

RESEARCH

Open Access



Eotaxin-2 and eotaxin-3 in malaria exposure and pregnancy

Cristina Mancebo-Pérez^{1,9}, Marta Vidal¹, Ruth Aguilar¹, Diana Barrios¹, Azucena Bardají^{1,2,3}, Maria Ome-Kaius⁵, Clara Menéndez^{1,2,3}, Stephen J. Rogerson⁴, Carlota Dobaño^{1,6}, Gemma Moncunill^{1,6*}† and Pilar Requena^{3,7,8*†}

Abstract

Background: Eotaxin-1 concentrations in plasma have been inversely associated with malaria exposure, malaria infection and pregnancy, but the effect of these conditions on the levels of the related chemokines eotaxin-2 and eotaxin-3 remains unknown.

Methods: Eotaxin-2 and -3 concentrations were measured in 310 peripheral or placental plasma samples from pregnant and non-pregnant individuals from Papua New Guinea (malaria-endemic country) and Spain (malaria-naïve individuals) with previous data on eotaxin-1 concentrations. Correlations between eotaxin concentrations were examined with the Spearman's test. Differences in eotaxin concentrations among groups were evaluated with the Kruskal–Wallis or Mann Whitney tests. The pairwise Wilcoxon test was performed to compare eotaxin-2 concentration between peripheral and placental matched plasmas. Univariable and multivariable linear regression models were estimated to assess the association between eotaxins and *Plasmodium* infection or gestational age.

Results: Eotaxin-2 concentrations in plasma showed a weak positive correlation with eotaxin-3 ($\rho = 0.35$, $p < 0.05$) concentrations. Eotaxin-2 concentrations in the malaria-exposed non-pregnant group were significantly lower than the in the malaria-naïve non-pregnant and the malaria-exposed pregnant groups. Eotaxin-3 plasma concentrations were lower in malaria-exposed than in non-exposed groups ($p < 0.05$), but no differences were found associated to pregnancy. Eotaxin-2 and eotaxin-3 plasma concentrations were negatively correlated with anti-*Plasmodium* IgG levels: PfDBL5E-IgG ($\rho_{E02} = -0.35$, $p = 0.005$; $\rho_{E03} = -0.37$, $p = 0.011$), and eotaxin-3 was negatively correlated with PfDBL3x-IgG levels ($\rho_{E03} = -0.36$; $p = 0.011$). Negative correlations of eotaxin-2 and 3 in plasma were also observed with atypical memory B cells ($\rho_{E02} = -0.37$, $p < 0.001$; $\rho_{E03} = -0.28$, $p = 0.006$), a B cell subset expanded in malaria-exposed individuals. In addition, a borderline negative association was observed between eotaxin-3 concentrations and *Plasmodium* infection (adjusted effect estimate, $\beta = -0.279$, 95% CI -0.605 ; 0.047 , $p = 0.091$). Moreover, eotaxin-2 placental concentrations were significantly increased compared to peripheral concentrations in the malaria-exposed pregnant group whereas the contrary was observed in the non-exposed pregnant group ($p < 0.005$).

†Gemma Moncunill and Pilar Requena contributed equally to the study

*Correspondence: gemma.moncunill@isglobal.org; prequena@ugr.es

¹ ISGlobal, Hospital Clínic- Universitat de Barcelona, Barcelona, Catalonia, Spain

³ Consorcio de Investigación Biomédica en Red de Epidemiología Y Salud Pública (CIBERESP), Barcelona, Spain

Full list of author information is available at the end of the article



Conclusion: Although a clear epidemiological negative association is observed between eotaxins concentrations and malaria exposure and/or infection, pregnancy may alter this association for eotaxin-2. Further research is required to understand the role of these chemokines in this disease and in combination with pregnancy.

Keywords: Eotaxin, Chemokine, Malaria, Placenta, Pregnancy

Background

The mechanisms involved in naturally acquired immunity (NAI) to malaria are not completely understood, but it is well accepted that they include both the cellular and humoral arms of the immune system [1, 2]. Of note, a subset of memory B cells (MBC) with an atypical phenotype (CD27⁻) has been repeatedly found to be expanded in individuals with large malaria exposure, including cohorts of pregnant women [3–6]. These atypical MBC seem to produce neutralizing antibodies against *Plasmodium falciparum* [7], but their impact on antibody-mediated immunity to malaria remains unclear. Moreover, marginal zone-like memory B cells (MZ-MBC) frequencies have been shown to be decreased in malaria-exposed individuals [6, 8], but its impact on NAI also needs to be elucidated.

Pregnant women have increased susceptibility to malaria infection and disease, a condition known as malaria in pregnancy (MiP) [9]. The immune response is modified during gestation in order to tolerate the presence of the fetus [6, 10–14], with a bias towards a Th2 response which may contribute to the increased susceptibility and severity of infections during pregnancy [15]. However, it is broadly accepted that a pro-inflammatory response is also essential for some pregnancy key processes like implantation or parturition. The role of many other biomarkers outside the classical inflammation-Th1-Th2 cytokine frame in MiP remains to be determined.

In a previous study, pregnancy and malaria exposure were associated with decreased plasma concentrations of the chemokine eotaxin-1 (CCL11), and its levels showed the highest (negative) correlation among thirty biomarkers with atypical MBC frequencies in a cohort from Papua New Guinea [6]. Furthermore, eotaxin-1 plasma concentrations were negatively associated with *Plasmodium vivax* infection during pregnancy and positively associated with haemoglobin levels at delivery in a multicenter study [15]. Additionally, studies in non-pregnant individuals have shown negative associations between plasma eotaxin-1 and malaria exposure, and with IgG plasma levels against the malarial antigen apical membrane antigen 1 (AMA-1; unpublished results). Moreover, a negative correlation between *P. falciparum* parasitaemia and eotaxin-1 plasma concentrations was found in two different cross-sectional studies performed in years of different malaria transmission intensity [16]. Interestingly,

a significant increase (instead of decrease) of eotaxin-1 levels has been reported in plasma from patients with other infectious diseases like dengue, *Clostridium difficile* infection, tuberculosis or HIV [17–21].

Eotaxin-1 concentrations seem to be also altered during pregnancy. One study has demonstrated that eotaxin-1 plasma levels decrease significantly during pregnancy, with the lowest found in the third trimester, and that these recover post-partum (after 6 weeks) [13]. The same result was observed in a cohort of pregnant women living in the tropics. Moreover, in that cohort, eotaxin 1 concentrations in the placenta was even lower than in peripheral blood at delivery [22].

Eotaxin-1 is a potent eosinophil chemoattractant involved in inflammatory diseases as well as parasitic infections [23]. Eotaxin-2 (CCL24) and eotaxin-3 (CCL26) are less well-studied, but they also stimulate eosinophil chemotaxis [24] and the three share the same receptor, CCR3, expressed selectively on eosinophils, basophils, mast cells, some Th2 cells and even in atypical MBC [6, 25–27]. The peripheral levels of eotaxin-2 and -3 have not been studied during pregnancy nor in malaria cohorts or any other infectious diseases.

The aim of the present study was to determine the plasma concentrations of eotaxin-2 and eotaxin-3 in malaria-exposed pregnant and non-pregnant individuals as well as in malaria-naive pregnant and non-pregnant individuals, and to determine their associations with pregnancy and malaria infection/exposure, as well as other parameters known to be affected by malaria exposure.

Methods

Study design and population

This study includes peripheral and placental plasma samples from pregnant and non-pregnant subjects from Papua New Guinea (PNG) who participated in the PregVax study [28] (FP7-HEALTH-201588). PNG is characterized by high levels of transmission of both *P. falciparum* and *P. vivax*, but transmission was much decreased in the period of recruitment of this cohort [28]. Peripheral blood samples from pregnant women obtained at recruitment (first antenatal visit occurring at any time during pregnancy except labor) or at delivery were used. Only 10 of the samples were matched. Malaria infection was assessed by microscopy and/or real-time

polymerase chain reaction (PCR) as previously reported [28], and was defined as a positive smear and/or PCR for any *Plasmodium* species.

To achieve the aims of this study, peripheral and placental plasma samples ($n=255$) from PregVax subjects from whom there was available data on other cytokine concentrations were used: 54 peripheral plasma samples from malaria-exposed pregnant women at enrolment (EP-e), 91 peripheral and 72 placental-matched samples at delivery (EP-d and EP-p, respectively) and 38 peripheral plasma samples from non-pregnant exposed women (ENP). In addition, peripheral plasma samples from malaria-naïve non-pregnant (NNP, $n=23$) as well as peripheral and placental-matched samples from malaria-naïve pregnant women (NP, $n=16$ and NP-p $n=16$) were taken from volunteers resident in Spain who had never travelled to malaria-endemic areas. Matched peripheral-placental blood samples were only analysed for eotaxin-2, based on the hypothesis explained in the results section.

Quantification of cytokines

Eotaxin-2 and eotaxin-3 concentrations were quantified using the enzyme-linked immunosorbent assay (ELISA) sandwich technique, with the following commercial kits: 'Human CCL24/Eotaxin-2/MPIF-2' kit (DY343) from R&D Systems (Madrid, Spain), and 'Human Eotaxin-3 (CCL26) Standard ELISA Development' kit (900-K167) from PeproTech (London, UK). Samples were tested directly or after dilution, depending on the group of subjects. Assays were conducted according to manufacturer's instructions, with the exception of eotaxin-2 ELISA in which half of the volume of each reagent was used to reduce the amount of plasma used without diluting it. Several tests were performed before the analysis to assess the sensitivity of the test under these conditions. Finally, the optical density was measured in an EPOCH instrument and the values fitted into a 4-parameter logistic regression curve automatically calculated by the GEN5 software, using the standards provided in the kit. Only eotaxin-3 measurements from 116 of all peripheral plasmas were available due to plasma volume limitations. Eotaxin-1 concentrations were previously determined in the same cohort by the Luminex technology using the Invitrogen™ Cytokine Magnetic 30-Plex Panel kit (Thermo Fisher Scientific, Madrid, Spain) [15, 22].

Quantification of antibodies

The malaria-specific IgG levels were measured in a previous study using the Luminex technology with an antigen panel developed in-house [6] and results are expressed as median fluorescence intensity (MFI). *Plasmodium* antigens included in the panel were PfMSP-1₁₉, PfEBA175, PfDBL3X, PfDBL5ε, PfDBL6ε, PfAMA-1, PvLP1 and

PvLP2, three *P. vivax* vir genes (Vir14, Vir2/15, Vir25), full-length PvMSP-5, Pv200L (PvMSP-1₁₂₁₋₄₁₆), PvMSP-1₁₉, PvDBP (RII), PvCSP-N, PvCSP-C, PvCSP-R, full-length PvCSP. Antibody data was not available for the exposed non-pregnant group (ENP).

Cellular assays

B cell subsets were measured from PBMC samples on a BD LSR Fortessa cytometer as part of a previous study [6] and data were analysed using FlowJo software (Tree Star). B cell subsets were determined according to CD19⁺ expression and a dump channel containing a viability marker, CD3, CD14 and CD16. A Boolean gate containing live B cells and not CD10⁺ was used to gate mature viable B cells (VBCs), which were further divided into IgD switched or unswitched populations and plasma cells (IgD⁻CD38^{high}). Furthermore, based on the expression of CD21 and CD27, switched and unswitched VBC populations were subdivided in naïve B cells (IgD⁺CD27⁻CD21⁺), MZ-like MBC (IgD⁺CD27⁺CD21⁺), active (IgD⁻CD27⁺CD21⁻) and resting (IgD⁻CD27⁺CD21⁺) classical MBC, and active (IgD⁻CD27⁻CD21⁻) and resting (IgD⁻CD27⁻CD21⁺) atypical MBC [6].

Statistical methods

Eotaxin concentrations and anti-malaria IgG levels were not normally distributed, therefore non-parametric statistical tests were used and the variables were log₁₀-transformed for regression analyses. Correlations of eotaxin-2 and eotaxin-3 concentrations with eotaxin-1 concentrations, antibody levels or cellular subset frequencies were examined with the Spearman's correlation test. Results of correlations were interpreted according to the absolute value of rho, as follows: |0–0.19|, 'very weak'; |0.20–0.39|, 'weak'; |0.40–0.59|, 'moderate'; |0.60–0.79|, 'strong'; |0.8–1|, 'very strong'. The Kruskal–Wallis and Mann Whitney tests were used to compare eotaxin concentrations between groups, followed by a Bonferroni Pairwise comparison to adjust the p-values for multiple testing.

Univariable linear regression models were performed to assess the associations between eotaxin-2 and eotaxin-3 concentrations (dependent variable) and *Plasmodium* infection, gestational age, age and haemoglobin levels (independent variables) for the malaria-exposed group. Models of eotaxin-2 or -3 and the variable *Plasmodium* infection were adjusted by gestational age when the latter was found to be significantly associated with eotaxin-2 or -3 in univariable models, respectively.

Pairwise Wilcoxon test was performed to compare the eotaxin-2 concentrations between peripheral and

placental matched plasmas, in malaria-exposed and non-exposed pregnant women. The Mann–Whitney test was used to compare the placental eotaxin-2 concentrations between malaria-exposed and non-exposed groups. The p-values < 0.05 were considered statistically significant and p-values < 0.1 were considered a trend. Missing data for any of the variables were excluded from the analysis. R Software (Version 3.6.1 [29]) was used for the statistical analysis and graphical representation as well as the packages dplyr [30], ggplot2 [31] and rstatix [32].

Results

Study population

Table 1 shows the characteristics of the participants involved in the study. Median age was highest for NNP (median [IQR] of 36 [29; 44] years) and lowest for EP (25 [18; 32] years) and there were males only in the NNP group. Most women in the EP group were multigravida. All samples in the NP group were collected at delivery, and the gestational age from NP women was unknown.

Correlations of peripheral plasma concentrations of eotaxin-1, eotaxin-2 and eotaxin-3

There was no correlation of eotaxin-1 with eotaxin-2 or eotaxin-3 levels in the EP group. Eotaxin-2 and eotaxin-3 presented a positive and significant correlation (Spearman's rho (R) = 0.35, $p < 0.001$) (Fig. 1).

Differences in eotaxin-2 and -3 peripheral plasma concentrations by malaria exposure and pregnancy

There were significant differences in the eotaxin-2 concentrations between groups ($p \leq 0.001$). When groups were compared two-by-two, there were not statistical differences in the plasma concentrations associated to

pregnancy in the malaria-naive group (NNP vs NP), but in the malaria-exposed groups, the ENP had lower levels than the EP group (Fig. 2A). With regards to the effect of malaria exposure, an effect was found only in the non-pregnant groups. Thus, significantly lower eotaxin-2 plasma concentrations were observed in the ENP compared to the NNP group, but no differences were observed between the two pregnant groups (Fig. 2A).

With regards to eotaxin-3, there were not statistical differences in the plasma concentrations associated to pregnancy (NNP vs NP or ENP vs EP, Fig. 2B). However, significantly lower eotaxin-3 plasma concentrations were observed in malaria-exposed groups, both in the non-pregnant (NNP vs ENP) and the pregnant (NP vs EP) individuals (Fig. 2B).

Correlations of eotaxin-2 and eotaxin-3 peripheral plasma concentrations with markers of malaria exposure

To further investigate the association between eotaxin-2 and -3 and *Plasmodium* exposure (*P. falciparum* or *P. vivax*), the correlations between the concentrations of these two eotaxins and IgG levels against 19 malaria antigens in peripheral plasma were assessed in the malaria-exposed group (Table 2). Most of the IgGs analysed are known markers of malaria exposure (like IgG to PfAMA-1) or MiP (like IgG to PfDBL5e or PfDBL3x). The greatest correlations observed were: PfDBL5e-IgG with eotaxin-2 and -3 ($R_{Eo2} = -0.35$, $p = 0.005$; $R_{Eo3} = -0.37$, $p = 0.011$) and PfDBL3x-IgG with eotaxin-3 ($R_{Eo3} = -0.36$, $p = 0.011$) (Table 2). Each of these associations are shown as scatterplots in Fig. 3.

Next, the correlation between eotaxin concentrations in peripheral plasma and the frequency of some B cell

Table 1 Description of the study participants

| | ENP | EP | NNP | NP |
|--|-------------|---|-------------|-------------|
| N | 38 | 145 | 23 | 16 |
| Age, years median (IQR) | 26 (20; 29) | 25 (18; 32) | 36 (29; 44) | 29 (26; 32) |
| Sex (females), n (%) | 38 (100) | 145 (100) | 9 (39.1) | 16 (100) |
| <i>Plasmodium</i> infection in peripheral blood, n (%) | 3 (7.69) | 20 (13.8) | na | na |
| Gravidity, n (%) | na | Primigravida 53 (36.8) 1–3 pregnancies 30 (20.8) 4+ pregnancies 61 (42.4) | na | nd |
| Sampling timepoint, n (%) | | | | |
| Recruitment (peripheral blood) | na | 54 (37.3) | na | 0 (0) |
| Delivery (peripheral and placental blood) | na | 91 (62.7) | na | 16 (100) |
| Gestational age by timepoint, weeks median (IQR) | | | | |
| Recruitment | na | 21 (12; 25) | na | nd |
| Delivery | na | 39 (35; 43) | na | nd |

ENP exposed non-pregnant, EP exposed pregnant, NNP naive non-pregnant, NP naive pregnant, na not applicable, nd not determined, IQR Interquartile range

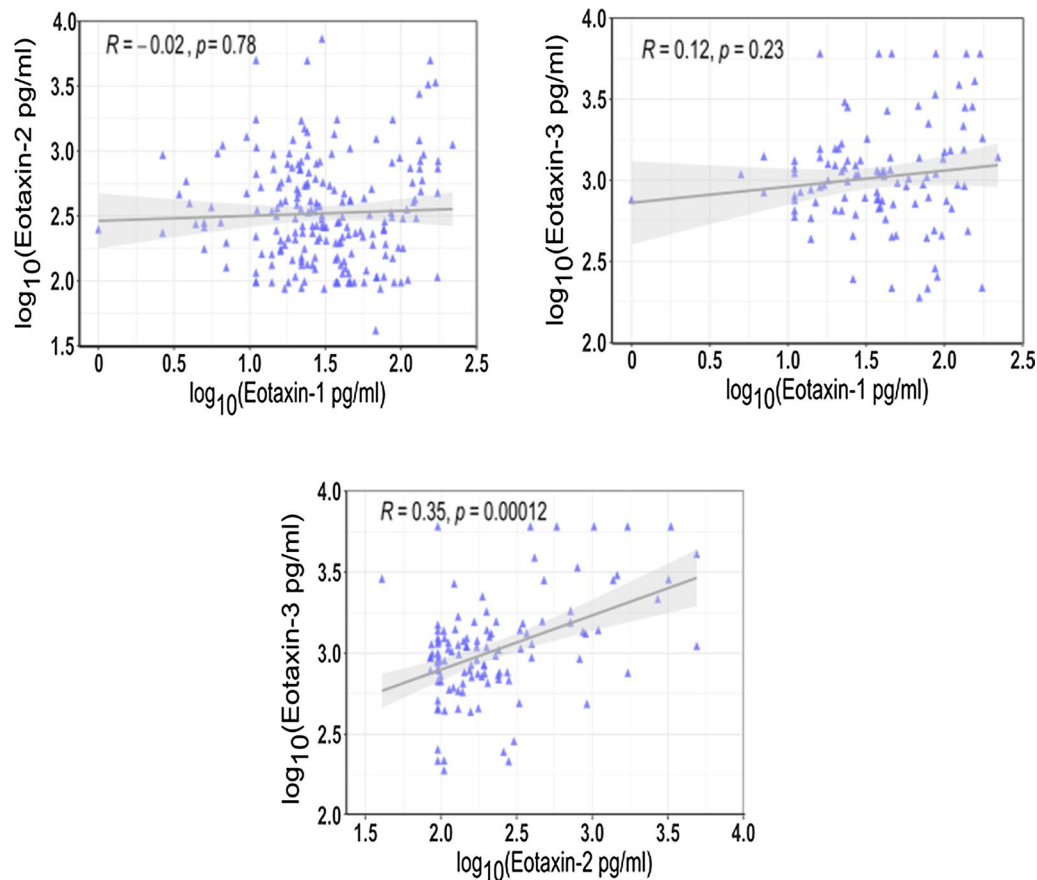


Fig. 1 Correlations between eotaxin-1, -2 and -3 plasma concentrations. Scatter plots showing the distribution of plasma eotaxins concentrations in all study subjects, $n_{Eo2,Eo1} = 202$, $n_{Eo3} = 116$. The p-value corresponds to Spearman's correlation test and R corresponds to the Spearman's coefficient

subsets, also known to be altered in malaria-exposed individuals and analysed in the same women as part of a previous study [6], were investigated. There was a significant negative correlation between the frequency of atypical MBC and the concentrations of both chemokines, especially eotaxin-2 (Table 3). A weaker but significant negative correlation was also observed between eotaxin-2 concentrations and active classical MBCs. In contrast, MZ-MBCs showed a positive and significant correlation with plasma concentrations of eotaxin-2. A scatter plot showing each of the strongest correlations is shown in Fig. 3.

Associations between eotaxin-2 and eotaxin-3 peripheral plasma concentrations and malaria infection

Regression models were estimated to evaluate the possible associations of eotaxin-2 and eotaxin-3 plasma concentrations with *Plasmodium* infection (*P. falciparum* or *P. vivax*), age, hemoglobin levels and gestational age (in the pregnant study groups). Neither age

nor hemoglobin levels had an association or trend with eotaxin-2 or -3 concentrations (data not shown), but gestational age was significantly associated with eotaxin-3 concentrations being higher at delivery (Table 4, $Coeff = 3.161$; 95%CI 0.322; 6.000, $p = 0.031$) and with eotaxin-2 when recruitment and delivery samples were analyzed together ($Coeff = 0.520$; 95%CI 0.199; 0.842, $p = 0.002$). Interestingly, in the exposed pregnant women, eotaxin-3 showed a trend of negative association with *Plasmodium* infection at recruitment in the crude analysis and after adjusting by gestational age (Table 4, adjusted effect estimate, $Coeff = -0.279$; 95%CI - 0.605–0.047, $p = 0.091$), but no association was observed at delivery. Because the prevalence of infection was relatively low at recruitment and delivery (14.19%), crude and adjusted regression models were also estimated with both pregnant groups together, but no effect was seen (Table 4).

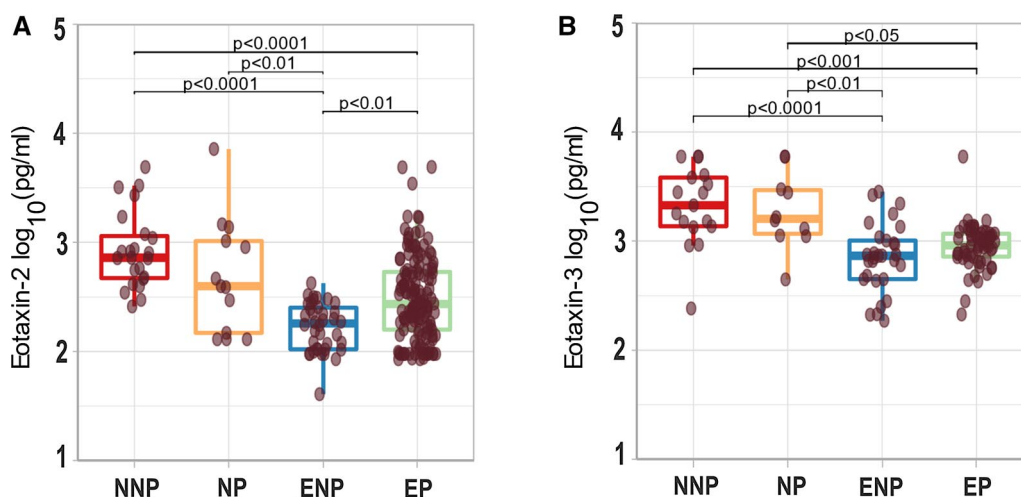


Fig. 2 Differences in eotaxin-2 and -3 peripheral plasma concentrations by malaria exposure and pregnancy. Comparison of eotaxin-2 and eotaxin-3 plasma concentrations in malaria exposed pregnant (EP, $n_{Eo2} = 145$, $n_{Eo3} = 60$), exposed nonpregnant (ENP, $n_{Eo2} = 38$, $n_{Eo3} = 29$), non-exposed pregnant (NP, $n_{Eo2} = 16$, $n_{Eo3} = 10$) and non-pregnant (NNP, $n_{Eo2} = 23$, $n_{Eo3} = 17$) individuals. The dotplots represent the eotaxin concentrations measured in each sample. The outliers are not shown. The boxplots correspond to the median and the 25th and 75th quartiles. The Mann Whitney test was performed for the two by two groups comparisons. P-values were adjusted for multiple comparisons using Bonferroni method. Only significant p-values are shown

Table 2 Correlations of eotaxin-2 and eotaxin-3 peripheral plasma concentrations with malaria IgG levels

| | Eo2 | Eo3 |
|-----------------------|--------------|--------------|
| PfMSP-1 ₁₉ | 0 | 0.04 |
| PfEBA | 0.08 | 0.05 |
| PfDBL6 _E | -0.23 | -0.04 |
| PfDBL5 _E | -0.35 | -0.37 |
| PfDBL3 _x | -0.19 | -0.36 |
| PfAMA-1 | 0.02 | -0.19 |
| PvLP2 | -0.02 | -0.01 |
| PvLP1 | 0.06 | -0.11 |
| Vir14 | 0 | -0.07 |
| Vir2/15 | 0.04 | -0.04 |
| Vir25 | 0.09 | -0.1 |
| PvMSP-5 | 0.21 | 0 |
| Pv200L | -0.14 | -0.02 |
| PvMSP-1 ₁₉ | -0.08 | -0.1 |
| PvDBP | -0.01 | 0.02 |
| PvCSP-R | 0.2 | 0.19 |
| PvCSP-N | -0.14 | 0 |
| PvCSP-C | 0.06 | 0.11 |
| PvCSP | -0.11 | 0.02 |

The specific Spearman's coefficient is displayed in the cells. Only the individuals of malaria exposed pregnant study group who had antibody data available are included in the analysis (EP, $n_{Eo2} = 85$, $n_{Eo3} = 56$). In bold if $p < 0.05$. Eo Eotaxin

Eotaxin-2 concentrations in peripheral and placental plasma in malaria exposed and non-exposed pregnant women

Placental eotaxin-1 was hypothesized to be decreased in placenta compared to periphery in a tropical cohort of pregnant women [22] to avoid receptor competition with eotaxin-2, shown to enhance decidualization in vitro [33]. So eotaxin-2 levels should be increased in the placenta. This hypothesis was reinforced by the fact that eotaxin-2 expression in the trophoblast is 3 times bigger than the expression of eotaxin-1 or eotaxin-3 [34]. Thus, eotaxin-2 levels in placental and peripheral plasma samples were compared separately in malaria-exposed and malaria-naive women. An important and significant increase in eotaxin-2 placental plasma concentrations compared with peripheral plasma concentrations was found in malaria-exposed women (Fig. 4A), while a decrease was observed in malaria-naive women (Fig. 4B). Also, there was a trend for increased eotaxin-2 levels in placental plasma samples of malaria-exposed women compared with malaria-naive (Fig. 5).

Discussion

In this study, the associations between malaria exposure/infection, pregnancy and the concentrations of eotaxin-2 and 3 were investigated. In non-pregnant participants, a significant decrease of the levels of eotaxin-2 and eotaxin-3 was detected in malaria-exposed groups

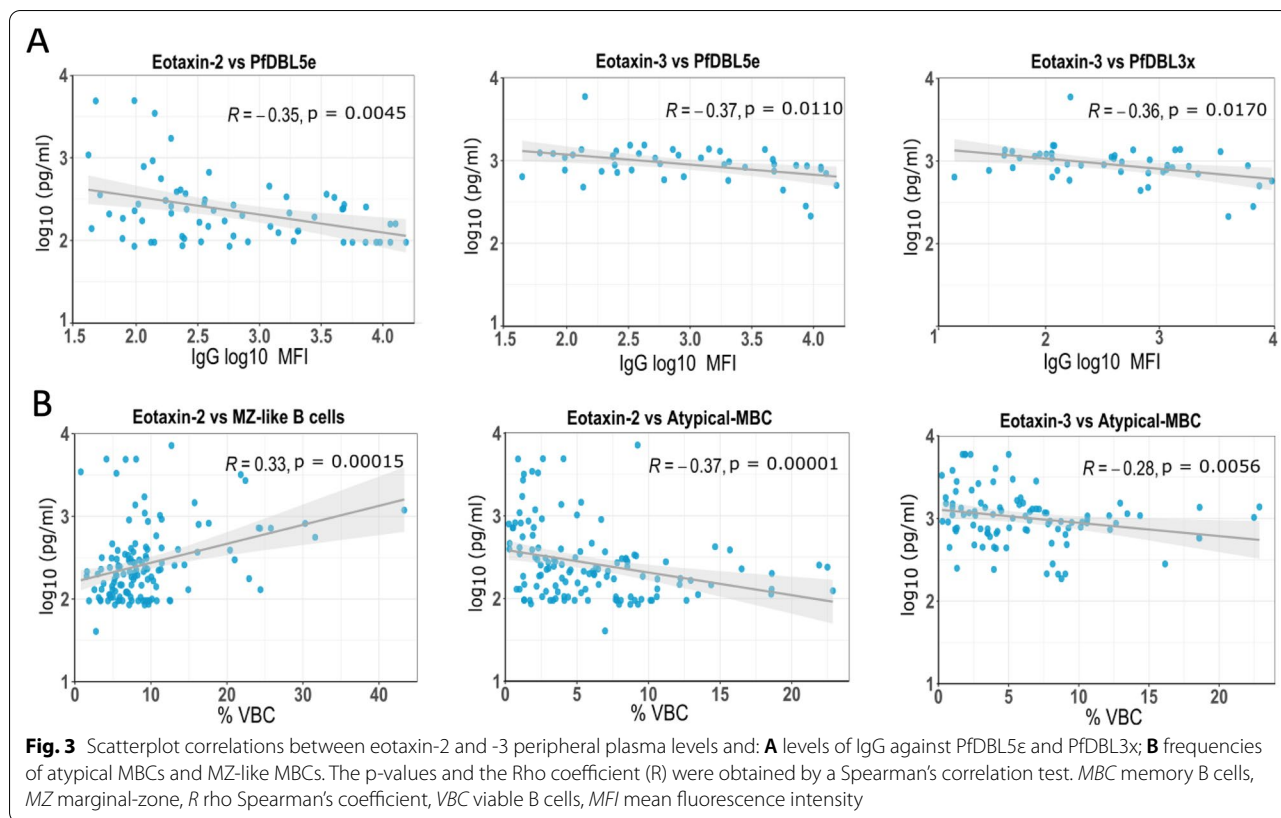


Table 3 Correlations of eotaxin-2 and eotaxin-3 peripheral plasma concentrations with B cell subsets frequencies

| | Eo2 | Eo3 |
|--------|--------------|--------------|
| MZ-MBC | 0.33 | 0.1 |
| Naïve | 0.03 | 0.08 |
| rcMBC | 0.09 | -0.01 |
| aaMBC | -0.37 | -0.28 |
| acMBC | -0.21 | -0.07 |

The specific Spearman's coefficient is displayed in the cells. Individuals of four study groups that had available B cell data are included: NNP ($n_{Eo2}=23$, $n_{Eo3}=17$), NP ($n_{Eo2}=13$, $n_{Eo3}=10$), ENP ($n_{Eo2}=38$, $n_{Eo3}=29$) and EP ($n_{Eo2}=82$, $n_{Eo3}=60$). In bold if $p < 0.05$.

MBC memory B cells, MZ-MBC marginal zone-like MBC, rcMBC resting classical MBC, aaMBC active atypical MBC, acMBC active classical MBC, Eo Eotaxin

compared with non-exposed groups; in the pregnant women, this difference was only observed for eotaxin-3. In addition, eotaxin-2 and eotaxin-3 concentrations were significantly and inversely correlated with some *Plasmodium*-specific IgG antibodies, i.e., PfDBL5e or PfDBL3x, as well as with atypical MBC, all of them considered markers of MiP [6]. In contrast, MZ-like B cells known to be diminished in malaria-exposed individuals showed a positive and significant correlation with eotaxin-2. Although these correlations were considered

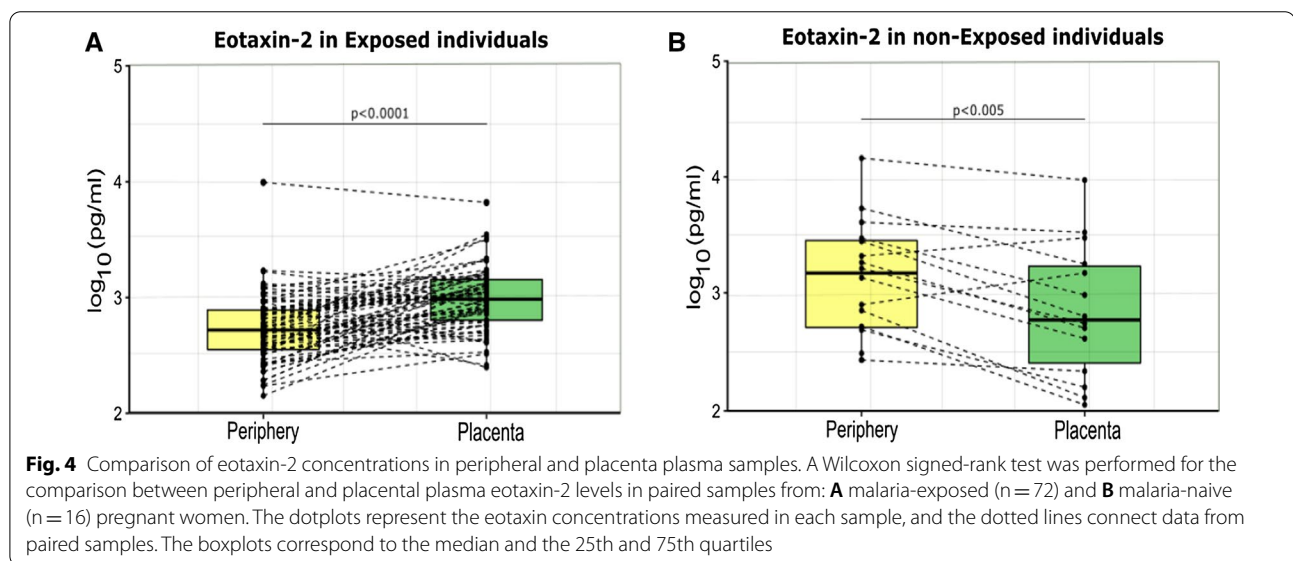
to be weak, the consistency of the results make us believe that malaria exposure has a negative association with eotaxin-2 and eotaxin-3 plasma concentrations, as previously observed with eotaxin-1 plasma levels ([6], unpublished results). Of note, other infections like tuberculosis or dengue have been associated with increased and not decreased eotaxins peripheral levels [18–20]. Further explanatory studies are necessary to unravel the causes of this phenomenon, i.e. to identify the cellular source of the three eotaxins after/during malaria infection.

Also, the association of current *Plasmodium* infection and not just previous exposure with eotaxin plasma concentrations in the EP group was analysed, as *P. vivax*-infection during pregnancy was previously shown to be related to lower levels of eotaxin-1 [15]. Because in this cohort there were very few infected individuals, *P. falciparum* and *P. vivax* infection could not be analysed separately. Eotaxin-3 plasma levels at recruitment showed a negative association with *Plasmodium* infection. The association was significant even after adjusting the model by gestational age. Interestingly, a negative association of eotaxin-2 with *Plasmodium* infection could not be found in this cohort of pregnant women, despite having found moderate correlations with anti-*Plasmodium* antibody levels, and atypical and MZ-like MBCs. Lack of statistical power may have limited the detection of associations

Table 4 Associations between plasma eotaxin-2 and eotaxin-3 concentrations and gestational age or *Plasmodium* infection

| | Eotaxin-2 | | | Eotaxin-3 | | |
|--------------------------|-------------|----------------|--------------|-------------|----------------|--------------|
| | Coefficient | 95% CI | p-value | Coefficient | 95% CI | p-value |
| Gestational age | | | | | | |
| Recruitment | 0.159 | − 0.343; 0.662 | 0.526 | 0.064 | − 0.229; 0.357 | 0.662 |
| Delivery | − 0.038 | − 1.264; 1.187 | 0.950 | 3.161 | 0.322; 6.000 | 0.031 |
| EP | 0.520 | 0.199; 0.842 | 0.002 | 0.063 | − 0.198; 0.326 | 0.629 |
| Plasmodium infection | | | | | | |
| Recruitment | − 0.218 | − 0.822; 0.386 | 0.471 | − 0.283 | − 0.601; 0.035 | 0.079 |
| Delivery | − 0.423 | − 0.964; 0.118 | 0.123 | − 0.256 | − 0.935; 0.424 | 0.44 |
| EP | − 0.296 | − 0.720; 0.129 | 0.170 | − 0.147 | − 0.570; 0.284 | 0.49 |
| Recruitment ^a | − 0.202 | − 0.813; 0.409 | 0.509 | − 0.279 | − 0.605; 0.047 | 0.091 |
| Delivery ^a | − 0.399 | − 0.963; 0.164 | 0.161 | − 0.454 | − 1.057; 0.149 | 0.130 |
| EP ^a | − 0.284 | − 0.698; 0.129 | 0.176 | − 0.121 | − 0.576; 0.333 | 0.588 |

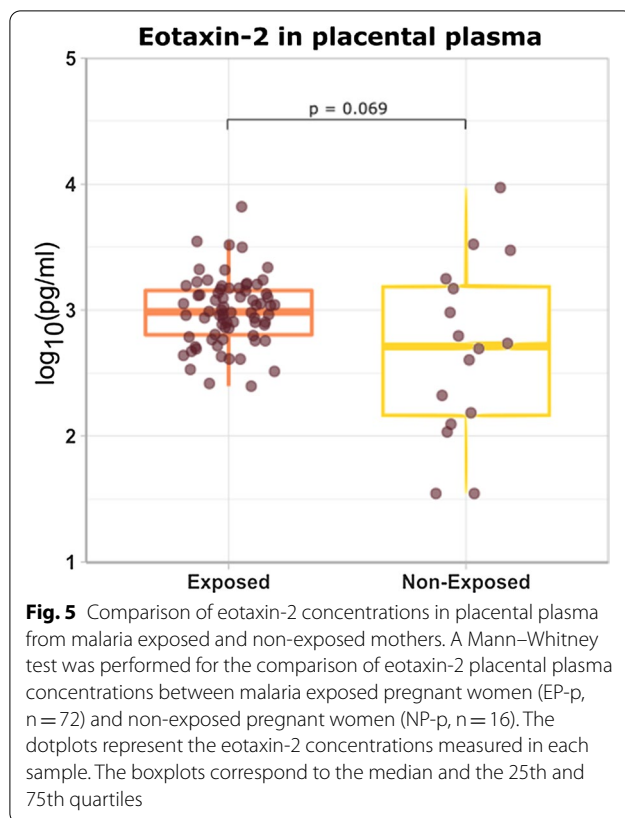
Crude or adjusted by gestational age (^a) linear regression models were estimated with eotaxin-2 and eotaxin-3 as dependent variables and gestational age or *Plasmodium* spp. infection as independent variables, for the malaria exposed pregnant women group EP ($n_{Eo2} = 145$, $n_{Eo3} = 60$), including recruitment ($n_{Eo2} = 54$, $n_{Eo3} = 40$) and delivery ($n_{Eo2} = 91$, $n_{Eo3} = 20$) timepoints. *Plasmodium* infection included *P. vivax* or *P. falciparum* infections. Bold = p-value < 0.1



of eotaxin-2 with infection. However, it should be noted that differences in the eotaxin-2 concentrations between the EP and NP groups were not found either. Altogether these results suggest that pregnancy may modify the effect of malaria on eotaxin-2 concentrations.

With regards to the association of eotaxins with pregnancy, there were no significant differences in eotaxin-3 concentrations between pregnant and non-pregnant individuals. In the case of eotaxin-2, the concentrations in the EP group were higher than in the ENP one, in contrast to eotaxin-1, for which a pregnancy-associated decrease of levels had been observed [6]. In line with this last result, eotaxin-2 placental concentrations were

higher than the peripheral ones in the malaria-exposed cohort, while eotaxin-1 followed exactly the opposite direction [15]. Moreover, eotaxin-2 placental levels were higher (although not statistically significant) in the malaria-exposed pregnant women compared to the malaria non-exposed pregnant ones. These results suggest again that eotaxin-1 and eotaxin-2 follow specific regulations in MiP. Thus, although in vitro studies have shown that the three eotaxins enhance the extravillous trophoblasts function [35], the effect of malaria infection and placental malaria on this function has yet to be determined.



This study presents some limitations. First, the measurements related to cells are not absolute counts but frequencies (proportions), thus some caution must be taken when interpreting the data. Second, cell samples were taken only from peripheral blood limiting the view of the immune response, therefore, what is seen as expansion/reduction could be a consequence of redistribution to other tissues. Third, the sample size was somehow small, especially for eotaxin-3 due to sample-volume limitations, limiting the power to detect statistical associations particularly with infection in adjusted models. Fourth, although samples from non-pregnant individuals were used as controls, a cohort of *Plasmodium*-infected non-pregnant individuals to ascertain the effect of malaria in eotaxin-2 and eotaxin-3 levels would be necessary.

Conclusion

Eotaxin-3 is inversely associated with malaria exposure and infection during pregnancy, as seen previously with eotaxin 1. Eotaxin-2 concentrations show an opposite pattern to eotaxin-1 in MiP, with increased levels in pregnant compared to non-pregnant malaria-exposed groups, increased placental levels compared to periphery, and enhanced placental concentrations in the malaria-exposed than in the malaria-naive women.

More research on the implication of all eotaxins in malaria and its interaction with pregnancy is required to elucidate the particular role of each of them and to clarify the immune mechanism behind their associations with B cell subsets.

Abbreviations

AMA1: Apical Membrane Antigen 1; CCR: C–C chemokine receptor; CD: Cluster of differentiation; CI: Confidence interval; CSP: Circumsporozoite protein; DBL: Duffy-Binding-like; DBP: Duffy binding protein; EBA: Erythrocyte binding antigen; ELISA: Enzyme-linked immunosorbent assay; ENP: Exposed Non-Pregnant; EP: Exposed pregnant; EP-d: Exposed pregnant at delivery; EP-e: Exposed pregnant at enrolment; EP-p: Placental plasma of exposed pregnant; HIV: Human immunodeficiency virus; IQR: Interquartile range; LP1/LP2: Long synthetic peptides containing conserved VIR sequences; MBC: Memory B cells; MFI: Mean fluorescence intensity; MiP: Malaria infection during pregnancy; MSP: Merozoite surface protein; MZ: Marginal zone; NAI: Naturally acquired immunity; NK cell: Natural killer cells; NNP: Non-exposed Non-Pregnant; NP: Non-exposed pregnant; PBMC: Peripheral blood mononuclear cells; PCR: Polymerase chain reaction; PNG: Papua New Guinea; Pv200L: *Plasmodium vivax* MSP-1 fragment 200L; R: Rho; RBC: Red Blood Cells; VAR2CSA: Variant surface antigen 2-CSA; VBC: Viable B cells; VCAM: Vascular cell adhesion protein 1; Vir: Antibody against VIR proteins; WHO: World Health Organization.

Acknowledgements

We thank all the volunteers who consented to participate in this study; the PNG-Institute of Medical Research staff involved in the field and laboratory work, Honor Rose, Ushtana Antia, Danielle Stanisic, Celine Barnadas, Sarah Hanieh, Leanne J. Robinson, Paula Samol, Alexandra J. Umbers, Regina Wangnapi and Holger Unger for contributing to the collection of samples and data in PNG; the PregVax team, Sócrates Herrera, Myriam Arevalo, Flor Ernestina Martínez-Espinosa, Norma Padilla, Swati Kochar, Sanjay Kochar, Chetan Chitnis, Ivo Müller, Mats Wahlgren, Carlo Serverini, Caterina Guinovart, Alfredo Mayor, Mireia Piqueras, Jaume Ordi, John J. Aponte, Meghna Desai, Hernando del Portillo for contributing to data collection of the main cohort; and Laura Moro, Alfons Jiménez, Pau Cisteró, and Laura Puyol for contributing to the collection of samples in Spain.

Author contributions

CMe, SJR, and CD conceived the Pregvax study (the large cohort that involves this study) and were responsible for funding acquisition. PR and CD were responsible of the immunological studies within PregVax. MoK and AB participated in the epidemiological studies. GM and PR designed the present study. CMa, MV, RA and DB performed the laboratory experiments. CMa performed the statistical analysis. CMa, GM and PR drafted the manuscript. All authors reviewed and approved the final manuscript.

Funding

The PREGVAX collaborative project was supported by European Union Seventh Framework Pro-gramme (FP7/2007–2013) under grant agreement number PREGVAX FP7-EALTH-201588. It also received co-funding from the Malaria in Pregnancy Consortium (MiPc) through a grant from the Bill & Melinda Gates Foundation [Grant No. 46099]. CD was affiliate and member of the European Union FP7 Network of Excellence EviMalaR, and Azucena Bardají was supported by the Government of Spain, Ramon y Cajal Fellowship [Grant No. RYC-2013–14512]. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. We acknowledge support from the Spanish Ministry of Science and Innovation and State Research Agency through the “Centro de Excelencia Severo Ochoa 2019–2023” Program (CEX2018-000806-S), and support from the Generalitat de Catalunya through the CERCA Program. This research is part of the ISGlobal’s Program on the Molecular Mechanisms of Malaria which is partially supported by the Fundación Ramón Areces.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Written informed consent was obtained from all study participants. This study was approved by the Medical Research Advisory Committee in PNG (MRAC 08.02), and by the Hospital Clinic of Barcelona Ethics Review Committee, Spain (Comitè d'Ètica de la Investigació amb medicaments, CEIm).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹ISGlobal, Hospital Clínic- Universitat de Barcelona, Barcelona, Catalonia, Spain. ²Manhiça Health Research Center (CISM), Manhiça, Mozambique. ³Consorcio de Investigación Biomédica en Red de Epidemiología Y Salud Pública (CIBERESP), Barcelona, Spain. ⁴Department of Infectious Diseases, University of Melbourne, the Doherty Institute, Melbourne, Australia. ⁵Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea. ⁶Consorcio de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC), Barcelona, Spain. ⁷Department of Preventive Medicine and Public Health, Universidad de Granada, Granada, Spain. ⁸Instituto de Investigación Biosanitaria (Ibs.GRANADA), Granada, Spain. ⁹Present Address: Infectious Diseases Department, Hospital Universitari Vall d'Hebron, Institut de Recerca (VHIR), Universitat Autònoma de Barcelona, Barcelona, Spain.

Received: 25 April 2022 Accepted: 7 November 2022

Published online: 15 November 2022

References

- Jeffery GM. Epidemiological significance of repeated infections with homologous and heterologous strains and species of *Plasmodium*. *Bull World Health Organ*. 1966;35:873–82.
- Doolan DL, Dobaño C, Baird JK. Acquired immunity to malaria. *Clin Microbiol Rev*. 2009;22:13–36.
- Weiss GE, Clark EH, Li S, Traore B, Kayentao K, Ongoiba A, et al. A positive correlation between atypical memory B cells and *Plasmodium falciparum* transmission intensity in cross-sectional studies in Peru and Mali. *PLoS ONE*. 2011;6: e15983.
- Scholzen A, Teirlinck AC, Bijker EM, Roestenberg M, Hermsen CC, Hoffman SL, et al. BAFF and BAFF receptor levels correlate with B cell subset activation and redistribution in controlled human malaria infection. *J Immunol*. 2014;192:3719–29.
- Illingworth J, Butler NS, Roetynck S, Mwacharo J, Pierce SK, Bejon P, et al. Chronic exposure to *Plasmodium falciparum* is associated with phenotypic evidence of B and T cell exhaustion. *J Immunol*. 2013;190:1038–47.
- Requena P, Campo JJ, Umbers AJ, Ome M, Wangnapi R, Barrios D, et al. Pregnancy and malaria exposure are associated with changes in the B cell pool and in plasma eotaxin levels. *J Immunol*. 2014;193:2971–83.
- Muellerbeck MF, Ueberheide B, Amulic B, Epp A, Fenyo D, Busse CE, et al. Atypical and classical memory B cells produce *Plasmodium falciparum* neutralizing antibodies. *J Exp Med*. 2013;210:389–99.
- Asito AS, Piriou E, Jura WGZO, Ouma C, Odada PS, Ogola S, et al. Suppression of circulating IgD+CD27+ memory B cells in infants living in a malaria-endemic region of Kenya. *Malar J*. 2011;10:362.
- Brabin BJ. An analysis of malaria in pregnancy in Africa. *Bull World Health Organ*. 1983;61:1005–16.
- Ziegler KB, Muzzio DO, Matzner F, Bommer I, Malinowsky K, Ehrhardt J, et al. Human pregnancy is accompanied by modifications in B cell development and immunoglobulin profile. *J Reprod Immunol*. 2018;129:40–7.
- Muzzio D, Zenclussen AC, Jensen F. The role of B cells in pregnancy: the good and the bad. *Am J Reprod Immunol*. 2013;69:408–12.
- Achidi EA, Apinjoh TO, Titanji VPK. Malaria parasitemia and systemic cytokine bias in pregnancy. *Int J Gynecol Obstet*. 2007;97:15–20.
- Kraus TA, Sperling RS, Engel SM, Lo Y, Kellerman L, Singh T, et al. Peripheral blood cytokine profiling during pregnancy and post-partum periods. *Am J Reprod Immunol*. 2010;64:411–26.
- Mor G, Cardenas I. The immune system in pregnancy: a unique complexity. *Am J Reprod Immunol*. 2010;64:425–33.
- Dobaño C, Bardaji A, Arévalo-Herrera M, Martínez-Espinosa FE, Bötto-Menezes C, Padilla N, et al. Cytokine signatures of *Plasmodium vivax* infection during pregnancy and delivery outcomes. *PLoS Negl Trop Dis*. 2020;14: e0008155.
- Aguilar R, Campo JJ, Chicuecue S, Cisteró P, Català A, Luis L, et al. Changing plasma cytokine, chemokine and growth factor profiles upon differing malaria transmission intensities. *Malar J*. 2019;5:406.
- Cui N, Liu R, Bin LuQ, Wang LY, Qin SL, Yang ZD, et al. Severe fever with thrombocytopenia syndrome bunyavirus-related human encephalitis. *J Infect*. 2015;70:52–9.
- De SousaTramontini Gomes CF, Baimukanova G, Lanteri MC, Keating SM, Moraes Ferreira F, Heitman J, et al. Serum from dengue virus-infected patients with and without plasma leakage differentially affects endothelial cells barrier function in vitro. *PLoS ONE*. 2017;12:e0178820.
- Yao X, Liu Y, Liu Y, Liu W, Ye Z, Zheng C, et al. Multiplex analysis of plasma cytokines/chemokines showing different immune responses in active TB patients, latent TB infection and healthy participants. *Tuberculosis*. 2017;107:88–94.
- Rao K, Erb-Downward JR, Walk ST, Micic D, Falkowski N, Santhosh K, et al. The systemic inflammatory response to clostridium difficile infection. *PLoS ONE*. 2014;9: e92578.
- Roberts L, Passmore JAS, Williamson C, Little F, Bebell LM, Mlisana K, et al. Plasma cytokine levels during acute HIV-1 infection predict HIV disease progression. *AIDS*. 2010;24:819–31.
- Dobaño C, Bardaji A, Kochar S, Kochar SK, Padilla N, López M, et al. Blood cytokine, chemokine and growth factor profiling in a cohort of pregnant women from tropical countries. *Cytokine*. 2020;125: 154818.
- Huber AK, Giles DA, Segal BM, Irani DN. An emerging role for eotaxins in neurodegenerative disease. *Clin Immunol*. 2018;189:29–33.
- Ahmadi Z, Hassanshahi G, Khorramdelazad H, Zainodini N, Koochakzadeh L. An overlook to the characteristics and roles played by eotaxin network in the pathophysiology of food allergies: allergic asthma and atopic dermatitis. *Inflammation*. 2016;39:1253–67.
- Marone G, Florio G, Petraroli A, Triggiani M, De Paulis A. Role of human FcεRI+ cells in HIV-1 infection. *Immunol Rev*. 2001;179:128–38.
- Walsh ER, Sahu N, Kearley J, Benjamin E, Boo HK, Humbles A, et al. Strain-specific requirement for eosinophils in the recruitment of T cells to the lung during the development of allergic asthma. *J Exp Med*. 2008;205:1285–92.
- Gangaplara A, Martens C, Dahlstrom E, Metidji A, Gokhale AS, Glass DD, et al. Type I interferon signaling attenuates regulatory T cell function in viral infection and in the tumor microenvironment. *PLoS Pathog*. 2018;14: e1006985.
- Bardaji A, Martínez-Espinosa FE, Arévalo-Herrera M, Padilla N, Kochar S, Ome-Kaius M, et al. Burden and impact of *Plasmodium vivax* in pregnancy: a multi-centre prospective observational study. *PLoS Negl Trop Dis*. 2017;11: e0005606.
- R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>. Accessed 5 Feb 2020.
- Wickham H, François R, Henry L, Müller K. dplyr: A grammar of data manipulation, 2020. <https://cran.r-project.org/web/packages/dplyr/index.html>. Accessed 20 May 2020.
- Wickham H. ggplot2: Elegant graphics for data analysis. Springer-Verlag New York; 2016. <https://ggplot2.tidyverse.org>. Accessed 3 July 2020.
- Alboukadel Kassambara. rstatix: Pipe-friendly framework for basic statistical tests. 2020. <https://github.com/kassambara/rstatix>. Accessed 15 May 2022.
- Li H, Huang Y-H, Li M-Q, Meng Y-H, Chen X, Shao J, et al. Trophoblast-derived chemokine CCL24 promotes the proliferation, growth and apoptosis of decidual stromal cells in human early pregnancy. *Int J Clin Exp Pathol*. 2013;6:1028–37.
- Tilburgs T, Crespo ÁC, van der Zwan A, Rybalov B, Raj T, Stranger B, et al. Human HLA-G+ extravillous trophoblasts: immune-activating

cells that interact with decidual leukocytes. *Proc Natl Acad Sci USA*. 2015;9:7219–24.

35. Chau SE, Murthi P, Wong MH, Whitley GS, Brennecke SP, Keogh RJ. Control of extravillous trophoblast function by the eotaxins CCL11, CCL24 and CCL26. *Hum Reprod*. 2013;28:1497–507.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

