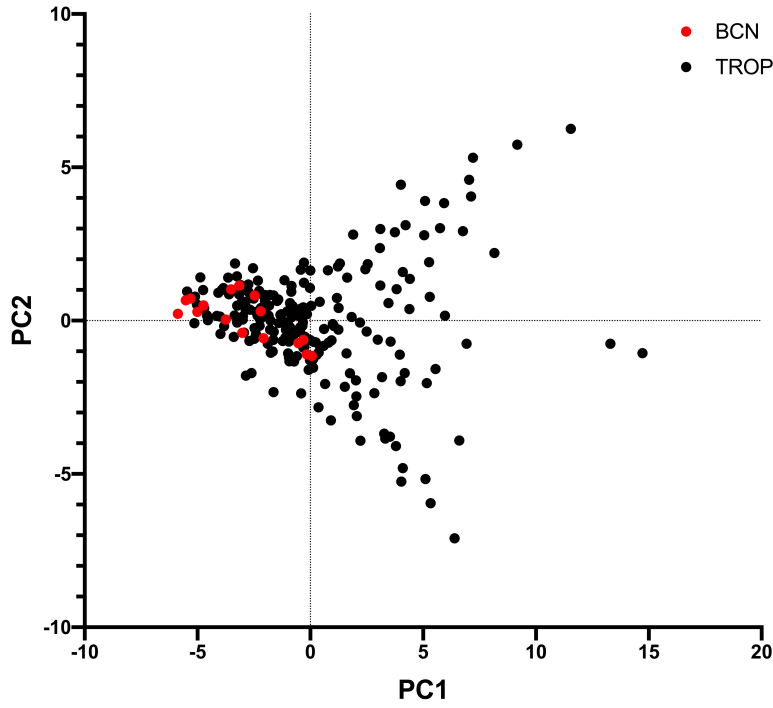
345

346

347

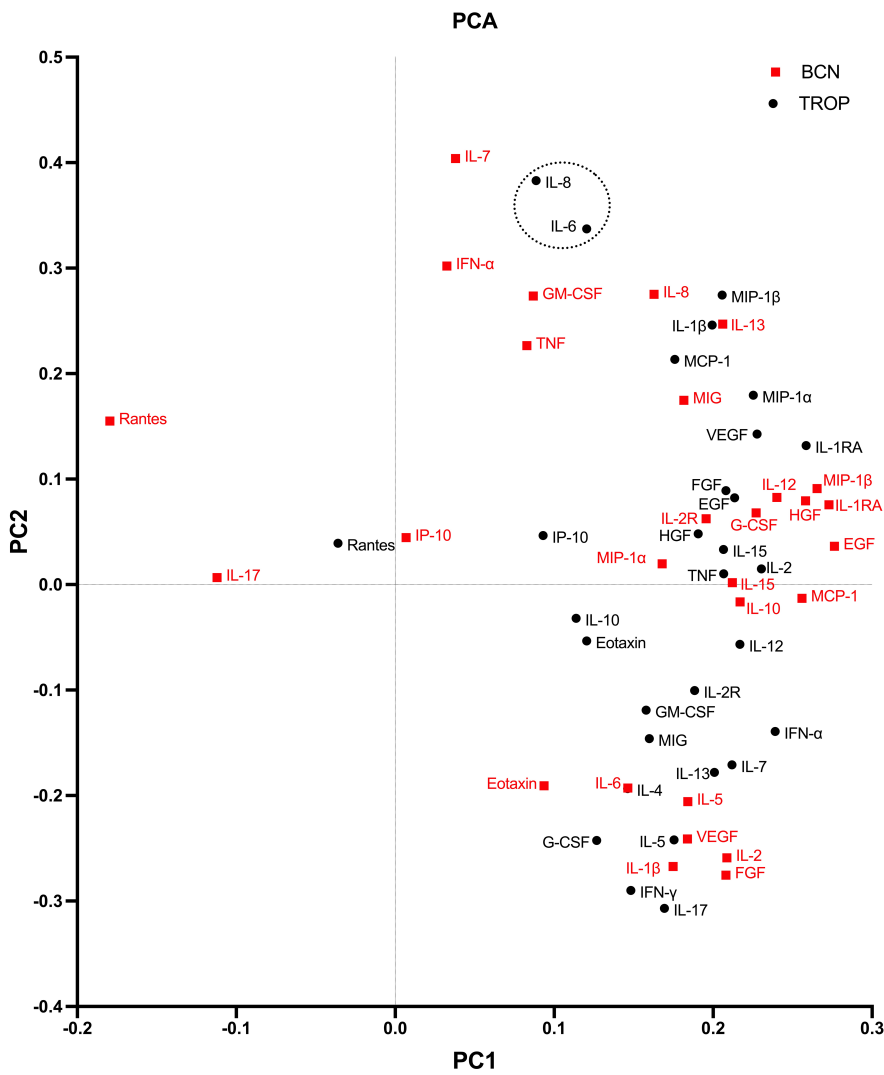
348

4A



349

4B



350

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

356

357 **3.5. Plasma cytokine concentrations correlate with *Plasmodium* antibody levels** 358 **at postpartum but not during pregnancy**

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

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

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

376 both species and anti-PfDBL6 ϵ antibodies, while IL-10, TGF- β and IL-1RA did not show
377 any biologically relevant correlations with antibody levels (Table 2).

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

381 Finally, the correlation pattern between antibodies and growth factors was very similar
382 to the T_H-related cytokines, with consistently negative moderate correlations between
383 several (remarkably G-CSF, GM-CSF and IL-7) and antibodies against merozoite
384 antigens of both species and PfDBL6 ϵ (Table 2).

385

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					Th1			Th2			Th17	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.

390 **4. Discussion**

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

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

415 higher concentrations of proinflammatory cytokines observed at delivery. The TGF- β
416 concentration and role have been assessed mainly in placenta/decidua at early
417 pregnancy and in cord blood at delivery. Two studies showed higher levels of this anti-
418 inflammatory cytokine in cord blood after spontaneous vaginal delivery compared to
419 elective caesarean section (with no labor) in term pregnancies (30,31). Thus, while in
420 early pregnancy TGF- β is known to inhibit trophoblast invasion of uterine arteries (32),
421 which may be associated with intrauterine growth retardation and preeclampsia, during
422 labor TGF- β may counteract the excessive inflammation in the placenta/fetal interface
423 (30,31). HGF has a role in cell proliferation, migration, and morphogenesis in different
424 tissues and its elevated levels in placenta are well recognized (33).

425 Our results show overall higher biomarker concentrations in both placenta (maternal
426 side) and cord blood than in periphery. This suggests that either they are produced by
427 the mother and traverse the placenta getting accumulated in the fetal circulation, or
428 they are in fact produced by the fetus (9). However, a few exceptions were found in
429 our study. First, MIG presented lower levels in cord and placenta. This chemokine is
430 silenced in the murine decidua (34) and elevated MIG levels have been associated
431 with poor outcomes in malaria-infected pregnant women (35,36) and in pregnant
432 women with autoimmune thyroiditis (37). This might be the reason for presenting lower
433 levels in the placenta and cord, compartments that are more related to the fetus.
434 Second, eotaxin concentration was remarkably lower in placental than in peripheral
435 plasma. The eotaxin receptor CCR3 is present in the placenta and the interaction with
436 eotaxin-2/CCL24 *in vitro* favors decidualization (38) that is essential in early
437 pregnancy. Thus, a reduced concentration of competitor eotaxin/CCL11 in the placenta
438 seems logical in the context of uncomplicated pregnancies. Unfortunately, we could
439 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 homogeneously 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

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

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

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

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

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

500

501 **Acknowledgments**

502 The authors thank all the volunteers who consented to participate in this study, the
503 staff involved in field and laboratory work at each institution; Tamara Tilburgs, Gemma
504 Moncunill and Ruth Aguilar for help with cytokine data analysis; and Mireia Piqueras,
505 Sam Mardell, and Laura Puyol for management and administrative support.

506

507 **Funding**

508 The PREGVAX project received funding from the European Union Seventh Framework
509 Programme (FP7/2007-2013) under grant agreement 201588, and co-funding from the
510 Ministerio de Economía y Competitividad (National R&D Internationalization
511 Programme, EUROSALUD 2008, Spain) under grant agreement EUS2009-03560.

512 The Latin American sites received co-funding from the Centers for Disease Control
513 and Prevention (CDC) Foundation, which received a grant from the Malaria in
514 Pregnancy Consortium (MiPc) which is partially funded through a grant from the Bill &

515 Melinda Gates Foundation to the Liverpool School of Tropical Medicine (46099). The
516 studies in PNG also received co-funding from the MiPc. CD was supported by a
517 fellowship from the Ministerio de Economía y Competitividad (RYC-2008-02631) and
518 was affiliate of the EU FP7 Network of Excellence EviMalaR, and IM was supported by
519 a National Health and Medical Research Council Senior Research Fellowship
520 (GNT1043345). The funders had no role in study design, data collection and analysis,
521 decision to publish, or preparation of the manuscript. ISGlobal is a member of the
522 CERCA Program, Generalitat de Catalunya.

523

524 **Declaration of interest**

525 The authors declare no conflicts of interest.

526

527 **Authorship contribution statement**

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

530 Azucena Bardají, Swati Kochar, Sanjay K. Kochar, Norma Padilla, Marta López, Holger
531 W. Unger, Maria-Ome-Kaius, Maria Eugenia Castellanos, Myriam Arévalo-Herrera,
532 Dhiraj Hans, Flor E. Martínez-Espinosa, Camila Bôtto-Menezes, Adriana Malheiros:
533 Conceptualization, Recruitment of study subjects, Data curation, Funding acquisition,
534 Writing - review & editing.,

535 Stephen Rogerson, Meghna Desai, Ivo Mueller, Chetan E. Chitnis, Clara Menéndez:
536 Conceptualization, Funding acquisition, Writing - review & editing.

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

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

540

541

542 **Figure legends**

543

544 **Figure 1.** Peripheral and placental plasma biomarker concentrations.

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

549

550 **Figure 2.** Peripheral and cord plasma biomarker concentrations.

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

555

556 **Figure 3.** Biomarker concentrations at delivery by country.

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

562

563 **Figure 4.** A principal component (PC) analysis of all biomarkers except TGF- β
564 analyzed in peripheral plasmas collected at delivery from the tropical cohort (TROP,
565 N=233) and Barcelona control cohort (BCN, N=16) was performed. A) Predicted PC1
566 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.

569

570 **Bibliography**

- 571 1. Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T.
572 Rate of Pregnancy-Related Relapse in Multiple Sclerosis. *N Engl J Med* (1998)
573 **339**:285–291. doi:10.1056/NEJM199807303390501
- 574 2. Ostensen M. Sex hormones and pregnancy in rheumatoid arthritis and
575 systemic lupus erythematosus. *Ann N Y Acad Sci* (1999) **876**:131–43;
576 discussion 144. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/10415601>
577 [Accessed March 3, 2017]
- 578 3. Champion EW, Kourtis AP, Read JS, Jamieson DJ. Pregnancy and Infection. *N*
579 *Engl J Med* (2014) **23370**:2211–8. doi:10.1056/NEJMra1213566
- 580 4. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine
581 interactions in the maternal-fetal relationship: is successful pregnancy a TH2
582 phenomenon? *Immunol Today* (1993) **14**:353–356. doi:10.1016/0167-
583 5699(93)90235-D
- 584 5. Chatterjee P, Chiasson VL, Bounds KR, Mitchell BM. Regulation of the Anti-
585 Inflammatory Cytokines Interleukin-4 and Interleukin-10 during Pregnancy.
586 *Front Immunol* (2014) **5**:1–6. doi:10.3389/fimmu.2014.00253
- 587 6. Kraus TA, Sperling RS, Engel SM, Lo Y, Kellerman L, Singh T, Loubeau M, Ge
588 Y, Garrido JL, Rodríguez-García M, et al. Peripheral blood cytokine profiling
589 during pregnancy and post-partum periods. *Am J Reprod Immunol* (2010)
590 **64**:411–26. doi:10.1111/j.1600-0897.2010.00889.x
- 591 7. Hanna N, Hanna I, Hleb M, Wagner E, Dougherty J, Balkundi D, Padbury J,
592 Sharma S. Gestational age-dependent expression of IL-10 and its receptor in

- 593 human placental tissues and isolated cytotrophoblasts. *J Immunol* (2000)
594 **164**:5721–8. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/10820249>
595 [Accessed November 9, 2018]
- 596 8. Stokkeland LMT, Giskeødegård GF, Stridsklev S, Ryan L, Steinkjer B,
597 Tangerås LH, Vanky E, Iversen AC. Serum cytokine patterns in first half of
598 pregnancy. *Cytokine* (2019) **119**:188–196. doi:10.1016/j.cyto.2019.03.013
- 599 9. Mir IN, Chalak LF, Liao J, Johnson-Welch S, Brown LS, Longoria C, Savani
600 RC, Rosenfeld CR. Fetal-placental crosstalk occurs through fetal cytokine
601 synthesis and placental clearance. *Placenta* (2018) **69**:1–8.
602 doi:10.1016/j.placenta.2018.07.006
- 603 10. Unal ER, Cierny JT, Roedner C, Newman R, Goetzl L. Maternal inflammation
604 in spontaneous term labor. in *American Journal of Obstetrics and Gynecology*,
605 223.e1–5. doi:10.1016/j.ajog.2011.01.002
- 606 11. Pappalardo JL, Hafler DA. The Human Functional Genomics Project:
607 Understanding Generation of Diversity. *Cell* (2016) **167**:894–896.
608 doi:10.1016/j.cell.2016.10.040
- 609 12. Requena P, Barrios D, Robinson LJ, Samol P, Umbers AJ, Wangnapi R, Ome-
610 Kaius M, Rosanas-Urgell A, Mayor A, López M, et al. Proinflammatory
611 responses and higher IL-10 production by T cells correlate with protection
612 against malaria during pregnancy and delivery outcomes. *J Immunol* (2015)
613 **194**:3275–85. doi:10.4049/jimmunol.1401038
- 614 13. Requena P, Campo JJ, Umbers AJ, Ome M, Wangnapi R, Barrios D, Robinson
615 LJ, Samol P, Rosanas-Urgell A, Ubillos I, et al. Pregnancy and malaria

- 616 exposure are associated with changes in the B cell pool and in plasma eotaxin
617 levels. *J Immunol* (2014) **193**:2971–83. doi:10.4049/jimmunol.1401037
- 618 14. Requena P, Rui E, Padilla N, Martínez-Espinosa FE, Castellanos ME, Bôtto-
619 Menezes C, Malheiro A, Arévalo-Herrera M, Kochar S, Kochar SK, et al.
620 Plasmodium vivax VIR Proteins Are Targets of Naturally-Acquired Antibody
621 and T Cell Immune Responses to Malaria in Pregnant Women. *PLoS Negl*
622 *Trop Dis* (2016) **10**:e0005009. doi:10.1371/journal.pntd.0005009
- 623 15. Weiss GE, Crompton PD, Li S, Walsh L a, Moir S, Traore B, Kayentao K,
624 Ongoiba A, Doumbo OK, Pierce SK. Atypical memory B cells are greatly
625 expanded in individuals living in a malaria-endemic area. *J Immunol* (2009)
626 **183**:2176–82. doi:10.4049/jimmunol.0901297
- 627 16. Illingworth J, Butler NS, Roetynck S, Mwacharo J, Pierce SK, Bejon P,
628 Crompton PD, Marsh K, Ndungu FM. Chronic exposure to Plasmodium
629 falciparum is associated with phenotypic evidence of B and T cell exhaustion. *J*
630 *Immunol* (2013) **190**:1038–47. doi:10.4049/jimmunol.1202438
- 631 17. Scholzen A, Teirlinck AC, Bijker EM, Roestenberg M, Hermsen CC, Hoffman
632 SL, Sauerwein RW. BAFF and BAFF Receptor Levels Correlate with B Cell
633 Subset Activation and Redistribution in Controlled Human Malaria Infection. *J*
634 *Immunol* (2014) **192(8)**:3719–29. doi:10.4049/jimmunol.1302960
- 635 18. Mazumdar S, Sachdeva S, Chauhan VS, Yazdani SS. Identification of
636 cultivation condition to produce correctly folded form of a malaria vaccine
637 based on Plasmodium falciparum merozoite surface protein-1 in Escherichia
638 coli. *Bioprocess Biosyst Eng* (2010) **33**:719–30. doi:10.1007/s00449-009-0394-
639 x

- 640 19. Kocken CHM, Withers-Martinez C, Dubbeld MA, van der Wel A, Hackett F,
641 Valderrama A, Blackman MJ, Thomas AW. High-level expression of the
642 malaria blood-stage vaccine candidate *Plasmodium falciparum* apical
643 membrane antigen 1 and induction of antibodies that inhibit erythrocyte
644 invasion. *Infect Immun* (2002) **70**:4471–6. Available at:
645 [http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=128198&tool=pmcen](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=128198&tool=pmcentrez&rendertype=abstract)
646 [trez&rendertype=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=128198&tool=pmcentrez&rendertype=abstract) [Accessed August 29, 2012]
- 647 20. Pandey KC, Singh S, Pattnaik P, Pillai CR, Pillai U, Lynn A, Jain SK, Chitnis
648 CE. Bacterially expressed and refolded receptor binding domain of
649 *Plasmodium falciparum* EBA-175 elicits invasion inhibitory antibodies. *Mol*
650 *Biochem Parasitol* (2002) **123**:23–33. Available at:
651 <http://www.ncbi.nlm.nih.gov/pubmed/12165386> [Accessed October 1, 2012]
- 652 21. Mayor A, Kumar U, Bardají A, Gupta P, Jiménez A, Hamad A, Sigauque B,
653 Singh B, Quintó L, Kumar S, et al. Improved pregnancy outcomes in women
654 exposed to malaria with high antibody levels against *Plasmodium falciparum*. *J*
655 *Infect Dis* (2013) **207**:1664–74. doi:10.1093/infdis/jit083
- 656 22. Valderrama-Aguirre A, Quintero G, Gómez A, Castellanos A, Pérez Y, Méndez
657 F, Arévalo-Herrera M, Herrera S. Antigenicity, immunogenicity, and protective
658 efficacy of *Plasmodium vivax* MSP1 PV200I: a potential malaria vaccine
659 subunit. *Am J Trop Med Hyg* (2005) **73**:16–24. Available at:
660 <http://www.ncbi.nlm.nih.gov/pubmed/16291762>
- 661 23. Devi YS, Mukherjee P, Yazdani SS, Shakri AR, Mazumdar S, Pandey S,
662 Chitnis CE, Chauhan VS. Immunogenicity of *Plasmodium vivax* combination
663 subunit vaccine formulated with human compatible adjuvants in mice. *Vaccine*

- 664 (2007) **25**:5166–74. doi:10.1016/j.vaccine.2007.04.080
- 665 24. Herrera S, Bonelo A, Perlaza BL, Valencia AZ, Cifuentes C, Hurtado S,
666 Quintero G, López JA, Corradin G, Arévalo-Herrera M. Use of long synthetic
667 peptides to study the antigenicity and immunogenicity of the *Plasmodium vivax*
668 circumsporozoite protein. *Int J Parasitol* (2004) **34**:1535–46.
669 doi:10.1016/j.ijpara.2004.10.009
- 670 25. Rui E, Fernandez-Becerra C, Takeo S, Sanz S, Lacerda MV, Tsuboi T, Del
671 Portillo H a. *Plasmodium vivax*: comparison of immunogenicity among proteins
672 expressed in the cell-free systems of *Escherichia coli* and wheat germ by
673 suspension array assays. *Malar J* (2011) **10**:192. doi:10.1186/1475-2875-10-
674 192
- 675 26. Singh S, Pandey K, Chattopadhyay R, Yazdani SS, Lynn A, Bharadwaj A,
676 Ranjan A, Chitnis C. Biochemical, biophysical, and functional characterization
677 of bacterially expressed and refolded receptor binding domain of *Plasmodium*
678 *vivax* duffy-binding protein. *J Biol Chem* (2001) **276**:17111–6.
679 doi:10.1074/jbc.M101531200
- 680 27. Bernabeu M, Lopez FJ, Ferrer M, Razaname A, Corradin G, Maier AG, Portillo
681 HA. Functional analysis of *Plasmodium vivax* VIR proteins reveals different
682 subcellular localizations and cytoadherence to the ICAM-1 endothelial receptor.
683 *Cell Microbiol* (2012) **14**:386–400. doi:10.1111/j.1462-5822.2011.01726.x
- 684 28. Requena P, Arévalo-Herrera M, Menegon M, Martínez-Espinosa FE, Padilla N,
685 Bôtto-Menezes C, Malheiro A, Hans D, Castellanos ME, Robinson L, et al.
686 Naturally acquired binding-inhibitory antibodies to *Plasmodium vivax* duffy
687 binding protein in pregnant women are associated with higher birth weight in a

- 688 multicenter study. *Front Immunol* (2017) **8**: doi:10.3389/fimmu.2017.00163
- 689 29. Shynlova O, Lee Y-H, Srihajan K, Lye SJ. Physiologic Uterine Inflammation
690 and Labor Onset. *Reprod Sci* (2013) **20**:154–167.
691 doi:10.1177/1933719112446084
- 692 30. Briana DD, Liosi S, Gourgiotis D, Boutsikou M, Marmarinos A, Baka S,
693 Hassiakos D, Malamitsi-Puchner A. Fetal concentrations of the growth factors
694 TGF- α and TGF- β 1 in relation to normal and restricted fetal growth at term.
695 *Cytokine* (2012) **60**:157–161. doi:10.1016/j.cyto.2012.06.005
- 696 31. Tutdibi E, Hunecke A, Lindner U, Monz D, Gortner L. Levels of cytokines in
697 umbilical cord blood in relation to spontaneous term labor. *J Perinat Med*
698 (2012) **40**:527–32. doi:10.1515/jpm-2011-0204
- 699 32. Cheng J-C, Chang H-M, Leung PCK. TGF- β 1 Inhibits Human Trophoblast Cell
700 Invasion by Upregulating Connective Tissue Growth Factor Expression.
701 *Endocrinology* (2017) **158**:3620–3628. doi:10.1210/en.2017-00536
- 702 33. Kauma S, Hayes N, Weatherford S. The Differential Expression of Hepatocyte
703 Growth Factor and Met in Human Placenta¹. *J Clin Endocrinol Metab* (1997)
704 **82**:949–954. doi:10.1210/jcem.82.3.3806
- 705 34. Nancy P, Tagliani E, Tay C-S, Asp P, Levy DE, Erlebacher A. Chemokine gene
706 silencing in decidual stromal cells limits T cell access to the maternal-fetal
707 interface. *Science* (2012) **336**:1317. doi:10.1126/SCIENCE.1220030
- 708 35. Fried M, Kurtis JD, Swihart B, Pond-Tor S, Barry A, Sidibe Y, Gaoussou S,
709 Traore M, Keita S, Mahamar A, et al. Systemic Inflammatory Response to
710 Malaria during Pregnancy Is Associated with Pregnancy Loss and Preterm

- 711 Delivery. *Clin Infect Dis* (2017) **65**:1729–1735. doi:10.1093/cid/cix623
- 712 36. Dong S, Kurtis JD, Pond-Tor S, Kabyemela E, Duffy PE, Fried M. CXC Ligand
713 9 Response to Malaria during Pregnancy Is Associated with Low-Birth-Weight
714 Deliveries. *Infect Immun* (2012) **80**:3034–3038. doi:10.1128/iai.00220-12
- 715 37. Aktas A, Berberoglu Z, Fidan Y, Yazıcı AC, Koc G, Aral Y, Ademoglu E,
716 Bekdemir H, Alphan Z. Higher levels of circulating CXCL-9 and CXCL-11 in
717 euthyroid women with autoimmune thyroiditis and recurrent spontaneous
718 abortions. *Gynecol Endocrinol* (2014) **30**:157–160.
719 doi:10.3109/09513590.2013.871514
- 720 38. Li H, Huang Y-H, Li M-Q, Meng Y-H, Chen X, Shao J, Tang C-L, Du M-R, Jin
721 L-P, Li D-J. Trophoblasts-derived chemokine CCL24 promotes the proliferation,
722 growth and apoptosis of decidual stromal cells in human early pregnancy. *Int J*
723 *Clin Exp Pathol* (2013) **6**:1028–37. Available at:
724 [http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3657354&tool=pmce](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3657354&tool=pmcentrez&rendertype=abstract)
725 [ntrez&rendertype=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3657354&tool=pmcentrez&rendertype=abstract)
- 726 39. Tilburgs T, Crespo ÂC, van der Zwan A, Rybalov B, Raj T, Stranger B, Gardner
727 L, Moffett A, Strominger JL. Human HLA-G+ extravillous trophoblasts: Immune-
728 activating cells that interact with decidual leukocytes. *Proc Natl Acad Sci U S A*
729 (2015) **9**:7219–24. doi:10.1073/pnas.1507977112
- 730 40. Keelan JA, Blumenstein M, Helliwell RJA, Sato TA, Marvin KW, Mitchell MD.
731 Cytokines, prostaglandins and parturition--a review. *Placenta* (2003) **24 Suppl**
732 **A**:S33-46. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/12842412>
733 [Accessed October 4, 2018]

- 734 41. Weiss G, Huppertz B, Siwetz M, Lang I, Moser G. Arterial endothelial cytokines
735 guide extravillous trophoblast invasion towards spiral arteries; An in-vitro study
736 with the trophoblast cell line ACH-3P and female non-uterine endothelial cells.
737 *Placenta* (2016) **38**:49–56. doi:10.1016/j.placenta.2015.12.010
- 738 42. Kaufmann P, Black S, Huppertz B. Endovascular Trophoblast Invasion:
739 Implications for the Pathogenesis of Intrauterine Growth Retardation and
740 Preeclampsia. *Biol Reprod* (2003) **69**:1–7. doi:10.1095/biolreprod.102.014977
- 741 43. Liu Y, Liu Y, Zhang R, Zhu L, Feng Z. Early- or mid-trimester amniocentesis
742 biomarkers for predicting preterm delivery: a meta-analysis. *Ann Med* (2017)
743 **49**:1–10. doi:10.1080/07853890.2016.1211789
- 744 44. Romero R, Gomez R, Ghezzi F, Yoon BH, Mazor M, Edwin SS, Berry SM. A
745 fetal systemic inflammatory response is followed by the spontaneous onset of
746 preterm parturition. *Am J Obstet Gynecol* (1998) **179**:186–93. Available at:
747 <http://www.ncbi.nlm.nih.gov/pubmed/9704786> [Accessed November 7, 2018]
- 748 45. Pantham P, Aye ILMH, Powell TL. Inflammation in maternal obesity and
749 gestational diabetes mellitus. *Placenta* (2015) **36**:709–715.
750 doi:10.1016/j.placenta.2015.04.006
- 751 46. Arruvito L, Billordo A, Capucchio M, Prada ME, Fainboim L. IL-6 trans-signaling
752 and the frequency of CD4+FOXP3+ cells in women with reproductive failure. *J*
753 *Reprod Immunol* (2009) **82**:158–165. doi:10.1016/j.jri.2009.04.010
- 754

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-1 α	25000	34
MIP-1 β	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 weight ^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/m²). 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-1 β	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-1 α	-0.0873	0.860
MIP-1 β	-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
PfDBL5e	-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
PfDBL6e	-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	EOTAXI N	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.