Malaria in pregnancy

In search of tools for improved prevention

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Chapter 6.
Peripheral and placental biomarkers in women with placental malaria: a systematic review.

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Abstract

Placental malaria (PM) causes significant morbidity in mothers and infants. Diagnosis of PM during pregnancy is however problematic due to placental sequestration of parasites. Host biomarkers may therefore be used as diagnostic method. In this systematic review most studies focused on inflammatory markers. A trend was observed for increased IL-10 and TNF-α in PM positives. These markers are however unspecific, thus a combination of multiple biomarkers involved in different pathophysiological pathways of PM is indicated. Of interest are inflammatory markers (TNF-R2, CXCL-13), markers of lipid metabolism (APO-B), angiogenesis (sFlt-1) and hormones (estradiol). As the majority of published studies tested biomarker levels only at delivery, more longitudinal cohort studies will be necessary to detect biomarkers during pregnancy that can predict PM.
Introduction

Malaria puts 50 million pregnant women at risk worldwide, of whom half in sub-Saharan Africa (SSA), and causes substantial morbidity and mortality in both mothers and their offspring.\(^1,2\) In fact, pregnant women are more prone to malaria infections compared with non-pregnant women\(^3\) and especially primigravidae are at risk.\(^4\) The exact mechanism to explain this phenomenon is unknown but it is generally accepted that it is due to the lack of antibodies to placental specific variant surface antigens of the parasitized red blood cells and to the altered immunological state during pregnancy.\(^5\) Normally, a delicate immunological balance, involving regulatory T cells and cytokines, is maintained during pregnancy to prevent rejection of the foetus.\(^6,7\) During malaria in pregnancy (MIP), parasitized red blood cells may sequester in the placenta and cause an inflammatory response with release of cytokines and attraction of mononuclear inflammatory cells into the intervillous space of the placenta, thereby disturbing this immunological balance.\(^8–10\)

This feature is mainly described for *Plasmodium falciparum*, but *Plasmodium vivax* is also found to induce inflammation in the placenta.\(^11\) Placental malaria (PM) is associated with anemia of the mother but also with low birth weight (LBW) in the offspring,\(^5,12–15\) indirectly causing around 100,000 infant deaths per year in SSA\(^15\) and possibly causing a substantial number of childhood deaths related to the increased risk of pneumonia and diarrhea in LBW children.\(^16,17\) Recent evidence also showed increased susceptibility to malaria and other infectious diseases in infants born from mothers with PM.\(^18,19\)

The maternal and fetal morbidity that is associated with malaria infection requires adequate detection and treatment of PM. However, antenatal detection of PM is problematic. First of all because in malaria endemic areas clinical symptoms in pregnant women can be rare or unspecific because of partial immunity, while the women and their babies are still exposed to the negative effects of the infection. Furthermore, diagnosis of PM in peripheral blood is unreliable and currently only post-partum diagnostic methods such as microscopy of placental blood smear (PBS) or impression smear (PIS), placental histology (HIS; can also diagnose past PM infections by detecting the malaria pigment hemozoin in fibrin), or polymerase chain reaction (PCR) on placental blood are used to detect PM. PCR on peripheral blood may also detect most of PM cases, but is less applicable in countries most affected by *P. falciparum* because of operational limitations. Peripheral blood smears, commonly used to diagnose malaria, show too many false negatives, because parasitemia may be extremely low or absent when parasites are sequestered in the placenta.\(^20\) Rapid diagnostic tests (RDTs) target malaria antigens circulating in the peripheral blood, but the sensitivity of these tests in pregnant women ranges between 78% and 95% if compared to placental blood microscopy.
as reference standard, and is only 57% if compared to placental histology.\textsuperscript{21} Therefore, other diagnostic methods to detect PM during pregnancy are needed.

Over the last couple of years there is an interest in detecting host biomarkers that may indicate PM. Cytokines involved in the inflammatory process in the placenta or proteins and cytokines involved in placentation or fetal growth are frequently studied for their association with PM. So far no overview of investigated markers and their association with PM has been published, while this could help in directing future research. The aim of this systematic review is to identify biomarkers during pregnancy that are specific for malaria. The focus is on host biomarkers that can fluctuate over time and thus can indicate the occurrence of an underlying pathophysiological process of PM. The focus is hereby on \textit{P. falciparum} as causative pathogen as it is most strongly related to PM pathology. PM caused by \textit{P. vivax} is beyond the scope of this review.

**Methods**

**Literature search and selection of articles**

A systematic search for available literature was performed in the databases of Medline, Embase and the Cochrane library (Supplementary information). Stepwise screening of the studies by inclusion and exclusion criteria was performed by ER and EL. Any discrepancies were discussed and if needed a third person was consulted to reach consensus (PFM). Studies were included if they measured biomarkers in our target population of women living in malaria endemic settings. Furthermore, studies needed to diagnose PM caused by \textit{P. falciparum}. To create a comprehensive review, all types of PM diagnosis were accepted (PBS, PIS, HIS, RDT, PCR). Studies that only screened for malaria in peripheral blood were excluded. Biomarkers were only included if protein level or protein-coding RNA expression was studied and if they represented the host response. Genetic markers and other markers in a permanent state were excluded, as well as animal studies, studies on antibodies, hemoglobin, glucose, cell proliferation and studies using less quantifiable methods such as immunohistochemistry for detection of biomarkers. Review Manager was used to create a flow chart.\textsuperscript{22}

**Risk of bias assessment**

Risk of bias was assessed in all articles by ER and EL according to criteria for diagnostic studies. Discrepant results were resolved by discussion, if needed a third reader was consulted to reach consensus (PFM). In addition it was recorded if studies adjusted for testing of multiple biomarkers or between multiple groups (multiple hypotheses correction).
Statistical analysis

All analyses were performed using Stata/SE 12.1. Authors of included manuscripts were contacted for raw data in case of insufficient reported results. Included studies presented their data either as means or medians. Weighted random effect meta-analysis of the mean difference (MD) was performed if at least three independent studies could be included. Data presented as medians were transformed to the natural logarithmic scale as to create the mean of the data on the log scale. The transformed standard deviation (SD) was calculated by dividing the log transformed interquartile range (IQR) by 1.35 [N. Nagelkerke, personal communication]. Normal distribution of the transformed data was verified by the log transformed upper and lower quartiles; if the difference between the mean and upper quartile and mean and lower quartile on the log scale was comparable (within 15% of each other’s range), the distribution was considered normal. The t-test formula from method 1 described in Higgins et al. was used to obtain the variance on the log scale. The calculation of the variance (transformed SD/ sample size) was slightly adapted as to account for the relative efficiency of the median by using the formula ‘transformed SD / (sample size * (2/π))’ [N. Nagelkerke, N. van Geloven, personal communication]. Subsequently the standard error (SE) of the MD could be calculated. Random effects meta-analysis of MD and corresponding SE on the log scale was performed. To attempt combining data presented as means and medians for meta-analysis, means were also transformed to the natural logarithmic scale by using method 1 from Higgins et al. Taylor series approximation from method 1 was used to calculate SE. If data were stratified in the studies, pooled means and SD were calculated where appropriate.

Results

Search results

The search initially resulted in 3615 articles, and 2424 unique articles remained after removal of duplicates. After the second screening step (Supplementary information), 47 articles were included for data extraction (Figure 1). Studies differed in various aspects, for example in diagnostic method for PM, in the classification of previously infected placentas (pigment in fibrin but no pigment or parasites in cells) and in biomarker test methods. Other differences between studies were found in the composition of the study population; for example the in- or exclusion of HIV-infected women and of women with (concurrent) peripheral malaria infection.
Figure 1. Flow chart of search strategy

1625 records identified in Pubmed
1656 records identified in Embase
134 records identified in the Cochrane Library
0 additional records identified through other sources

2424 records after duplicates were removed

2424 of records screened

2201 of records were excluded after title and abstract were screened for in- and exclusion criteria

85 of full-text records excluded:
- 25 abstracts of conferences (unsufficient information or separate full text article already screened)
- 18 no comparison with PM
- 26 no PM diagnosis
- 20 other reasons

133 of full-text records assessed for eligibility

47 records included in qualitative synthesis

All records checked for 'cited by' and references in WoS. 0 new relevant studies

Of all 47 records included in review, 3 records included in meta-analysis.

WoS = web of science
## Risk of bias

Table 1 shows an overview of the risk of bias for each of the included studies. In most studies a case-control design was used based on PM diagnosis, but occasionally it was based on peripheral malaria infection, HIV infection, gestational age at delivery or birth weight, which all imposes a risk of patient selection bias. For some studies with a case-control design not based on PM diagnosis, results on biomarkers were presented for pooled data. These studies were not excluded, but pooling is indicated as (p) in Table 1. Most studies did not report anything on correction of multiple hypotheses testing, nine used the Bonferroni or Bonferroni-Dunn method,\textsuperscript{26–28,30,36,37,44,46,52} three studies used the Tukey method\textsuperscript{28,36,56} and one used the Holm-Bonferroni method.\textsuperscript{38} Methods used and the corresponding \(p\) values (if presented) for each outcome can be found in the Supplementary information.

### Table 1. Risk of bias summaries

| Article                        | Selection patients | Index test standardized | Index test blinded | Reference test standardized | Reference test blinded | Same reference test for all | Same reference test only | Time bias per. biomarkers | Time bias plac. biomarkers | Missing data per. biomarkers | Missing data plac. biomarkers | Notes patient selection     |
|-------------------------------|--------------------|-------------------------|--------------------|----------------------------|------------------------|-----------------------------|--------------------------|-----------------------------|------------------------------|-----------------------------|-------------------------------|--------------------------------|
| Abramsa\textsuperscript{26}   | -                  | +                       | +                  | +                          | +                      | +                           | +                        | -                           | -                            | +                           | +                             | Case-control based on HIV and preterm versus full term (p). |
| Abrams\textsuperscript{a,27}  | -                  | +                       | ?                  | +                          | +                      | NA                         | +                        | NA                          | +                            | NA                          | ?                             | Case-control based on peripheral infection (p). |
| Avery\textsuperscript{28}     | -                  | +                       | +                  | +                          | NA                     | NA                         | +                        | NA                          | +                            | NA                          | ?                             | Case-control                   |
| Bayoumi\textsuperscript{29}   | -                  | ?                       | ?                  | ?                          | NA                     | NA                         | ?                        | ?                           | ?                            | NA                          | ?                             | Case-control                   |
| Boeuf\textsuperscript{30}     | -                  | ?                       | ?                  | +                          | +                      | NA                         | NA                       | +                           | +                            | NA                          | +                             | Case-control                   |
| Boeuf\textsuperscript{a,31}   | -                  | +                       | +                  | +                          | +                      | +                          | +                        | NA                          | +                            | NA                          | ?                             | Case-control                   |
| Bouyou-Akotet\textsuperscript{32} | ?          | +                       | +                  | +                          | +                      | +                          | +                        | +                           | ?                            | +                           | ?                             | Case-control                   |
| Chaisavaneeyakorn\textsuperscript{a,33} | ?       | ?                       | ?                  | +                          | NA                     | NA                         | +                        | NA                          | ?                            | NA                          | ?                             | Case-control                   |
| Chaisavaneeyakorn\textsuperscript{b,34} | ?         | ?                       | ?                  | +                          | NA                     | NA                         | ?                        | ?                           | ?                            | NA                          | ?                             | Case-control                   |
| Chaisavaneeyakorn\textsuperscript{b,35} | ?         | +                       | ?                  | +                          | NA                     | NA                         | +                        | NA                          | +                            | +                           | +                             | Case-control                   |
Table 1. Risk of bias summaries (continued)

| Article | Index test standardized | Index test blinded | Reference test standardized | Reference test blinded | Same reference test for all | Time bias plac. biomarkers | Time bias plac. biomarkers | Missing data plac. biomarkers | Missing data plac. biomarkers |
|---------|-------------------------|---------------------|----------------------------|-----------------------|----------------------------|-----------------------------|-----------------------------|-------------------------------|-------------------------------|
| Chua36  | ?                       | +                   | ?                          | +                     | +                         | +                          | ?                          | ?                             | ?                             |
| Conroy37| ?                       | +                   | ?                          | ?                     | +                         | +                          | ?                          | ?                             | ?                             |
| Conroy38| -                       | +                   | +                          | ?                     | +                         | NA                         | NA                         | NA                            | ?                             |
| Conroy39| ?                       | +                   | ?                          | ?                     | +                         | +                          | ?                          | ?                             | ?                             |
| Diallo40| -                       | +                   | ?                          | ?                     | +                         | +                          | ?                          | ?                             | ?                             |
| Diouf41 | -                       | +                   | ?                          | ?                     | +                         | NA                         | ?                          | NA                            | ?                             |
| Dong42  | +                       | +                   | ?                          | ?                     | +                         | +                          | -                          | -                             | -                             |
| Fievet43| -                       | +                   | ?                          | ?                     | ?                         | NA                         | NA                         | -                             | Case-control based on peripheral infection (p). |
| Flanagan44| ?                     | +                   | ?                          | ?                     | +                         | NA                         | -                          | NA                            | Case-control based on rapid immunochromatographic test |
| Fried45  | +                       | +                   | ?                          | ?                     | ?                         | +                          | +                          | NA                            | Case-control based on peripheral infection (p). |
| Jakobsen46| ?                   | +                   | ?                          | ?                     | +                         | NA                         | ?                          | ?                             | ?                             |
| Kabyemela47| +                  | +                   | ?                          | ?                     | +                         | +                          | +                          | ?                             | ?                             |

+ = low risk of bias, - = high risk of bias, ? = unknown risk of bias, NA = not applicable, periph. = peripheral, plac. = placental, biom. = biomarkers, (p) = case-control data were pooled for PM+ PM-comparison, a-e = studies with same letter are based on same study.

Meta-analysis

Random effect meta-analyses were performed for placental levels of biomarkers IL-10, IFN-γ, TNF-α and IL-4, as these were investigated in a minimum of three studies with available outcome data. Meta-analysis of untransformed mean levels of IL-4 in three studies showed no significant difference (Supplementary information). All other meta-analyses revealed high heterogeneity between studies (both for analyses of means and MD of log transformed medians). Meta-analyses on the log transformed scale for medians and means combined resulted in extremely
higher assigned weights for studies with original medians compared to studies with original means. These meta-analyses were discarded because this would not reflect the actual relevant data that were available.

Overview of biomarkers

All studied biomarkers can be found in the Supplementary information. Because of the extensive numbers, results are only presented for the biomarkers at protein or cytokine level that were most studied (Tables 2 – 5) and for other markers if in at least one study a significant difference for PM+ in peripheral blood was shown for primigravidae or all gravidities combined (Table 6). A complete overview of results can be found in the Supplementary information. Nearly all biomarkers were tested at delivery and compared with PM diagnosis. Only two studies related peripheral biomarker levels during pregnancy (zinc protoporphyrin (ZPP), estradiol, progesterone and human placental lactogen (HPL)) to a diagnosis of PM at delivery.

Table 2. IL-10 as biomarker for placental malaria

| Reference | Per. levels in PM+ primigravidae | Per. levels in PM+ secundigravidae | Per. levels in PM+ multigravidae | Per. levels in PM+ all gravidities | Plac. levels in PM+ primigravidae | Plac. levels in PM+ secundigravidae | Plac. levels in PM+ multigravidae | Plac. levels in PM+ all gravidities | Notes |
|-----------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|-------|
| Avery28    | ↑                               |                                 |                                 |                                 |                                 |                                 |                                 |                                 | For PM diagnosed by PBS. |
| Bayoumi29  | ↓                               | ↑                               |                                 |                                 |                                 |                                 |                                 |                                 | Past versus no placental infection. |
| Diouf41    |                                 |                                 |                                 |                                 |                                 |                                 |                                 |                                 |                   |
| Fievet43   |                                 |                                 |                                 |                                 |                                 |                                 |                                 | x                               | Cultures of blood, villi, or villi + blood |
| Flanagan44 | x                               |                                 |                                 |                                 |                                 |                                 |                                 |                                 | Culture supernatants. |
| Fried45    |                                 | x                               | x                               | x                               | x                               | x                               | x                               |                                 | Cultures of medium, PHA, CD3, PPD, malarial antigen. Primi-/secundigravidae combined in one group. |
| Moore50*   |                                 | x                               | x                               | x                               |                                 |                                 |                                 |                                 |                                 |
| Moore51#   |                                 |                                 |                                 |                                 | x                               |                                 |                                 |                                 | Cultures of medium, PHA, CD3, PPD, malarial antigen. Includes the subgroup described in Moore 1999 [50]. |
| Suguitan66#|                                 |                                 |                                 |                                 |                                 |                                 |                                 | ↑                               | Significantly increased for PM+ versus PM- in both preterm and full term deliveries. |
Table 2. IL-10 as biomarker for placental malaria (continued)

| Reference      | Per. levels in PM+ primigravida | Per. levels in PM+ secundigravida | Per. levels in PM+ multigravidae | Per. levels in PM+ all gravidities | Plac. levels in PM+ primigravidae | Plac. levels in PM+ secundigravidae | Plac. levels in PM+ multigravidae | Plac. levels in PM+ all gravidities | Notes                                                                 |
|----------------|---------------------------------|-----------------------------------|----------------------------------|-----------------------------------|-----------------------------------|-------------------------------------|-----------------------------------|-----------------------------------|-----------------------------------------------------------------------|
| Suguitan⁶⁷#    |                                  |                                   |                                  |                                   |                                   |                                     |                                   | ±                                 | Significantly increased in PM+ versus PM- in placental plasma direct measurement and WBC culture, NS after 24 hour villous tissue culture. |
| Only HIV- included                                |                                  |                                   |                                  |                                   |                                   |                                     |                                   |                                                 |                                                                         |
| Diallo⁴⁰       | ↑                                |                                   |                                   |                                   |                                   |                                     |                                   |                                  | Increased but not significant.                                        |
| Jakobsen⁶⁵     |                                  |                                   |                                   |                                   |                                   |                                     |                                   |                                  |                                                                         |
| Kabyemela⁶⁷    | ↑†                              |                                   | ↑†                                |                                   |                                   |                                     |                                   | ↑†††††                          |                                                                         |
| Perkins⁵⁷      |                                  |                                   |                                   |                                   |                                   |                                     |                                   | ±                                 | Significantly decreased in placentas with 10 - 25% pigment versus placentas with 1 - 10% or no pigment after 48 hours of culture. |

*/#= studies with partial overlap in participants.

Peripheral IL-10 is increased in women with active PM infection (3 studies), but not with past PM infections (1 study) or after culture of the samples (1 study). Placental IL-10 levels show variable results without a very clear trend possibly relating to the use of cultures, past PM infections and other baseline differences (Supplementary information).

↑ = significantly increased in PM+, ↓ = significantly decreased in PM+, ± = significant difference in one or multiple subgroups, x = no significant differences, Per. = peripheral, Plac. = placental, NS = not significant, PBS = placental blood smear, PIS = placental impression smear, HIS = histology.

Table 3. TNF-α as biomarker for placental malaria (subscript)

*/#= studies with partial overlap in participants.

Peripheral TNF-α levels are increased in half of the studies (3 studies) measuring peripheral levels. No consensus can be found in study population or test methods between studies with significant or without significant results. For direct measurements of placental TNF-α many studies showed increased levels (5 studies), culture of placental samples showed variable results but a trend of increased levels in PM+ patients can be noted.

↑ = significantly increased in PM+, ↓ = significantly decreased in PM+, ± = significant difference in one or multiple subgroups, x = no significant differences, Per. = peripheral, Plac. = placental, NS = not significant, PBS = placental blood smear, PIS = placental impression smear, HIS = histology.
| Reference | Per. level PM+ primigravidae | Per. level PM+ secundigravidae | Per. level PM+ multigravidae | Plac. level PM+ primigravidae | Plac. level PM+ secundigravidae | Plac. level PM+ multigravidae | Plac. level PM+ all gravidities | Notes |
|-----------|-----------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|--------------------------------|--------------------------------|-------|
| Abrams\textsuperscript{26} | x                            |                               |                               |                               |                                |                                |                                | Pooled case-control data (preterm and full term delivery). |
| Avery\textsuperscript{28}    |                               | ↑                             |                               |                               |                                |                                |                                | Significant increase in PM+ versus PM- by PBS. NS for placentas negative by PBS but positive by PCR versus PM-. |
| Diouf\textsuperscript{41}    | x                            |                               |                               |                               |                                |                                |                                | Only significant increase in direct serum measurements. NS in cultures of villi, villi + blood, or blood. |
| Fievet\textsuperscript{43}   |                               |                               |                               |                                |                                |                                |                                | Measured in culture supernatants. |
| Flanagan\textsuperscript{44}  | x                           |                               |                               |                               |                                |                                |                                | Significant increase for paucigravidae in medium, PHA, CD3, PPD, decrease in malaria antigen cultures. Significant decrease in medium in multigravidae. |
| Fried\textsuperscript{45}      |                               | ↑                             | x                             |                               |                                |                                |                                | Significantly increased in cultures of medium, PHA, CD3 and malarial antigen. NS for PPD cultures. Includes subgroup from Moore 1999 \textsuperscript{50}. |
| Moore\textsuperscript{50*}    |                               |                               | ±                             | ±                             | ±                              |                                |                                | Significantly decreased in placentas with 10 - 25% pigment versus placentas with 1 - 10% and no pigment after 48 hours culture. |
| Moore\textsuperscript{51*}    |                               |                               | ±                             | ±                             | ±                              |                                |                                | |
| Rogerson\textsuperscript{58}  | ↑                           |                               |                               |                                |                                |                                |                                | Significantly increased in PM+ versus PM- preterm deliveries, NS for full term deliveries. |
| Suguitan\textsuperscript{66#} |                               |                               |                               |                                |                                |                                |                                | Borderline significant increase in PM+ in direct plasma measurement, after culture NS for villous tissue, significant increase in WBC culture. |
| Suguitan\textsuperscript{67#} |                               |                               |                               |                                |                                |                                |                                | |

**Only HIV+ included**

| Reference | Per. level PM+ primigravidae | Per. level PM+ secundigravidae | Per. level PM+ multigravidae | Plac. level PM+ primigravidae | Plac. level PM+ secundigravidae | Plac. level PM+ multigravidae | Plac. level PM+ all gravidities | Notes |
|-----------|-----------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|--------------------------------|--------------------------------|-------|
| Diallo\textsuperscript{40}   | ↑                           |                               |                               |                               |                                |                                |                                | Significantly decreased in placentas with 10 - 25% pigment versus placentas with 1 - 10% and no pigment after 48 hours culture. |
| Kabyemela\textsuperscript{47} | ↑ ↑ ↑                       |                               |                               |                               |                                |                                |                                | |
| Perkins\textsuperscript{57}   | ↑ ↑ ↑                       | x                             |                               |                               |                                |                                |                                | |
Table 4. IFN-γ as biomarker for placental malaria

| Reference | Per. level PM+ primigravidae | Per. level PM+ secundigravidae | Per. level PM+ multigravidae | Per. level PM+ all gravidities | Plac. level PM+ primigravidae | Plac. level PM+ secundigravidae | Plac. level PM+ multigravidae | Plac. level PM+ all gravidities | Notes |
|-----------|-----------------------------|--------------------------------|-----------------------------|-------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|-------|
| Bayoumi   | ↓                           |                                |                             |                               |                                |                                |                                |                                |       |
| Diou       | x                           |                                |                             |                               |                                |                                |                                |                                |       |
| Fievet     | ±                           |                                |                             |                               |                                |                                |                                |                                |       |
| Flanagan   | x                           |                                |                             |                               |                                |                                |                                |                                |       |
| Fried      | x                           | x                              | x                           | ±                             |                                |                                |                                |                                |       |
| Moore      | x                           | x                              | x                           | ±                             |                                |                                |                                |                                |       |
| Moore      | x                           |                                |                             |                               |                                |                                |                                |                                |       |
| Rogerson   | x                           |                                |                             |                               |                                |                                |                                |                                |       |
| Suguitan   | x                           |                                |                             |                               |                                |                                |                                |                                |       |
| Suguitan   | x                           |                                |                             |                               |                                |                                |                                |                                |       |

**HIV+ and HIV-included or unknown**

- Past versus no placental infection.
- Significant increase in cultures of villi or blood. NS in serum or cultures of villi + blood.
- Measured in culture supernatants.
- Paucigravidae NS. In multigravidae significant decrease in Med, PHA, CD3 and PPD cultures.
- Cultures of medium, PHA, CD3, PPD, malarial antigen. Includes subgroup described in Moore 1999 [50].
- Significant increase in plasma, but only detectable in 4% of total samples (8 samples).
- Significant increase in PM+ versus PM- in full term deliveries. NS for preterm deliveries.
- Borderline significantly increased in plasma direct measurement and in 24 hours villous tissue culture, NS in WBC culture.

**Only HIV- included**

- =significantly increased in PM+, ↓ = significantly decreased in PM+, = = significant difference in one or multiple subgroups, ≠ = no significant differences, Per. = peripheral, Plac. = placental, NS = not significant, PBS = placental blood smear, PIS = placental impression smear, HIS = histology, Med = medium, PHA= phytohemagglutinin, CD3 = anti-CD3 monoclonal antibody, PPD = purified protein derivative.
**Table 5. IL-4 as biomarker for placental malaria**

| Reference       | Per. level PM+ primigravida | Per. level PM+ secundigravida | Per. level PM+ multigravida | Plac. level PM+ primigravida | Plac. level PM+ secundigravida | Plac. level PM+ multigravida | Notes                                                                 |
|-----------------|-----------------------------|-------------------------------|-----------------------------|-----------------------------|-------------------------------|-----------------------------|------------------------------------------------------------------------|
| Bayoumi29       | ↓                            |                               |                             |                             |                               |                             | Past versus no placental infection.                                   |
| Fievet33        |                             |                               |                             |                             |                               |                             |                                                                        |
| Flanagan44      |                             |                               |                             |                             |                               |                             |                                                                        |
| Fried45         |                             |                               |                             |                             |                               |                             | Significant decrease for paucigravidae in malarial antigen cultures,  |
| Moore30*        |                             |                               |                             |                             | x                             | x                           | NS in medium, PHA, CD3 and PPD cultures and for multigravidae.         |
| Moore31*        |                             |                               |                             |                             | x                             |                            | Cultures of medium, PHA, CD3, PPD, malarial antigen. Includes subgroup  |
| Suguitan46#     |                             |                               |                             |                             | x                             |                            | NS between PM+ and PM- in groups with preterm and full term deliveries.|
| Suguitan47#     |                             |                               |                             |                             | x                             |                            | NS in placental plasma direct measurement, WBC culture and villous     |

*Only HIV- included*

| DiaIlo40        | x                            |                               |                             |                             |                               |                             |                                                                        |
| Kabyemela47     | x                            | x                             |                             |                             |                               |                             |                                                                        |

*/# = studies with partial overlap in participants

There seems to be no difference in IL-4 levels with PM infection.

↑ = significantly increased in PM+, ↓ = significantly decreased in PM+, ± = significant difference in one or multiple subgroups, x = no significant differences, Per. = peripheral, Plac. = placental, NS = not significant, PBS = placental blood smear, PIS = placental impression smear, HIS = histology.
Table 6. Overview of biomarkers for placental malaria

| Biomarker | Per. level PM+ primigravidae | Per. level PM+ secundigravidae | Per. level PM+ multigravidae | Per. level PM+ all gravidities | Plac. level PM+ primigravidae | Plac. level PM+ secundigravidae | Plac. level PM+ multigravidae | Plac. level PM+ all gravidities | Notes |
|-----------|------------------------------|-------------------------------|-----------------------------|-------------------------------|-----------------------------|-------------------------------|-------------------------------|-------------------------------|-------|
| Alanine   | ±                            | x                            | ↓                           |                               | x                           | ↓                             |                               | ± Significant increase for PM with intervillosis vs PM- | 31    |
| Ang-1     | ±                            |                               | x                            | ↓                             | x                           | ↓                             | ↓                             | ± Significant decrease for PM+ versus PM- in LBW group. NS in NBW | 38,39 |
| Ang-2     | ±                            |                               | x                            | ↑                             | ↑                           | ↑                             | ↑                             | ↑                             | ± Significant increase for PM+ LBW/NBW versus PM- NBW, NS versus PM- LBW | 63    |
| Ang-2     | ±                            |                               |                               |                               | ∆                            | ↑                             | ↑                             | ↑                             | ↓                             | Significant decrease for active or past PM vs PM- (but influenced by anaemia) | 31    |
| APO-A1    | ±                            |                               | ↓                            |                               |                             |                               |                               |                               | ↑                             | Significant increase for PM without intervillosis vs PM- | 64    |
| APO-B     | ↓                            |                               |                               |                               |                             |                               |                               |                               | ↓                             | Significant increase for PM without intervillosis vs PM- | 64    |
| Asparagine| ±                            |                               |                               |                               |                             |                               |                               |                               | ± Significant increase for PM without intervillosis vs PM- | 31    |
| Aspartic acid | ↑                            |                               |                               |                               |                             |                               |                               |                               | ↑                             | Significant increase for PM if peripheral C5a levels >100 ng/mL | 38    |
| C5a**     | ↑                            |                               |                               |                               |                             |                               |                               |                               | ↑                             | Significant increase for PM+ LBW/NBW versus PM- NBW, NS versus PM- LBW | 39    |
| C5a**     | ↑                            |                               |                               |                               |                             |                               |                               |                               | ↑                             | Significant increase for PM without intervillosis vs PM- | 39    |
| C5a*      | ↑                            | x                             | x                            |                               | x                           | x                             | x                             | ↑                             | x                             | Significant higher percentage of PM+ if peripheral C5a levels >100 ng/mL | 37    |
| CCL18     | ↑                            | x                             | x                            | x                             | x                           | x                             | x                             | ↑                             | x                             | Increase in PM+ with or without anemia compared with PM- without anemia | 42    |
| CRP       | ↑                            |                               |                               |                               |                             |                               |                               |                               | ↑                             | Significant increase for PM without intervillosis vs PM- | 47    |
| CRP       | ±                            |                               |                               |                               |                             |                               |                               |                               | ±                             | Significant increase for PM without intervillosis vs PM- | 47    |
| CXCL1     | ↑                            | x                             | x                            | x                             | x                           | x                             | x                             | x                             | ↑                             | Significant increase for PM without intervillosis vs PM- | 31    |
| CXCL13*   | ↑                            | x                             |                               |                               |                             |                               |                               |                               | ↑                             | Significant increase for PM without intervillosis vs PM- | 42    |
| Cystine   | ↑                            | ↑                             | ↑                             |                               | x                           |                               |                               | x                             | ↑                             | Significant increase for PM without intervillosis vs PM- | 47    |
| Ferritin  | ↑                            | ↑                             | ↑                             | x                             | ↑                           | x                             | x, ↑                          | ↑                             | x                             | Significant increase for PM without intervillosis vs PM- | 47    |
| Ferritin  | ↑                            | ↑                             | ↑                             | x                             | ↑                           | x                             |                               | ↑                             | x                             | Significant increase for PM without intervillosis vs PM- | 47    |
Inflammatory biomarkers

Tables 2 - 5 present the most frequently investigated (anti-)inflammatory cytokines. IL-10 was increased in peripheral and placental blood in most studies (Table 2), but this difference was not always significant and the SDs or IQRs were often large.\textsuperscript{29,41,45–48,50,51,57,66,67} The levels of IL-10 in the different studies are within the same range, both for peripheral and placental measurements (culture measurements are more variable).\textsuperscript{53,44,50,51} These findings are however contradicted by Bayoumi \textit{et al.}: they showed high peripheral and placental IL-10 levels, but with an adverse effect (PM- women showed significantly higher levels than women with past placental infections).\textsuperscript{29} A difference in this study is the comparison of PM- with past infections, while past infections were considered PM- in the other IL-10 studies except for Fievet \textit{et al.}.\textsuperscript{43} Contradicting results were also observed by Perkins \textit{et al.}, who studied IL-10 in comparison to the level of pigmented cells in the placenta. IL-10 levels were increased in moderately pigmented placentas in culture, but significantly decreased in severely pigmented placentas compared with moderate and nonpigmented placentas.\textsuperscript{57} The authors suggested that cytokine synthesis might be suppressed in immune cells with excessive ingestion of hemozoin.\textsuperscript{57} With respect to subgroups, PM+ primigravidae showed a trend for higher placental IL-10
levels compared with other gravidities.\textsuperscript{45,47} Higher placental IL-10 levels were also found in women with preterm deliveries, especially in PM+ cases.\textsuperscript{66} HIV infection did not influence IL-10 levels in culture supernatants of PM+ and PM-.\textsuperscript{51}

For TNF-\(\alpha\) more variable levels were found: up to a 10-fold difference in placental direct measurements (Table 3) as is seen for Kabyemela \textit{et al.} (PM+: mean 263.2 – 512.3 pg/ml; PM-: mean 263.6 – 324.1 pg/ml, depending on gravidity)\textsuperscript{47} versus Rogerson \textit{et al.} (PM+: 10.0 pg/ml; PM-: 3.3 pg/ml).\textsuperscript{58} However, these studies differed in biomarker test method, PM diagnostic method, inclusion of HIV patients and peripheral infection in PM- (Supplementary information). Several studies found significantly increased peripheral and placental TNF-\(\alpha\) levels\textsuperscript{23,40,43,45,47,48,50,51,58,66,67} As for IL-10, Perkins \textit{et al.} also showed an increase in TNF-\(\alpha\) level in moderately pigmented placentas in culture, but a significant decreased level in severely pigmented placentas compared with moderate and nonpigmented placentas.\textsuperscript{57}

IFN-\(\gamma\) is occasionally significantly elevated (Table 4), but like TNF-\(\alpha\), the magnitude of levels were variable. In two and three studies high levels of IFN-\(\gamma\) were found in peripheral blood (100 – 360 pg/ml)\textsuperscript{29,40} and placental blood (25 – 280 pg/ml) respectively,\textsuperscript{29,40,45} whereas levels of this cytokine were often undetectable or very low in other studies.\textsuperscript{43,47,48,58} There is no single explanation for these differences as all studies varied in design (Table 1). HIV infection impaired the IFN-\(\gamma\) response.\textsuperscript{51}

IL-4 was the only biomarker for which meta-analysis was possible for three studies; this showed no significant difference between PM+ and PM- which is generally confirmed by the remaining studies on IL-4 that were not included in the meta-analysis (Table 5).

In Table 6 less frequently studied inflammatory biomarkers with significant peripheral changes are shown. For both IL-1 and C-reactive protein (CRP) a significant increase was seen in only one of two studies. TNF receptors 1 and 2 (TNF-R1/ TNF-R2) also showed significantly increased peripheral levels in PM+, for TNF-R2 this was consistent in both reporting studies.\textsuperscript{48,69} Furthermore, the significant increased peripheral TNF-R2 level was found for both peripheral malaria positive and peripheral malaria negative women with PM.\textsuperscript{59} Another increased inflammatory marker was suPAR (soluble urokinase-type plasminogen activator receptor).\textsuperscript{56} In PM infected women, suPAR levels correlated negatively with birth weight.\textsuperscript{56} CCL-18, CXCL13, CXCL1 and CCL2 (also known as MCP-1) are all chemokines induced by TNF-\(\alpha\) or both TNF-\(\alpha\) and IFN-\(\gamma\) (CCL2). These chemokines were significantly increased in primigravidae with PM, although for CCL2 (MCP-1) this was not substantiated by other studies.\textsuperscript{26,32,67} CXCL13 was furthermore associated with reduced birth weight in primigravidae, but after multivariate analyses this independent association was lost.\textsuperscript{62} C3a and C5a of the complement system were investigated by one group.\textsuperscript{37–39} C5a was significantly increased in placental blood of PM+ women of all gravidities, but in peripheral blood only for PM+ primigravidae.\textsuperscript{37–39} Besides an
association with PM, one of the studies showed an association between high C5a levels and higher odds of delivering a small for gestational age baby. Noninflammatory biomarkers

Several other groups of proteins and cytokines were studied (Table 6 & Supplementary information). Ferritin, hepcidin, iron, TIBC, sTfR, TS, and ZPP were markers involved in hemoglobin and iron metabolism that were studied. Only ferritin was significantly increased in peripheral blood in one of two studies, while the other showed a trend of increased levels. Within the group of angiogenesis markers (Ang-1, Ang-2, sTie-2, sEng, sFlt-1, and VEGF) only angiopoietin 1 and 2 (Ang-1/Ang-2) and sFlt-1 showed significant changes, but the angiopoietins were dependent on birth weight and sFlt-1 on hypertension or genotype (stratified data). In contrast, all markers involved in lipid metabolism (apolipoprotein A1 (APO-A1), apolipoprotein B (APO-B), and leptin) showed significant decreases in primigravidae or all gravidities. Unfortunately the single study on apolipoproteins used a case-control design based on severe anemia, and APO-A1 levels in the PM- group were much influenced by the severely anemic women. APO-B levels did not differ between anemic or non-anemic groups. One study focused only on amino acids and found increased levels for alanine, asparagine, aspartic acid, cystine, leucine, phenylalanine, and tryptophan. Possibly, as hypothesized by the authors, this was related to reduced placental transport of these amino acids. Finally, the hormone estradiol showed promising results with significant decreased levels throughout pregnancy in women with pigmented placentas at delivery.

Expression of biomarkers

Many biomarkers were tested for RNA expression levels in the placenta, but often by only one study (Supplementary information). The inflammatory markers IL-10 and IFN-γ and angiogenesis marker sFlt-1 did not show a consistent trend of increased placental expression levels. TNF-α did show significant increased expression in placental tissue, as well as chemokines CCL18 and CXCL13 which were also increased in protein levels. Unfortunately no RNA expression levels were tested for the other discussed protein/cytokine markers. Biomarkers not tested at protein level but with significant changes in placental RNA expression are not discussed separately but can be found in the Supplementary data.
Discussion

Although many cytokines, proteins and RNA expression levels have been studied for their association with PM, no single biomarker with proven diagnostic potential has been identified so far. For diagnostic potential, biomarkers need not only be sensitive and specific, but should also be easily detected in peripheral blood. In our review we have therefore mostly focused on peripheral cytokines or proteins. The cytokines IL-10 and TNF-α showed a general trend of increased levels in PM+ women. These levels were measured at delivery, however, for IL-10 significantly increased levels were also found during pregnancy for peripherally infected women and these levels normalised after treatment. Despite these trends, the changes in PM+ for IL-10 and TNF-α were not consistent. The observed variability in results between studies is probably a result of the variability in study populations, biomarker test methods and diagnostic methods for PM. For example, in Conroy et al. all negative tested placentas with blood smear microscopy were afterwards checked with histology. About half of these placentas had evidence of PM on histology. This disagreement in reference standards for PM may explain the heterogeneity in biomarker levels.

The variability can also be explained by the fact that cytokine release can be triggered by multiple pathways and many other factors outside of PM may cause shifts in the cytokine levels. This could be the cause of between study variability (because study populations differed in the presence of peripheral malaria infection, HIV prevalence and risk of other co-infections) and within study variability (large SDs and IQRs). This large range in data and resulting overlap between PM+ and PM- will impair the use of a single biomarker as diagnostic tool for PM; the required cut off value will likely result in many false positives and false negatives.

Combining biomarkers could overcome this problem of low sensitivity and specificity. For this purpose, IL-10 and TNF-α might still be suitable. IL-10 is a Th2 type cytokine and is normally increased in women during pregnancy, although higher levels have been related to preeclampsia. Higher levels were associated with PM in this review, which is consistent with the trend of increased levels in malaria infected nonpregnant individuals. TNF-α is a Th1 type cytokine and increased levels have been associated with pregnancy complications. In accordance with literature on nonpregnant individuals, this review showed a trend of increased levels in malaria infected women, although this finding was not consistent. Studying a combination of the two markers may reflect the underlying immunological balance in PM.

Other, less frequently, studied cytokines and proteins may also be used as biomarkers in a combination model. Markers with most potential due to pathophysiological explanations or additional clinical evidence are discussed here. TNF-R2 is
a cell surface receptor for TNF-α binding. Increased soluble levels of TNF-R2 will therefore inhibit the actions of TNF-α. Besides being significantly increased at delivery in peripheral blood of PM+, an association between TNF-R2 and peripheral malaria infection during pregnancy was also found in Thévenon et al. Moreover, peripheral parasitemia levels directly correlated with TNF-R2 and even sub-microscopic infections resulted in significant increases. After clearance of infection, TNF-R2 levels also normalised. TNF-R2 has also been found to be increased in non-pregnant individuals with malaria.

Another interesting inflammatory marker is CXCL13, because of its association with PM and its trend towards association with low birth weight. This chemokine and its receptor (CXCR5) are shown to attract B-cells that produce natural antibodies in mice and might thus be important for early immunity. In Muehlenbachs et al. the increased placental expression of CXCL13 was associated with massive chronic intervillositis and the authors suggest that the role of B cells has thus far been underappreciated in chronic PM. CXCL13 has also been related to other causes of inflammation in pregnancy like villitis of unknown origin, but this was only shown for RNA expression.

C5a of the complement system was significantly increased in PM+ patients and has previously been linked to the pathogenesis of cerebral malaria. Furthermore, expression of its receptor was shown to be increased in placental inflammation in Muehlenbachs et al. Increased levels of suPAR, a receptor involved in the recruitment of leucocytes to sites of inflammation, were associated with PM and with lower birth weight. Furthermore, suPAR levels correlated with parasitemia levels in children with malaria. However, levels of suPAR are also significantly increased in many other conditions like preeclampsia and tuberculosis and have been correlated to tuberculosis and HIV prognosis. It is therefore likely to be an unspecific biomarker, but this applies to a greater or lesser extent for all of the above inflammatory biomarkers. Even a combination might not be sufficient to distinguish PM from other inflammatory conditions.

Therefore, noninflammatory markers involved in PM pathogenesis may increase the specificity of a combination model; for example markers of angiogenesis, as there are indications of impaired vascularization in PM and of increased endothelial activation in nongestational malaria. Unfortunately, in the studies included in our review Ang-1, Ang-2 and sFlt-1 in peripheral blood were stratified for other factors than PM and were not significantly different in all strata. Nevertheless, sFlt-1 is also used as biomarker for preeclampsia and because of several pathophysiological similarities between preeclampsia and PM this marker could still be of interest.
Furthermore, in normal pregnancy there is a state of hyperlipidemia. In contrast, in PM+ women APO-A1 (component of high-density lipoproteins [HDL]) and APO-B (component of low-density lipoproteins [LDL]) were significantly decreased in a case-control study based on anemia. In this study, APO-A1 levels were also decreased with severe anemia, independent of PM status. In contrast, APO-B levels were uninfluenced by anemia and might therefore be more specific as PM biomarker. A recent review also showed changes in lipid metabolism in non-gestational malaria by decreased levels of LDL and HDL. Pathophysiological explanations are still hypothetical, but are related to the metabolic needs of the parasite and the use of lipids in hemoglobin formation.

One other non-inflammatory marker is the hormone estradiol; the only marker tested during pregnancy that showed an association with PM at delivery. Normally, estradiol levels increase gradually during pregnancy, whereas women with indications of PM showed a reduced increase. Decreased estradiol concentrations are considered to induce a Th1 response, in this review there are indications of both Th1 and Th2 responses in PM.

Finally, as primigravidae generally showed more significantly different biomarker levels between PM+ and PM- and because this is the group most severely affected by PM, it could be that a combination model of biomarkers for PM is only valid in primigravidae, or it might be necessary to include gravidity in a prediction model. Furthermore, antibody levels to the specific placental malaria antigen (VAR2CSA) may also indicate an increased vulnerability of pregnant women for PM and could therefore be used as biomarkers; the association between antibody levels and PM in primigravidae has recently been reviewed.

A limitation of the current review was the scarce availability of data for comparison. Many studies presented their results in medians and IQRs, which impaired comparison with studies presenting means. Furthermore, some studies did not present the exact data but only reported conclusions. Requests sent to authors for the raw data results remained often unanswered or raw data could not be accessed anymore. The diversity in study population and test methods made it more difficult to present overall conclusions for biomarkers. For example HIV infection can have an impact on biomarker levels itself and therefore studies with differences in the number of HIV infected participants cannot easily be compared.

Moreover, most studies did not test for other concurrent infectious diseases, therefore it cannot be ruled out that PM+ women also experienced other infections more often, due to behavioural or genetic susceptibility. The observed changes in inflammatory biomarkers may therefore not be related to malaria.

In addition, many studies tested multiple biomarkers, or multiple groups of patients, and the chance of finding a significant result with p value ≤ 0.05 is conse-
quently more likely to occur. Nevertheless, the majority of studies did not report any correction for multiple hypotheses testing. Furthermore, there is no consensus on the correct way of adjusting for this bias. For example, conservative tests like the Bonferroni method will increase the chance of finding false negative test results.\(^{100}\) It therefore remains important to interpret not only \(p\)-values but also the means and ranges of biomarker levels.

Our review was directed at biomarkers that were associated with PM rather than only peripheral infection, because PM is more strongly associated with detrimental pregnancy outcomes\(^ {101}\) and peripheral infection is not a good indicator of PM.\(^ {102}\) The selection for studies that compared biomarkers with proven PM resulted in very few studies on antenatal levels of biomarkers and may have caused some loss of valuable information, but no selection would have resulted in even more variability in studies.

Besides these limitations, some implications for future research can be given. Longitudinal studies are needed that focus on peripheral biomarkers during pregnancy related to PM outcome. This is methodologically more challenging due to time bias between index test (during pregnancy) and reference test (at delivery). Therefore, PCR confirmed diagnosis of peripheral malaria during pregnancy can be used as an additional indicator as to when a woman was probably infected and when she cleared the infection. This type of research is essential to estimate if biomarkers can be used for antenatal detection of PM.

To find out more about the specificity of biomarkers for detecting PM, it would also be interesting to retrieve more information about biomarker levels in nonpregnant women in future studies.

Furthermore, combinations of biomarkers need to be studied in future research. This could be a group of inflammatory markers, or more interestingly, inflammatory markers with markers of placental angiogenesis, lipid metabolism, hormones or clinical characteristics. A proposed combination of markers could be IL-10, TNF-R2, CXCL13, sFlt-1, APO-B and estradiol. Additionally, studies using proteomic-based techniques may identify new potential biomarkers and could therefore facilitate the search for the holy grail of biomarkers.

Almost all included studies in this review were situated in SSA. Malaria transmission patterns are different in other parts of the world where \(P.\) \textit{vivax} co-infections are, for example, more common. This means that if a biomarker or combination of biomarkers is found that shows good distinctive capacities for \(P.\) \textit{falciparum} PM in women in SSA, it will not necessarily be suitable for other parts in the world. If potential biomarkers are finally found, they should therefore be extensively studied in different areas in the world and in patients with different types of comorbidity.
Conclusion

None of the frequently studied biomarkers showed significant differences between PM+ and PM-, to be used as a single biomarker to predict PM. Nevertheless, a combination of biomarkers may increase specificity and could subsequently have diagnostic value, or could help in identifying women at risk for developing PM. For a useful combination of biomarkers researchers should focus on different pathophysiological pathways in PM, such as the proposed combination in our discussion. New insights in nongestational malaria and in mechanisms of fetal growth retardation could help in identifying other pathways and suitable biomarkers. More longitudinal studies will finally be needed to show which biomarkers will be suited to predict PM.

Future perspective

Diagnosis or risk group identification will become increasingly important with the reported change in malaria endemicity patterns in the last few years. In the upcoming era of decreasing malaria prevalence or even elimination, the current strategy of scheduled presumptive treatment of all pregnant women will likely become inefficient. Screening for PM with the use of biomarkers can enable more individually targeted care. Biomarker data during pregnancy will furthermore enhance our understanding of the complicated balance of (anti-)inflammatory cytokines during pregnancy and will inform on levels of non-inflammatory proteins in normal pregnancy and in pregnancies challenged by malaria infection.

Abbreviations

IQR: interquartile range; LBW: low birth weight; md: mean difference; MIP: malaria in pregnancy; NBW: normal birth weight; PBS: placental blood smear; PIS: placental impression smear; PM: placental malaria; PM+: placental malaria positives; PM-: placental malaria negatives; RDT: rapid diagnostic test; PCR: polymerase chain reaction; SD: standard deviation; SE: standard error; SSA: sub-Saharan Africa;
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## Supplementary information

### Table - Database search syntax.

Last search on 26th of February 2014.

| Database       | Syntax                                                                                                                                                                                                 |
|----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Medline        | (((((“Biological Markers”[Mesh] OR biomarker*[tiab] OR marker*[tiab] OR “Cytokines”[Mesh] OR cytokine*[tiab] OR chemokine*[tiab] OR complement*[tiab] OR proteins[Mesh] OR proteins[tiab] OR protein[tiab] OR RNA[tiab] OR RNA[Mesh] OR mRNA[tiab] OR level*[tiab] OR concentration*[tiab])) AND (“Placenta”[Mesh] OR “Placenta Diseases”[Mesh] OR Pregnancy[Mesh] OR Pregnancy Complications, Infectious[Mesh] OR placenta*[tiab] OR pregnant*[tiab])) AND (“Malaria”[Mesh] OR malaria*[tiab] OR “Plasmodium falciparum”[Mesh] OR plasmodium falciparum[tiab] OR p falciparum[tiab]))) AND |
| Excluded article | Reason for exclusion |
|------------------|----------------------|
| Abrams 2003 J Immunol | Comparison with MIP, no separate comparison with PM. |
| Adam 2007 Int J Gynaecol Obstet | No PM diagnosis. |
| Adam 2009 Int J Gynecol Obstet | Abstract. Full text Bayoumi 2009. |
| Adegnika 2006 Am J Trop Med Hyg | Comparison with MIP, no separate comparison with PM. |
| Agudelo 2013 Am J Trop Med Hyg | Abstract. Personal communication with authors: full text submitted for publication in Malaria Journal (Agudelo et al. 2014 Malaria Journal). |
| Akanbi 2010 Asian Pac J Trop Med | No PM diagnosis. |
| Akanbi 2010 Afr J Reprod Health | No PM diagnosis. |
| Ayoola 2012 Malaria J | Comparison with peripheral malaria, no separate comparison with PM. |
| Ayoola 2010 Horm Res Paediatr | Abstract. Full text Ayoola 2012. |
| Bayoumi 2009 Ann Trop Med Parasitol | No PM diagnosis. |
| Boeuf 2012 Placenta | Abstract. Full text Boeuf 2013. |
| Böstrom 2011 Scand J Immunol | Abstract. Full text Böstrom 2012 |
| Böstrom 2011 Am J Reprod Immunol | Abstract. Full text Böstrom 2012. |
| Böstrom 2012 PLoS One | Comparison with MIP, no separate comparison with PM. |
| Bouyou-Akotet 2004 Clin Infect Dis | No PM diagnosis. |
| Bouyou-akotet 2005 Microbes Infect | No PM diagnosis. |
| Bouyou-akotet 2009 Exp Parasitol | Unclear if comparison with MIP or with PM. Only two cases of PM detected. |
| Chaisavaneeyakorn 2005 Infect Immun | Expression of biomarker on stained histological sections. |
| Excluded article     | Reason for exclusion                                                                 |
|---------------------|---------------------------------------------------------------------------------------|
| Chaiyaroj 2004 Acta Trop | Unclear how and in which compartment malaria was diagnosed.                         |
| Chandrasiri 2014 J Infect Dis | No PM diagnosis.                                                                      |
| Conroy 2010 Am J Trop Med Hyg | Abstract. Full text Conroy 2013.                                                       |
| Conroy 2013 Reprod Sci | Abstract. Full text Conroy 2013.                                                       |
| Danquah 2008 J Infect Dis | No PM diagnosis.                                                                      |
| Dong 2007 Am J Trop Med Hyg | Abstract. Full study described in Dong 2012?                                           |
| Dong 2013 Am J Trop Med Hyg | Abstract. Full text Engle-Stone 2013 J Nutr – no comparison with PM.                  |
| Engelmann 2005 J Infect Dis | Only comparison with level of cytokine active cells.                                  |
| Engle-Stone 2013 Ann Nutr Metab | Abstract. Full study described in Dong 2012?                                           |
| Fievet 1997 Clin Exp Immunol | No PM diagnosis.                                                                      |
| Fievet 2002 Malaria J | Comparison of biomarkers measured 3 months after delivery with PM.                    |
| Fowkes 2013 Am J Trop Med Hyg | Abstract. Full text has yet been published.                                            |
| Hercberg 1987 Br J Nutr | No PM diagnosis.                                                                      |
| Howard 2007 Am J Trop Med Hyg | No PM diagnosis.                                                                      |
| Jones 2007 J Placenta | Expression of biomarker on stained histological sections. Not quantitative.           |
| Joubert 2010 PLoS One | Unclear if markers were compared with MIP (including only peripheral infections) or PM. |
| Karumanchi 2008 Proc Natl Acad Sci | Commentary.                                                                          |
| Kfutwah 2009 PLoS One | No comparison between PM+ and PM-, only between HIV+ and HIV-.                         |
| Khattab 2013 Placenta | Comparison with MIP, no separate data for PM.                                          |
| Koura 2011 Med Trop (Mars) | No access.                                                                            |
### Table - Excluded records (continued)

| Excluded article | Reason for exclusion |
|------------------|----------------------|
| Lokossou 2010 Am J Trop Med Hyg | Abstract. Full study described in Lokossou 2013. |
| Lokossou 2013 BMC Infect Dis | No comparison of biomarker levels with PM (but with gene polymorphisms). |
| Ludlow 2014 PloS One | No PM diagnosis. |
| Malhotra 2005 Infect Immun | No comparison biomarker levels with PM. |
| Malhotra 2006 J infect Dis | Biomarkers measured in cord blood. |
| Malhotra 2008 J Immunol | Biomarkers measured in cord blood. |
| Matangila 2011 Trop Med Int Health | Abstract. No full text found. |
| Matteelli 1994 Trop Med Int Health | No separate comparison with PM. |
| McDonald 2013 Am J Trop Med Hyg | Abstract. No full text found. |
| McFarlane 1970 Trans R Soc Trop Med Hyg | No PM diagnosis. |
| Mockenhaupt 2000 Trop Med Int Health | No PM diagnosis. |
| Mockenhaupt 2000 Trans R soc Trop Med hyg | No PM diagnosis. |
| Moncunill 2011 Trop Med Int Health | Abstract. No full text found. |
| Moore 2004 Infect Immun | Same cohort as Moore 1999 and Moore 2000, but only PM positive placentas included. |
| Muehlenbachs 2009 (Int J Gyn Obs) | Abstract. Full text article in Muehlenbachs 2006. |
| Muehlenbachs 2009 Am J Trop Med Hyg | Abstract. Full text Muehlenbachs 2010. |
| Muehlenbachs 2010 J Inf Dis | Biomarkers compared to inflammation of all PM+ placentas. |
| Muehlenbachs 2010 (Lab Invest) | Abstract. Full text Muehlenbachs 2010 J Inf Dis. |
| Excluded article | Reason for exclusion |
|------------------|----------------------|
| Nmorsi 2010 Asian Pac J Trop Med | Comparison with MIP, no separate comparison with PM. |
| Obi 1981 Trop Geogr Med | No access. |
| Oesterholt 2010 Am J Trop Med Hyg | Abstract. Full study described in Boström 2012. |
| Othoro 2008 Infect Immun | PM status compared to number of cytokine producing cells. |
| Ouedraogo 2011 Trop Med Int Health | Abstract. Full study described in Ouedraogo 2012/2013. |
| Ouedraogo 2012 Am J Trop Med Hyg | Abstract. Full study described in Ouedraogo 2012/2013. |
| Ouedraogo 2012 Malaria J | Comparison of biomarkers with MIP, no separate comparison with PM. |
| Ouedraogo 2013 Am J Trop Med Hyg | Only influence of PM on hemoglobin. |
| Pearson 2007 PLoS Med | Correspondence, no original study. |
| Reinhardt 1978 Helv Paediat Acta Suppl | No access. |
| Saad 2012 Trans R Soc Trop Med Hyg | No PM diagnosis. |
| Samba 2013 J Health Popul Nutr | No PM diagnosis. |
| Sartetel 2000 Histophatology | Immunostaining. |
| Shulman 1996 Trans R Soc Trop Med Hyg | No PM diagnosis. |
| Silver 2009 Am J Trop Med Hyg | Abstract. Full study described in Silver 2010. |
| Silver 2011 PLoS one | Malawian cohort described in Conroy 2011/2013, Cameroon cohort no comparison with PM. |
| Stuetz 2006 Sci Total Environ | No PM diagnosis. |
| Sugiyama 2001 Placenta | Immunostaining. |
Table - Excluded records (continued)

| Excluded article | Reason for exclusion |
|------------------|----------------------|
| Suguitan 2004    | No comparison biomarkers with PM, this was published in Suguitan 2003 articles. |
| Tagbor 2008      | No PM diagnosis. |
| Thévenon 2009    | Abstract. Full text Thévenon 2010. |
| Tagbor 2008      | No PM diagnosis. |
| Thévenon 2009    | No PM diagnosis. |
| Tagbor 2008      | Abstract. No full text found. |
| Tagbor 2008      | No PM diagnosis. |
| Umbers 2011      | Abstract. Full study described in Umbers 2011. |
| Umbers 2013      | Comparison with peripheral malaria, not with PM. |
| Umeschandra 2012 | No PM diagnosis. |
| Vleugels 1987    | No PM diagnosis. |
| Vleugels 1989    | No PM diagnosis. |
| Wilson 2010      | No PM diagnosis. |
| Wilson 2009      | Abstract. Full study described in Wilson 2010. |

MIP = peripheral and/or placental malaria
Table - Baseline differences in studies

| Article                          | PM diagnosis | Past infection* | Peripheral PM+/ PM-** | HIV infections PM+/PM- *** | Biomarker test method       |
|---------------------------------|--------------|-----------------|-----------------------|-----------------------------|------------------------------|
| Abrams 2004 [26]                | PBS/ HIS     | UK              | Mix/ mix              | 92%, matched                | ELISA                        |
| Abrams 2005 [27]                | PBS/ HIS     | UK              | Mix/ mix              | Neg                         | ELISA                        |
| Avery 2012 [28]                 | PBS / PCR    | UK              | Mix/ mix              | Mix, % UK                   | ELISA                        |
| Bayoumi 2008 [29]               | HIS          | Past            | Mix/ mix              | UK                          | ELISA                        |
| Boeuf 2008 [30]                 | HIS          | Exc             | Mix/ mix              | Neg                         | PCR                          |
| Boeuf 2013 [31]                 | HIS          | UK              | Mix/ neg              | UK                          | ELISA, PCR, RP-UPLC          |
| Bouyou-Akotet 2004 [32]         | PBS/ HIS     | UK              | Mix /mix              | UK                          | ELISA                        |
| Chaisavaneeakorn 2002 [33]      | PBS          | UK              | Mix/ mix              | 38% / 34%                   | ELISA                        |
| Chaisavaneeakorn 2002 [34]      | PBS          | UK              | Mix/ mix              | 35% / 50%                   | ELISA                        |
| Chaisavaneeakorn 2003 [35]      | PBS          | UK              | Mix/ mix              | 35% / 50%                   | ELISA                        |
| Chua 2013 [36]                  | HIS          | UK              | Mix/ Neg              | UK                          | ELISA                        |
| Conroy 2009 [37]                | PBS          | UK              | Mix/ mix              | Neg                         | ELISA                        |
| Conroy 2011 [38]                | PBS          | Neg             | Neg/ neg              | UK                          | ELISA                        |
| Conroy 2013 [39]                | PBS          | Neg             | Mix/ neg              | UK                          | ELISA                        |
| Diallo 2008 [40]                | HRPII/ PBS   | UK              | Mix/ mix              | Neg                         | Flow-cytometry               |
| Diouf 2007 [41]                 | PBS          | UK              | Pos/ neg              | UK                          | Flow-cytometry               |
| Dong 2012 [42]                  | PBS          | UK              | Mix/ mix              | Neg                         | Bead-based assay             |
| Fievet 2001 [43]                | PBS          | Pos             | Mix/ mix              | UK                          | ELISA                        |
| Flanagan 2010 [44]              | HIS          | UK              | Mix / mix             | UK                          | Bead-based assay             |
| Fried 1998 [45]                 | PBS          | Neg             | Mix/ mix              | UK                          | ELISA                        |
| Jakobsen 1998 [46]              | PBS/ HIS     | Past            | Mix/ mix              | UK                          | ELISA                        |
| Kabyemela 2008 [47]             | PBS          | UK              | Mix/ mix              | Neg                         | Bead-based assay             |
| Kabyemela [48]                  | PBS          | UK              | Mix/mix               | Neg                         | Bead-based assay             |
| Kumar 2012 [49]                 | HIS          | Neg             | Mix/ mix              | Pos                         | Bead-based assay             |
| Moore 1999 [50]                 | PBS          | UK              | Mix/ mix              | Mix, % UK                   | ELISA                        |
| Moore 2000 [51]                 | PBS          | UK              | Mix/ mix              | 40% / 30%                   | ELISA                        |
| Moormann 1999 [52]              | CBS          | Exc             | Mix/ neg              | Neg                         | RPA                          |
### Table - Baseline differences in studies (continued)

| Article               | PM diagnosis | Past infection* | Peripheral PM+/ PM-** | HIV infections PM+/PM- *** | Biomarker test method |
|-----------------------|--------------|-----------------|------------------------|---------------------------|-----------------------|
| Muehlenbachs 2006 [53]| PBS          | Neg             | Mix/ mix               | Neg                       | ELISA/ PCR            |
| Muehlenbachs 2007 [54]| PBS          | UK              | Mix/ mix               | Neg                       | PCR                   |
| Muehlenbachs 2008 [55]| PBS          | UK              | Mix/ mix               | Neg                       | ELISA/ PCR            |
| Ostrowski 2007 [56]   | HIS          | Past            | Mix/ mix               | 32% / 25%                 | ELISA                 |
| Perkins 2003 [57]     | PBS          | UK              | Mix/ mix               | Neg                       | ELISA                 |
| Rogerson 2003 [58]    | HIS          | UK              | Mix/ neg               | Mix, % UK                 | ELISA                 |
| Santen 2011 [59]      | PIS          | UK              | Neg/ neg               | UK                        | Variety****            |
| Sarr 2010 [60]        | HRPII/ HIS   | Past            | Mix/ neg               | Mix, % UK                 | PCR                   |
| Senga 2011 [61]       | HIS          | Past            | Mix/ mix               | UK                        | ELISA                 |
| Senga 2012 [62]       | PBS          | UK              | Mix/ mix               | UK                        | Hematofluorometer     |
| Silver 2010 [63]      | PBS/ PIS     | UK              | Mix/ mix               | UK                        | ELISA                 |
| Simpson 2010 [64]     | PBS/ HIS     | Past/neg        | Mix/ mix               | UK                        | ELISA                 |
| Singh 2012 [65]       | PBS/ PIS     | UK              | Pos/ neg               | UK                        | ELISA                 |
| Suguitan 2003 [66]    | PBS/PIS/HIS  | UK              | Mix/ mix               | UK                        | ELISA                 |
| Suguitan 2003 [67]    | PBS/PIS/HIS  | UK              | Mix/ mix               | UK                        | ELISA                 |
| Thévenon 2009 [68]    | PIS/ HIS     | UK              | Mix/ mix               | UK                        | ELISA                 |
| Thévenon 2010 [69]    | PIS/ HIS     | UK              | Mix/ mix               | ELISA                     |
| Tkachuk 2001 [70]     | CBS          | Exc             | Mix / neg              | UK                        | Riboprobe assay       |
| Umbers 2011 [71]      | HIS          | Exc             | Mix/ mix               | UK                        | PCR                   |
| Watkinson 1985 [72]   | HIS          | UK              | Mix/ mix               | UK                        | Radio-immunoassay     |

PBS = placental blood smear, PIS = placental impression smear, HIS = histology, CBS = cord blood smear, RP-UPLC = reversed phase ultra-performance liquid chromatography, HRPII = rapid diagnostic test detecting histidine-rich protein II, RPA = ribonuclease protection assay

*Classification of past infections. UK = unknown (no diagnosis or unclear), neg = included as negative for PM, pos = included as positive for PM, past = analysed as separate ‘past infected’ group, exc = excluded from analysis.

**Concurrent peripheral infection. Mix = patients with both positive and negative peripheral infection included (or unclear), pos = only patients with peripheral infection included, neg = only patients without peripheral infection included.

***Co-infection with HIV. Mix = both patients with and without HIV infection included (% presented if known), UK = unknown, pos = only patients with HIV infection included, neg = only patients without HIV infection included.

****cation-exchange chromatography, time-of-flight mass spectrometry, colorimetric method, immunologic agglutination detection, immunometric assay, immunonephelometrics.
Placental IL-4 levels (original means, all gravidities, no culture, HIV+/-mix populations)

| Study | Mean (95% CI) | Weight |
|-------|---------------|--------|
| 1     | 0.09 (0.0, 0.19) | 41.96  |
| 17    | 0.15 (0.07, 0.38) | 27.71  |
| 78    | 0.04 (0.01, 0.07) | 36.50  |

Overall: p (Hetero) = 0.10, p = 0.57

NOTE: weights are non-central t-effect analysis
### Table - Summary of studied biomarkers

**Studied biomarkers**

**Proteins and cytokines**

| Alanine [31]          | hepcidin [59] |
|-----------------------|---------------|
| APO-A1 [64]           | histidin [31] |
| APO-B [64]            | HPL [72]      |
| Ang-1 [38,39,63]      | sICAM-1 [28,46] |
| Ang-2 [38,39,63]      | IFN-α [44]    |
| arginine [31]         | IFN-γ [29,40,41,43–45,47,48,50,51,58,66,67] |
| asparagine [31]       |                |
| aspartic acid [31]    | IGF1 [71]     |
| C3a [38,39]           | IGF2 [71]     |
| C5a [37–39]           | IL-1 [43,47,48] |
| CCL2 [42]             | IL-1β [31,43] |
| CCL18 [42]            | IL-2 [40,43–45,67] |
| CCL20 [42]            | sIL-2R [46]   |
| sCD163 [28,36]        | IL-4 [29,40,43–45,47,48,50,51,66,67] |
| cortisol [27]         | sIL-4R [46]   |
| CRH [27]              | IL-5 [40,47,48] |
| CRP [38,59]           | IL-6 [26,28,43–45,47,48] |
| CXCL1 [42]            | IL-8 [26,44,67] |
| CXCL9 [42]            | IL-10 [28,29,40,41,43–48,50,51,57,66,67] |
| CXCL13 [42]           | IL-12 [33,44,67] |
| cysteine [31]         | IL-13 [44]    |
| D dimer [28]          | IL-17 [44]    |
| sEng [38,39]          | IL-18 [33]    |
| sE selectin [46]      | IP-10 [33,49,67] |
| estradiol [72]        | iron [59]     |
| ferritin [47,48,59,61] | isoleucine [31] |
| sFlt-1 [38,39,53,55]  | leptin [38,47,48] |
| glutamic acid [31]    | leucine [31]  |
| glutamine [31]        | lysine [31]   |
| glycine [31]          | MBL [68]      |
| GM-CSF [43,67]        | MCP-1 [26,32,42,67] |
| heparin [31]          | methionine [31] |
| histidin [31]         | MIF [34,65]   |
| IFN-γ [29,40,41,43–45,47,48,50,51,58,66,67] | MIP-1α [26,35,67] |
| prolyl hydroxylase domain (PHD) [35] | MIP-1β [35,67] |
| arginine [31]         | PAI-1 [28]    |
| asparagine [31]       | PGE2 [57]     |
| aspartic acid [31]    | phenylalanine [31] |
| C3a [38,39]           | progesterone [72] |
| C5a [37–39]           | proline [31]  |
| CCL2 [42]             | RANTES [67]   |
| CCL18 [42]            | serine [31]   |
| CCL20 [42]            | SuPAR [56]    |
| sCD163 [28,36]        | TAT complex [28] |
| cortisol [27]         | sTfR [58,61]  |
| CRH [27]              | threonine [31] |
| CRP [38,59]           | TGF-β [26,44,67] |
| CXCL1 [42]            | TGF-β1 [45]   |
| CXCL9 [42]            | TGF-β2 [44]   |
| CXCL13 [42]           | stTie-2 [38,39] |
| cysteine [31]         | TIBC [59]     |
| D dimer [28]          | tissue factor [38] |
| sEng [38,39]          | TNF-α [26,28,40,41,43–45,47,48,50,51,57,66,67] |
| sE selectin [46]      | sTNF-R1 [46–48,69] |
| estradiol [72]        | sTNF-R2 [47,48,69] |
| ferritin [47,48,59,61] | tryptophan [31] |
| sFlt-1 [38,39,53,55]  | ts [59]       |
| glutamic acid [31]    | tyrosine [31] |
| glutamine [31]        | VEGF [38,39]  |
| glycine [31]          | sVCAM [46]    |
| GM-CSF [43,67]        | ZPP [62]      |
Table - Summary of studied biomarkers (continued)

| Studied biomarkers | RNA expression |
|--------------------|----------------|
| BAFF [54]          | HEPC [54]      |
| CCL4 [54]          | HIF-1α [30]    |
| CCL5 [54]          | IFN-γ [52,54,55]|
| CCL18 [54]         | IGF1 [71]      |
| CCR3 [70]          | IGF2 [71]      |
| CCR5 [70]          | IGF1R [71]     |
| COX-1 [60]         | IGF2R [71]     |
| COX-2 [60]         | IGGH [54]      |
| CXCL9 [54]         | IGMH [54,55]   |
| CXCL10 [54]        | IL-1α [52]     |
| CXCL13 [54]        | IL-1β [52,54]  |
| CXCL16 [54]        | IL-2 [52]      |
| CXCR4 [70]         | IL-4 [52]      |
| (s)Flt-1 [30,53]   | IL-5 [52]      |
| GM-CSF [52]        | IL-6 [52]      |
|                    | IL-8 [52]      |
|                    | IL-10 [52,60]  |
|                    | IL-12 [52]     |
|                    | IL-18 [54]     |
|                    | 15-LOX [60]    |
|                    | PIGF [30]      |
|                    | SNAT-1 [31]    |
|                    | SNAT-2 [31]    |
|                    | TNF-α [52,54]  |
|                    | TNF-β [52]     |
|                    | TGF-β1 [52]    |
|                    | VEGF [30,53]   |
|                    | + many more in Muehlenbachs et al. (supplemental data) [54] |
### Table – Summary of all studied biomarkers

| Article   | Biomarker | Peripheral levels in PM+ primigravid | Peripheral levels in PM+ secundigravid | Peripheral levels in PM+ multigravid | Peripheral levels in PM+ all gravidities | Placental levels in PM+ primigravid | Placental levels in PM+ secundigravid | Placental levels in PM+ multigravid | Placental levels in PM+ all gravidities | Correction for multiple comparisons | Notes                                                                 |
|-----------|-----------|--------------------------------------|----------------------------------------|--------------------------------------|-----------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|---------------------------------------|--------------------------------------|--------------------------------------|
| Boeuf 2013 | Alanine   | ±                                    |                                        |                                      |                                         |                                      |                                      |                                      |                                       |                                      | Only significantly increased for PM with intervillositis compared with uninfected placentas ($p \leq 0.01$). |
| Conroy 2011 | Ang-1     | NS                                   |                                        |                                      |                                         |                                      |                                      |                                      |                                       |                                      | Decreased, $p$ value 0.037. NS after Holm’s correction. |
| Conroy 2013 | Ang-1     | ↓                                    | ↓                                      |                                      |                                         |                                      |                                      |                                      |                                       |                                      | $p$ values 0.006 and 0.024 respectively |
| Silver 2010 | Ang-1     | ±                                    |                                        |                                      |                                         |                                      |                                      |                                      |                                       |                                      | Data stratified for LBW, ANG-1 levels were significantly decreased in PM+ with LBW compared to PM- with LBW ($p$ value 0.002). |
| Conroy 2011 | Ang-2     | NS                                   |                                        |                                      |                                         |                                      |                                      |                                      |                                       |                                      | ($p$ value 0.555) $p$ values 0.039 and 0.011 respectively |
| Conroy 2013 | Ang-2     | ↑                                    | ↑                                      |                                      |                                         |                                      |                                      |                                      |                                       |                                      | Data stratified for LBW, ANG-2 levels were significantly increased for PM+ regardless of birth weight ($p$ values 0.0171, <0.0001) compared to PM- with NBW. NS for PM+ regardless of birth-weight compared with PM- with LBW. |
| Silver 2010 | Ang-2     | ±                                    |                                        |                                      |                                         |                                      |                                      |                                      |                                       |                                      | $p$ values 0.006 and 0.024 respectively |
| Article       | Biomarker | Peripheral levels in PM+ primigravid | Peripheral levels in PM+ secundigravid | Peripheral levels in PM+ all gravidities | Placental levels in PM+ primigravid | Placental levels in PM+ secundigravid | Placental levels in PM+ all gravidities | Correction for multiple comparisons | Notes |
|--------------|-----------|--------------------------------------|----------------------------------------|-----------------------------------------|--------------------------------------|----------------------------------------|----------------------------------------|-----------------------------------------|--------|
| Simpson 2010 | APO-A1    | ↓                                     |                                        |                                         |                                     |                                        |                                        |                                         | significantly decreased for active (p value 0.05) or past placental infection (p value 0.0008) versus no placental infection (but influenced by severe anaemia). |
| Simpson 2010 | APO-B     | ↓                                     | ↓                                      |                                        |                                     |                                        |                                        |                                         | p value 0.0008 primigravidae, <0.0001 multigravidae (pooled for severe anaemia versus no anaemia because no difference in APO-B between these groups) |
| Boeuf 2013   | Arginine  | NS                                   |                                        |                                         |                                     |                                        |                                        |                                         | not significant for either PM with or without intervillositis compared with uninfected placentas. |
| Boeuf 2013   | Asparagine| ±                                     |                                        |                                         |                                     |                                        |                                        |                                         | only significantly increased for PM without intervillositis compared with uninfected placentas (0.01 < p ≤ 0.05). |
| Boeuf 2013   | Aspartic acid | ↑                                 |                                        |                                         |                                     |                                        |                                        |                                         | significant for either PM with or without intervillositis compared with uninfected placentas (0.01 < p ≤ 0.05). |
| Conroy 2011  | C3a       | NS                                   |                                        |                                         |                                     |                                        |                                        |                                         | p value 0.704 |
| Conroy 2013  | C3a       | NS                                   | NS                                     |                                         |                                     |                                        |                                        |                                         | p values 0.076 and 0.746 respectively |
Table – Summary of all studied biomarkers (continued)

| Article       | Biomarker | Peripheral levels in PM+ primigravid | Peripheral levels in PM+ secundigravid | Peripheral levels in PM+ multigravid | Placental levels in PM+ primigravid | Placental levels in PM+ secundigravid | Placental levels in PM+ multigravid | Correction for multiple comparisons | Notes |
|---------------|-----------|--------------------------------------|----------------------------------------|--------------------------------------|--------------------------------------|----------------------------------------|--------------------------------------|--------------------------------------|-------|
| Conroy 2011   | C5a       | NS                                   |                                        |                                      |                                      |                                        |                                      | Holm                                 | Decreased, p value 0.307             |
| Conroy 2013   | C5a       | ±                                     | ↑                                      | ↑                                    | UK                                   |                                        |                                      | p values 0.01 and 0.0264 respectively |
| Conroy 2009   | C5a       | ↑                                     | ↑                                      |                                      | Bonferroni                           |                                        |                                      |                                      |
| Dong 2012     | CCL18     | ↑                                     | NS                                     | NS                                   | NS                                   | NS                                     | NS                                   | UK                                   |
| Dong 2012     | CCL20     | NS                                   | ↓                                      | NS                                   | NS                                   | NS                                     | NS                                   | UK                                   |
| Avery 2012    | sCD163    | ↑                                     |                                        |                                      | Tukey/Dunn                           |                                        |                                      |                                      |
| Chua 2013     | sCD163    | NS                                   | NS                                     | ±                                    | ±                                    | Tukey/Dunn                            |                                      |                                      |
| Abrams 2005   | Cortisol  |                                        |                                        |                                      | Bonferroni                           |                                        | slight increase (p value 0.357)      |                                      |
| Abrams 2005   | CRH       |                                        |                                        |                                      | Bonferroni                           |                                        | slight increase (p value 0.421)      |                                      |
| Conroy 2011   | CRP       | ↑                                     |                                        |                                      | Holm                                 |                                        |                                      | p value 0.002, still significant after Holm's correction |

Significant higher percentage of PM+ if peripheral C5a levels >100 ng/mL. Placental p values <0.001 and 0.002 respectively.

For PM diagnosed by PBS (p value 0.0002).

Significantly increased in placental blood of PM+ with intervillitis compared to PM-, (p <0.01) not significant for PM+ without intervillitis compared to PM-.
| Article          | Biomarker | Peripheral levels in PM+ primigravid | Peripheral levels in PM+ secundigravid | Peripheral levels in PM+ multigravid | Correction for multiple comparisons | Notes                                                                 |
|-----------------|-----------|--------------------------------------|----------------------------------------|------------------------------------|-----------------------------------|-----------------------------------------------------------------------|
| Santen 2011     | CRP       | ±                                    |                                        |                                    |                                   | Stratified for anemia. CRP increased in PM+ with (p < 0.05) or without (p < 0.01) anemia compared with PM- without anemia. |
| Dong 2012       | CXCL1     | ↑ NS NS NS                           |                                        |                                    |                                   | p value 0.03.                                                        |
| Dong 2012       | CXCL9     | NS NS NS                             |                                        |                                    |                                   | placental p values <0.0001, 0.002, and <0.0001 respectively.         |
| Dong 2012       | CXCL13    | ↑ NS NS                             |                                        |                                    |                                   | significant increased values (p values 0.003, <0.0001, and 0.009), all other p values >0.05. |
| Boeuf 2013      | Cystine   | ±                                    |                                        |                                    |                                   | Only significantly increased for PM without intervillitis compared with uninfected placentas (0.01 < p ≤ 0.05). |
| Avery 2012      | D dimer   | ↑                                    |                                        |                                    | Tukey / Dunn                     | Significant increase for PM diagnosed by PBS (p value 0.025) or PBS and PCR combined (p value 0.0008). |
| Conroy 2011     | sEng      | NS                                  |                                        |                                    | Holm                              | p value 0.819                                                          |
| Conroy 2013     | sEng      | NS                                  |                                        |                                    |                                   | Increased levels, p values 0.057 and 0.001 respectively               |
| Jakobsen 1998   | sE selectin | NS                             |                                        |                                    | Bonferroni                        | No association.                                                       |
**Table – Summary of all studied biomarkers (continued)**

| Article            | Biomarker | Peripheral levels in PM+ primigravid | Peripheral levels in PM+ secundigravid | Peripheral levels in PM+ multigravid | Placental levels in PM+ primigravid | Placental levels in PM+ secundigravid | Placental levels in PM+ multigravid | Correction for multiple comparisons | Notes                                                                 |
|--------------------|-----------|--------------------------------------|----------------------------------------|--------------------------------------|--------------------------------------|----------------------------------------|--------------------------------------|-------------------------------------|--------------------------------------------------------------------------------|
| Watkinson 1985     | Estradiol | ↑                                    |                                        |                                      |                                      |                                        |                                      |                                     | Significantly increased at 32 - 35 weeks \((p <0.025)\) and 36+ weeks \((p <0.005)\) in women who had pigmented placentas at delivery compared to women with non-pigmented placentas. NS earlier in gestation. |
| Kabyemela 2008 (1) | Ferritin  | ↑                                    | ↑                                      | ↑                                    | ↑                                    | ↑                                      | NS                                   |                                     | Peripheral levels \(p\) values \(<0.0001\), \(<0.0001\) and 0.004 respectively. Placental levels \(p\) values 0.0002, NS, 0.0007 respectively. |
| Kabyemela (2)      | Ferritin  | ↑                                    | ↑                                      |                                      |                                      |                                        |                                      |                                     | \(p\) values \(<0.0001\) and \(<0.0001\) respectively. |
| Santen 2011        | Ferritin  | NS                                   |                                        |                                      |                                      |                                        |                                      |                                     | \(p\) value 0.003 (after Holm’s correction still significant \(p\) value \(<0.05\)) |
| Conroy 2011        | sFlt-1    | ↓                                    |                                        |                                      |                                      |                                        |                                      |                                     | \(p\) values 0.045 and                                      |
| Conroy 2013        | sFlt-1    | ↑                                    | ↑                                      |                                      |                                      |                                        |                                      |                                     | Only significantly elevated for primigravids with PM but without hypertension compared to uninfected primigravids \((p\) value 0.005\). |
| Muehlenbachs 2006  | sFlt-1    | ±                                     | NS                                     |                                      |                                      |                                        |                                      |                                     |                                                |
| Article                  | Biomarker | Notes                                                                                                                                 |
|-------------------------|-----------|---------------------------------------------------------------------------------------------------------------------------------------|
| Muehlenbachs 2008       | sFlt-1    | Stratified for newborn genotype and only significantly increased for PM+ versus PM- in two out of three genotypes (p < 0.05).       |
| Boeuf 2013              | Glutamic acid | Not significant for either PM with or without intervillositis compared with uninfected placentas.                                  |
| Boeuf 2013              | Glutamine  | Not significant for either PM with or without intervillositis compared with uninfected placentas.                                  |
| Boeuf 2013              | Glycine    | Not significant for either PM with or without intervillositis compared with uninfected placentas.                                  |
| Fievet 2001             | GM-CSF     | Cytokine production in cultures of villi, villi + blood, blood or serum.                                                             |
| Suguitan 2003 (2)       | GM-CSF     | NS in placental plasma direct measurement, WBC culture and villous tissue culture.                                                     |
| Santen 2011             | Hepcidin   | Unknown p value                                                                                                                      |
| Boeuf 2013              | Histidin   | Not significant for either PM with or without intervillositis compared with uninfected placentas.                                  |
| Article       | Biomarker | Peripheral levels in PM+ primigravid | Peripheral levels in PM+ secundigravid | Peripheral levels in PM+ multigravid | Placental levels in PM+ primigravid | Placental levels in PM+ secundigravid | Placental levels in PM+ multigravid | Correction for multiple comparisons | Notes                                                                 |
|--------------|-----------|--------------------------------------|---------------------------------------|-------------------------------------|-------------------------------------|---------------------------------------|-------------------------------------|----------------------------------|------------------------------------------------------------------------------------------------|
| Watkinson 1985 | HPL       | NS                                   | UK                                   |                                     |                                     |                                       |                                     |                                  | NS during gestation between women with pigmented placentas at delivery compared with women with non-pigmented placentas at delivery. |
| Avery 2012   | sICAM-1   | ↑                                    |                                       |                                     |                                     |                                       |                                     | Tukey/Dunn                      | For PM diagnosed by PBS ($p <0.0001$)                                              |
| Jakobsen 1998 | sICAM-1   |                                       |                                       |                                     |                                     |                                       | Bonferroni                        | Increase in active infections versus no infections but NS ($p$ value 0.06)            |
| Flanagan 2010 | IFN-α     | NS                                   | Bonferroni                           |                                     |                                     |                                       |                                     | Levels low or absent.             |                                                                                      |
| Bayoumi 2008 | IFN-γ     | ↓                                    | ↓                                    | UK                                 |                                     |                                       |                                     | $p$ values 0.01 and 0.04 respectively. | Past versus uninfected placentas, $p$ values 0.01 and 0.04 respectively.                  |
| Diallo 2008  | IFN-γ     | ↑                                    | ↓                                    | UK                                 |                                     |                                       |                                     | $p$ values <0.01 and <0.05 respectively. | $p$ values <0.01 and <0.05 respectively.                                           |
| Diouf 2007   | IFN-γ     | NS                                   | Bonferroni                           |                                     |                                     |                                       |                                     | (p value 0.34)                   | Significantly increased in PM+ versus PM- in cultures of blood ($p = 0.002$). NS in serum or cultures of villi or villi + blood. |
| Fievet 2001  | IFN-γ     | ±                                    | UK                                   |                                     |                                     |                                       |                                     |                                  |                                                                                      |
| Flanagan 2010 | IFN-γ   |                                        |                                        | Bonferroni                         |                                     |                                       |                                     | Measured in culture supernatants.       |                                                                                      |
| Fried 1998   | IFN-γ     | NS                                   | NS                                   | ↑                                   | UK                                 |                                       |                                     | $p$ values 0.4, 0.9 and 0.001 respectively. |                                                                                      |
| Article       | Biomarker | Peripheral levels in PM+ primigravid | Peripheral levels in PM+ secundigravid | Peripheral levels in PM+ multigravid | Placental levels in PM+ primigravid | Placental levels in PM+ secundigravid | Placental levels in PM+ multigravid | Notes                                                                 |
|--------------|-----------|--------------------------------------|----------------------------------------|--------------------------------------|--------------------------------------|----------------------------------------|--------------------------------------|-----------------------------------------------------------------------|
| Kabyemela 2008 (1) | IFN-γ     | NS NS NS                             |                                        |                                       |                                       |                                        |                                       | Placental levels p values 0.004, NS, and 0.001 respectively.          |
| Kabyemela 2008 (2) | IFN-γ     | NS NS                               |                                        |                                       |                                       |                                        |                                       | Primiparous-secundigravid combined in one group. Result for all gravidities stratified for HIV status (NS). |
| Moore 1999    | IFN-γ     | NS NS NS                             |                                        |                                       |                                       |                                        |                                       | No significant differences between PM+ and PM− in cultures of medium, PHA, CD3, PPD, malarial antigen. Includes the subgroup described in Moore 1999. |
| Moore 2000    | IFN-γ     | NS                                  |                                        |                                       |                                       |                                        |                                       | Significantly increased in placental plasma (p value 0.0001), however only detectable in 4% of total samples (5 PM+ women, 3 PM− women) |
| Rogerson 2003 | IFN-γ     | NS                                  |                                        |                                       |                                       |                                        |                                       | Stratified for preterm and fullterm deliveries. Significantly increased in PM+ versus PM− in full term deliveries (p value 0.0001), NS for preterm deliveries. |
| Suguitan 2003 (1) | IFN-γ    | ±                                    |                                        |                                       |                                       |                                        |                                       |                                                                       |
| Article          | Biomarker | Notes                                                                 |
|------------------|-----------|----------------------------------------------------------------------|
| Suguitan 2003 (2) | IFN-γ     | Borderline significantly increased in placental plasma direct measurement (0.05 ≤ p < 0.07), NS in WBC culture, significantly increased for PM+ versus PM- after 24 hour villous tissue culture (0.001 ≤ p < 0.01). |
| Umbers 2011      | IGF1      | NS for PM+ without monocyte infiltration versus PM-, significantly decreased for PM+ with monocyte infiltration versus PM- (p value 0.007). |
| Umbers 2011      | IGF2      | NS UK                                                                |
| Fievet 2001      | IL-1      | Cytokine production in cultures of villi, villi + blood, blood or serum |
| Kabyemela 2008 (2) | IL-1      | p value 0.0018. Multigravid women are women with second and later pregnancies. |
| Boeuf 2013       | IL-1β     | Only significantly increased for PM with intervillitis compared with uninfected placentas. |
| Fievet 2001      | IL-1β     | NS UK                                                                |
| Diallo 2008      | IL-2      | NS UK                                                                |
**Table 2:** Biomarker levels in PM+ pregnancy (PM = placental malaria).

| Article        | Biomarker | Notes                                                                 |
|----------------|-----------|----------------------------------------------------------------------|
| Fievet 2001    | IL-2      | NS UK Cytokine production in cultures of villi or serum, levels always very low |
| Flanagan 2010  | IL-2      | NS Bonferroni Measured in culture (SEB) supernatants.                 |
| Fried 1998     | IL-2      | NS NS NS UK NS in placental plasma direct measurement, WBC culture and villous tissue culture. |
| Suguitan 2003  | IL-2      | NS UK                                                                |
| Jakobsen 1998  | sIL-2R    | NS Bonferroni                                                        |
| Bayoumi 2008   | IL-4      | ↓ ↓ UK Past versus uninfected placentas                               |
| Diallo 2008    | IL-4      | NS ↓ UK p <0.01 (placental)                                         |
| Fievet 2001    | IL-4      | NS UK Cytokine production in cultures of villi, villi + blood, blood or serum |
| Flanagan 2010  | IL-4      | NS Bonferroni Measured in culture (SEB) supernatants.                 |
| Fried 1998     | IL-4      | NS NS NS UK                                                         |
| Kabyemela 2008 | IL-4      | NS NS UK Multigravid women are women with second and later pregnancies. |

*Multigravid women are women with second and later pregnancies.*
| Article            | Biomarker | Notes                                                                 |
|-------------------|-----------|-----------------------------------------------------------------------|
| Moore 1999        | IL-4      | Significantly decreased in PM+ primi-/secundigravid in soluble malarial antigen cultures (p value 0.041). NS for multi-gravid and medium, PHA, CD3 and PPD cultures. Primi-secundigravid combined in one group. |
| Moore 2000        | IL-4      | No significant differences between PM+ and PM- in cultures of medium, PHA, CD3, PPD, malarial antigen. Includes the subgroup described in Moore 1999. |
| Suguitan 2003 (1) | IL-4      | Stratified for preterm deliveries and fullterm deliveries. No significant differences between PM+ and PM- in these groups. |
| Suguitan 2003 (2) | IL-4      | NS in placental plasma direct measurement, WBC culture and villous tissue culture. |
| Jakobsen 1998     | sIL-4R    | Bonferroni                                                             |
| Diallo 2008       | IL-5      | Data presented in graphs, no exact data provided after request.        |
| Article           | Biomarker | Peripheral levels in PM+ primigravid | Peripheral levels in PM+ multigravid | Placental levels in PM+ primigravid | Placental levels in PM+ multigravid | Notes                                      |
|-------------------|-----------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------------|
| Kabyemela 2008 (2) | IL-6      | NS                                   | NS                                   | Bonferroni                           |                                      | UK                                         |
| Abrams 2004       | IL-6      | NS                                   | NS                                   | Bonferroni                           | Tukey / Dunn                        | For PM diagnosed by PBS.                   |
| Avery 2012        | IL-6      | ↑                                    |                                      |                                      |                                      | Cytokine production in cultures of villi, villi + blood, blood or serum |
| Fievet 2001       | IL-6      | NS                                   |                                      |                                      | UK                                   | Measured in culture (SEB) supernatants.    |
| Flanagan 2010     | IL-6      | NS                                   |                                      | Bonferroni                           |                                      |                                            |
| Fried 1998        | IL-6      | NS                                   |                                       |                                      |                                      |                                            |
| Kabyemela 2008 (2) | IL-6      | NS                                   | NS                                   | Bonferroni                           | Increased peripheral levels with p value 0.05, but NS after Bonferroni adjustment |
| Abrams 2004       | IL-8      | NS                                   | NS                                   | Bonferroni                           |                                      | UK                                         |
| Flanagan 2010     | IL-8      | NS                                   |                                      | Bonferroni                           | Measured in culture (SEB) supernatants.|
| Suguitan 2003 (2)  | IL-8      | NS                                   |                                       |                                      |                                      | NS in placental plasma direct measurement, WBC culture and villous tissue culture. |
| Avery 2012        | IL-10     | ↑                                    |                                      |                                      | Tukey / Dunn                        | For PM diagnosed by PBS.                   |
| Bayoumi 2008      | IL-10     | ↓                                    | ↓                                    | UK                                   | Past versus uninfected placentas     |
| Diallo 2008       | IL-10     | ↑                                    | ↑                                    | UK                                   | p values <0.001, <0.05               |
| Diouf 2007        | IL-10     | ↑                                    |                                      | UK                                   | p value 0.0001                       |
### Table – Summary of all studied biomarkers (continued)

| Article       | Biomarker | Peripheral levels in PM+ primigravid | Peripheral levels in PM+ secundigravid | Peripheral levels in PM+ multigravid | Placental levels in PM+ primigravid | Placental levels in PM+ secundigravid | Placental levels in PM+ multigravid | Correction for multiple comparisons | Notes |
|---------------|-----------|--------------------------------------|----------------------------------------|--------------------------------------|--------------------------------------|----------------------------------------|--------------------------------------|--------------------------------------|-------|
| Fievet 2001   | IL-10     | NS                                   |                                        |                                      |                                      |                                        |                                      |                                      |       |
| Flanagan 2010 | IL-10     | NS                                   |                                        |                                      |                                      |                                        | Bonferroni                           | Measured in culture (SEB) supernatants. |       |
| Fried 1998    | IL-10     | NS                                   |                                        |                                      |                                      |                                        | Bonferroni                           |                                      |       |
| Jakobsen 1998 | IL-10     |                                       |                                        |                                      |                                      |                                        |                                      | Peripheral p values <0.0001, <0.0001, <0.0001. Placental p values <0.0001, <0.0001. |       |
| Kabyemela 2008 (1) | IL-10 | ↑ ↓ ↑ ↑ ↑ ↑ ↑ |                                        |                                      |                                      |                                        |                                      |                                      |       |
| Kabyemela 2008 (2) | IL-10 | ↑ ↓ ↑ |                                        |                                      |                                      |                                        |                                      |                                      | p values <0.0001, <0.0001. |
| Moore 1999    | IL-10     | NS                                   |                                        |                                      |                                      |                                        |                                      | No significant differences between PM+ and PM- in cultures of medium, PHA, CD3, PPD, malarial antigen. Primi-/secundigravid combined in one group. |       |
| Moore 2000    | IL-10     | NS                                   |                                        |                                      |                                      |                                        |                                      | No significant differences between PM+ and PM- in cultures of medium, PHA, CD3, PPD, malarial antigen. Includes the subgroup described in Moore 1999. |       |
| Article                  | Biomarker | Notes                                                                                                                                 |
|-------------------------|-----------|----------------------------------------------------------------------------------------------------------------------------------------|
| Perkins 2003            | IL-10     | Significantly decreased in placentas pigmented 10 - 25% versus placentas pigmented 1 - 10% (p value 0.01) and versus non pigmented placentas (p value 0.03) after 48 hours of medium culture. |
| Suguitan 2003 (1)       | IL-10     | Stratified for preterm deliveries and fullterm deliveries. Significantly increased for PM+ versus PM- in both preterm (p value < 0.0001) and full term deliveries (p value 0.003) |
| Suguitan 2003 (2)       | IL-10     | Significantly increased in PM+ versus PM- in placental plasma direct measurement (p <0.001) and WBC culture (0.01 ≤ p < 0.05), NS after 24 hour villous tissue culture. |
| Chaisavanneekorn 2002 (iii) | IL-12    | Significantly increased for HIV negative PM+ versus PM- for medium, PPD and PHA cultures. Marginally significantly increased for PM+ versus PM- women in HIV positive group in PPD culture, NS for PHA and medium culture. |
### Table – Summary of all studied biomarkers (continued)

| Article                          | Biomarker | Notes                                      |
|----------------------------------|-----------|--------------------------------------------|
| Flanagan 2010                    | IL-12     | NS Bonferroni                              |
|                                  |           | Measured in culture (SEB) supernatants.    |
| Suguitan 2003 (2)                 | IL-12     | NS UK                                      |
|                                  |           | NS in placental plasma direct measurement, WBC culture and villous tissue culture. |
| Flanagan 2010                    | IL-13     | NS Bonferroni                              |
|                                  |           | Measured in culture (SEB) supernatants.    |
| Flanagan 2010                    | IL-17     | NS Bonferroni                              |
|                                  |           | Measured in culture (SEB) supernatants.    |
| Chaisavaneeeyakorn 2002 (III)    | IL-18     | NS UK                                      |
|                                  |           | Stratified for HIV status, NS in medium, PPD and PHA cultures. |
| Chaisavaneeeyakorn 2002 (III)    | IP-10     | ± UK                                       |
|                                  |           | In medium cultures significantly increased in HIV positive PM+ women compared to PM- women and HIV negative PM+ women. In PHA cultures only significantly increased in PM+ versus PM- women in the HIV positive group. |
| Kumar 2012                       | IP-10     | ↑ No                                       |
|                                  |           | NS in placental plasma direct measurement and villous tissue culture, significantly increased in WBC culture (0.01 ≤ p < 0.05). |
| Suguitan 2003 (2)                | IP-10     | ± UK                                       |
|                                  |           | NS in placental plasma direct measurement and villous tissue culture, significantly increased in WBC culture (0.01 ≤ p < 0.05). |
### Table 1: Biomarker Levels in PM+ Gravidities

| Article                | Biomarker | Peripheral levels in PM+ primigravid | Peripheral levels in PM+ secundigravid | Peripheral levels in PM+ multigravid | Placental levels in PM+ primigravid | Placental levels in PM+ secundigravid | Placental levels in PM+ multigravid | Placental levels in PM+ all gravidities | Correction for multiple comparisons | Notes                                                                 |
|------------------------|-----------|--------------------------------------|----------------------------------------|--------------------------------------|--------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|---------------------------------------|--------------------------------------|
| Santen 2011            | Iron      | NS                                   |                                        |                                      |                                      |                                        |                                        |                                        |                                      | Not significant for either PM with or without intervillitis compared with uninfected placentas. |
| Boeuf 2013             | Isoleucine| NS                                   |                                        |                                      |                                      |                                        |                                        |                                        |                                      |                                      |
| Conroy 2011            | Leptin    | ↓                                    | Holm                                   |                                      |                                      |                                        |                                        |                                        |                                      |                                      |
| Kabyemela 2008 (1)     | Leptin    | ↓                                    | NS, NS                                 | NS                                   | NS                                   | NS                                    | NS                                    |                                        |                                      | Peripheral p values 0.0004 for primigravidae. |
| Kabyemela 2008 (2)     | Leptin    | ↓                                    | NS                                    |                                       |                                       |                                       |                                       |                                        |                                      | p value 0.0245                          |
| Boeuf 2013             | Leucine   | ↑                                    |                                       |                                       |                                       |                                       |                                       |                                        |                                      | Only significantly increased for PM with intervillitis compared with uninfected placentas. |
| Boeuf 2013             | Lysine    | NS                                   |                                        |                                      |                                      |                                        |                                        |                                        |                                      | Not significant for either PM with or without intervillitis compared with uninfected placentas. |
| Thévenon 2009          | MBL       | NS                                   |                                        |                                      |                                      |                                        |                                        |                                        |                                      |                                      |
| Abrams 2004            | MCP-1     | NS                                   |                                        |                                      |                                      |                                        |                                        |                                        |                                      | Bonferroni                            |
| Bouy-ou-Akotet 2004    | MCP-1     | ↑                                    | NS                                     | ↑                                    | NS                                   |                                        |                                        |                                        |                                      | Higher in PM+ primigravid but NS. Multivariate analysis showed significant association with PM (all gravidities). |
| Dong 2012              | MCP-1     | ↑                                    | NS                                     | ↑                                    | NS                                   |                                        |                                        |                                        |                                      | p values 0.003, 0.007                  |
Table – Summary of all studied biomarkers (continued)

| Article                  | Biomarker | Notes                                                                                                                                 |
|--------------------------|-----------|---------------------------------------------------------------------------------------------------------------------------------------|
| Suguitan 2003 (2)        | MCP-1     | NS in placental plasma direct measurement and villous tissue culture, significantly increased in WBC culture \(0.001 \leq p < 0.01\). |
| Boeuf 2013               | Methionine| Not significant for either PM with or without intervillitis compared with uninfected placenta.                                        |
| Chaisavaneeyakorn 2002 (IV) | MIF      | Significantly increased for PM+ HIV-compared with PM- irrespective of HIV status. PM+HIV+ only significantly increased compared with PM-HIV-. |
| Singh 2012               | MIF       | NS \(p < 0.001\) (placental), PM positives and PM negatives matched for low birth weight, stillbirths and gravidity.                  |
| Abrams 2004              | MIP-1α    | Bonferroni                                                                                                                               |
| Chaisavaneeyakorn 2003   | MIP-1α    | Stratified for HIV status and no overall significant difference between PM+ and PM- was presented.                                     |
| Article               | Biomarker | Notes                                                                                                                                 |
|----------------------|-----------|----------------------------------------------------------------------------------------------------------------------------------------|
| Suguitan 2003 (2)    | MIP-1α    | ± UK NS in placental plasma direct measurement and villous tissue culture, significantly increased in WBC culture (0.01 ≤ p < 0.05). |
|                      |           |                                                                                                                                       |
| Chaisavaneeanyakorn 2003 | MIP-1β  | ↑ UK Stratified for HIV status. Significantly increased for PM+ compared with PM- irrespective of HIV status.                           |
|                      |           |                                                                                                                                       |
| Perkins 2003         | PGE2      | ↓ ± UK Only significantly increased for PM with intervillositis compared with uninfected placentas.                                     |
| Boeuf 2013           | Phenylalanine | ± UK Only significantly increased for PM with intervillositis compared with uninfected placentas.                                     |
Table – Summary of all studied biomarkers (continued)

| Article          | Biomarker       | Notes                                                                 |
|------------------|-----------------|----------------------------------------------------------------------|
| Watkinson 1985   | Progesterone    | NS during gestation between women with pigmented placentas at delivery compared with women with non-pigmented placentas at delivery. |
| Boeuf 2013       | Proline         | Not significant for either PM with or without intervillositis compared with uninfected placentas. |
| Suguitan 2003 (2) | RANTES          | NS in placental plasma direct measurement, WBC culture and villous tissue culture. |
| Boeuf 2013       | Serine          | Not significant for either PM with or without intervillositis compared with uninfected placentas. |
| Ostrowski 2007   | SuPAR           | Significantly increased for active versus no placental infection and active versus past placental infection. |
| Avery 2012       | TAT complex     | Significant increase for PM diagnosed by PBS or PCR.                  |
| Santen 2011      | sTfR            | UK                                                                  |

Table – Summary of all studied biomarkers (continued)
| Article          | Biomarker | Peripheral levels in PM+ primigravid | Peripheral levels in PM+ secondigravid | Peripheral levels in PM+ multigravid | Peripheral levels in PM+ all gravidities | Placental levels in PM+ primigravid | Placental levels in PM+ secondigravid | Placental levels in PM+ multigravid | Correction for multiple comparisons |
|------------------|-----------|--------------------------------------|----------------------------------------|--------------------------------------|-----------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| Senga 2011       | sTfr : log ferritin | NS                                  | ±                                      | ±                                    |                                         |                                     |                                     |                                     |                                    |
| Boeuf 2013       | Threonine  | NS                                  |                                         |                                      |                                         |                                     |                                     |                                     |                                    |
| Abrams 2004      | TGF-β     | NS                                  |                                         | NS                                   | Bonferroni                              |                                     |                                     |                                     |                                    |
| Fievet 2001      | TGF-β     | NS                                  |                                         |                                      |                                         |                                     |                                     |                                     |                                    |
| Suguitan 2003 (2)| TGF-β     | NS                                  |                                         |                                      |                                         |                                     |                                     |                                     |                                    |
| Fried 1998       | TGF-β1    | NS                                  | NS                                     | ↑                                    |                                         |                                     |                                     |                                     |                                    |
| Fievet 2001      | TGF-β2    | NS                                  |                                         |                                      |                                         |                                     |                                     |                                     |                                    |
| Conroy 2011/2013 | sTie-2    | NS                                  | NS                                     | NS                                   |                                         |                                     |                                     |                                     | Holm/                                 |
| Santen 2011      | TIBC      | NS                                  |                                         |                                      |                                         |                                     |                                     |                                     |                                    |

Notes:
- sTfr : log ferritin ratio of >1.6 was considered iron deficiency. Significant decreased OR for all infected (active/chronic/past) versus non infected in all gravidities combined and multigravidae separately.
- Not significant for either PM with or without intervillitis compared with uninfected placentas.
- Decreased placental values with p value 0.03, but NS after Bonferroni adjustment.
- Cytokine production in cultures of villi, villi + blood, blood or serum.
- NS in placental plasma direct measurement, WBC culture and villous tissue culture.
- NS for both direct cytokine levels as for cytokine levels after culturing.

Correction for multiple comparisons
| Article      | Biomarker | Peripheral levels in PM+ primigravid | Peripheral levels in PM+ secundigravid | Peripheral levels in PM+ multigravid | Placental levels in PM+ primigravid | Placental levels in PM+ secundigravid | Placental levels in PM+ multigravid | Correction for multiple comparisons | Notes |
|-------------|-----------|--------------------------------------|----------------------------------------|-------------------------------------|-------------------------------------|--------------------------------------|-------------------------------------|-----------------------------------|-------|
| Conroy 2011 | Tissue factor | NS                                   |                                        |                                     |                                     |                                      |                                     | Holm                              |       |
| Avery 2012  | TNF-α     | ↑                                    |                                        |                                     |                                     |                                      | Tukey / Dunn                       | Case-control for preterm and fullterm delivery, groups are pooled here and stratified for PM status. |
| Abrams 2004 | TNF-α     | NS                                   |                                        | NS                                  |                                     |                                     | Bonferroni                         | Only significantly increased in serum for PM+ versus PM- (p = 0.005). NS in cultures of villi, villi + blood, or blood. |
| Diallo 2008 | TNF-α     | ↑                                    |                                        |                                      |                                      |                                      | NS UK                              | p <0.05 (peripheral)               |
| Diouf 2007  | TNF-α     | NS                                   |                                        |                                     |                                     |                                      | UK                                 |                                   |
| Fievet 2001 | TNF-α     | ±                                    |                                        |                                     |                                     |                                      | UK                                 |                                   |
| Flanagan 2010 | TNF-α   | NS                                   |                                        |                                     |                                     |                                      | Bonferroni                         | Measured in culture (SEB) supernatants. |
| Fried 1998  | TNF-α     | ↑                                    |                                        | NS                                  |                                     |                                      | UK                                 |                                   |
| Kabyemela 2008 (1) | TNF-α | ↑ ↑ ↑ ↑ | ↑ ↑ ↑ | NS ↑ | UK | Peripheral p values <0.0001, 0.0003, 0.01. Placental p values 0.005, NS, 0.005. |
| Kabyemela (2) | TNF-α | ↑ ↑ ↑ | ↑ | NS ↑ | UK | p values <0.0001, 0.0002. |
| Article       | Biomarker | Peripheral levels in PM+ primigravid | Peripheral levels in PM+ secundigravid | Peripheral levels in PM+ multigravid | Placental levels in PM+ primigravid | Placental levels in PM+ secundigravid | Placental levels in PM+ multigravid | Correction for multiple comparisons | Notes                                                                                                                                 |
|--------------|-----------|--------------------------------------|----------------------------------------|--------------------------------------|--------------------------------------|---------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------------------------------------------------|
| Moore 1999   | TNF-α     | ±                                    | ±                                      | ±                                    | UK                                   |                                       |                                      |                                      | Significant increase in PM+ paucigravidae medium ($p$ value 0.045), PHA ($p$ value 0.001), CD3 ($p$ value 0.017) and PPD cultures ($p$ value 0.034), significant decrease in malarial antigen cultures ($p$ value 0.04) and in PM+ multigravidae medium cultures ($p$ value 0.024). |
| Moore 2000   | TNF-α     | ±                                    |                                       |                                       | UK                                   |                                       |                                      |                                      | Significantly increased in PM+ versus PM- in cultures of medium, PHA, CD3 (all $p$ values <0.014) and malarial antigen ($p$ value 0.025), NS for PPD cultures. |
| Rogerson 2003| TNF-α     | ↑                                    |                                       |                                       |                                       |                                       |                                      |                                      | $p$ values 0.026, <0.0001                                                                  |
| Perkins 2003 | TNF-α     | ±                                    |                                       |                                       | UK                                   |                                       |                                      |                                      | Significantly decreased in placenta pigmented 10 - 25% versus placenta pigmented 1 - 10% ($p$ value 0.05) and almost versus non pigmented placenta ($p$ value 0.07) after 48 hours medium culture. |
**Table – Summary of all studied biomarkers (continued)**

| Article          | Biomarker | Notes                                                                 |
|------------------|-----------|----------------------------------------------------------------------|
| Suguitan 2003 (1)| TNF-α     | ± UK  
Stratified for preterm deliveries and fullterm deliveries. Significantly increased in PM+ versus PM- preterm deliveries ($p$ value 0.012), NS for fullterm deliveries. |
| Suguitan 2003 (2)| TNF-α     | ± UK  
Borderline significantly increased in PM+ in placental plasma direct measurement ($0.05 \leq p < 0.07$), NS after 24 hour villous tissue culture, significantly increased in WBC culture ($0.01 \leq p < 0.05$). |
| Kabayemela 2008 (2)| TNF-R1 | ↑ NS  UK  
$p$ value 0.0003  
Significantly increased in peripheral blood from PM+ women with peripherally detectable parasitemia versus PM- ($p < 0.001$), NS in peripheral blood from PM+ women without peripherally detectable parasitemia versus PM-. |
| Jakobsen 1998    | sTNF-R1   | NS  Bonferroni  
$p$ values $<0.0001$, $<0.0001$ |
| Thévenon 2010    | sTNF-R1   | ±  NS  UK  
Significantly increased in peripheral blood from PM+ women with peripherally detectable parasitemia versus PM- ($p < 0.001$), NS in peripheral blood from PM+ women without peripherally detectable parasitemia versus PM-. |
| Kabayemela 2008 (2)| TNF-R2 | ↑  ↑  UK  
$p$ values $<0.0001$, $<0.001$ |
| Article       | Biomarker | Peripheral levels in PM+ primigravid | Peripheral levels in PM+ secundigravid | Peripheral levels in PM+ multigravid | Placental levels in PM+ primigravid | Placental levels in PM+ secundigravid | Placental levels in PM+ multigravid | Notes                                                                 |
|--------------|-----------|---------------------------------------|----------------------------------------|--------------------------------------|--------------------------------------|----------------------------------------|--------------------------------------|-----------------------------------------------------------------------|
| Thévenon 2010 | sTNF-R2   | ↑                                     |                                        |                                      |                                      |                                        |                                      | p value 0.005 for peripheral levels                                    |
| Boeuf 2013   | Tryptophan| ±                                     |                                        |                                      |                                      |                                        |                                      | Only significantly increased for PM+ without intervillitis compared with PM- |
| Santen 2011  | TS        |                                        |                                        |                                      |                                      |                                        |                                      | UK                                                                    |
| Boeuf 2013   | Tyrosine  |                                        |                                        |                                      |                                      |                                        |                                      | UK                                                                    |
| Conroy 2011/2013 | VEGF    | NS                                    |                                        |                                      | NS                                   |                                        |                                      | Holm/Bonferroni                                                       |
| Jakobsen 1998 | sVCAM     |                                        |                                        |                                      |                                      |                                        |                                      | Bonferroni                                                            |
| Senga 2012   | ZPP       |                                        |                                        |                                      | NS                                   |                                        |                                      | UK                                                                    |

**RNA**

| Muehlenbachs 2007 | BAFF  |                                        |                                        |                                      |                                      |                                        |                                      | Increased, p value <0.006 for PM+, p value <0.001 for PM+ with intervillitis versus PM- |
| Muehlenbachs 2007 | CCL4   |                                        |                                        |                                      |                                      |                                        |                                      | p value <0.001 for PM+ and PM+ with intervillitis versus PM-           |
| Muehlenbachs 2007 | CCL5   |                                        |                                        |                                      |                                      |                                        |                                      | Increased, p value 0.034 for PM+, p value <0.001 for PM+ with intervillitis versus PM- |
| Muehlenbachs 2007 | CCL18  |                                        |                                        |                                      |                                      |                                        |                                      | p value <0.001 for PM+ and PM+ with intervillitis versus PM-           |
Table – Summary of all studied biomarkers (continued)

| Article          | Biomarker | Peripheral levels in PM+ primigravid | Peripheral levels in PM+ secundigravid | Peripheral levels in PM+ multigravid | Placental levels in PM+ primigravid | Placental levels in PM+ secundigravid | Placental levels in PM+ multigravid | Correction for multiple comparisons | Notes |
|------------------|-----------|--------------------------------------|----------------------------------------|--------------------------------------|-------------------------------------|---------------------------------------|-------------------------------------|-------------------------------------|-------|
| Tkachuk 2001     | CCR3      | ±                                    | UK                                     | p value 0.03                         |                                     |                                       |                                     |                                     |       |
| Tkachuk 2001     | CCR5      | †                                    | UK                                     | p value 0.01                         |                                     |                                       |                                     |                                     |       |
| Sarr 2010        | COX-1     | ±                                    | UK                                     |                                       |                                     |                                       |                                     |          |       |
| Sarr 2010        | COX-2     | ±                                    | UK                                     |                                       |                                     |                                       |                                     |          |       |
| Muehlenbachs 2007| CXCL9     | ±                                    | UK                                     |                                       |                                     |                                       |                                     |          |       |
| Muehlenbachs 2007| CXCL10    | ±                                    | UK                                     |                                       |                                     |                                       |                                     |          |       |

Significantly increased for active infections versus no placental infection (p value 0.03) and for HRP2+ placentas with no other indications of PM versus no placental infection (p value 0.003). NS for chronic or past infection versus no placental infection.

Significantly increased for active (p value 0.0005), chronic (p value 0.01) and past infections (p value 0.0006) versus no placental infection. NS for HRP2+ placentas with no other indications of PM versus any of the PM+ or non infected placentas.

Increased, p value 0.006 for PM+, p value <0.001 for PM+ with intervillositis versus PM-.

NS for PM+, p value 0.003 for PM+ with intervillositis versus PM-.
| Article                  | Biomarker | Peripheral levels in PM+ gravidities | Placental levels in PM+ gravidities | Notes                                                                 |
|-------------------------|-----------|--------------------------------------|-------------------------------------|----------------------------------------------------------------------|
| Muehlenbachs 2007       | CXCL13    | ↑ UK                                 |                                     | p value <0.001 for PM+ and PM+ with intervillositis versus PM-       |
| Muehlenbachs 2007       | CXCL16    | ↑ UK                                 |                                     | p value <0.001 for PM+ and PM+ with intervillositis versus PM-       |
| Tkachuk 2001            | CXCR4     | NS                                   | UK                                  |                                                                       |
| Boeuf 2008              | (s)Flt-1  | NS                                   | Bonferroni                          |                                                                       |
| Muehlenbachs 2008       | sFlt-1    | ± UK                                 |                                     | Significantly elevated in PM infected women compared to PM negative women without hypertension. Significantly elevated for PM+ with inflammation versus PM- without inflammation. |
| Moormann 1999           | GM-CSF    | NS                                   | Bonferroni                          |                                                                       |
| Muehlenbachs 2007       | HEPC      | ↑ UK                                 |                                     | p value <0.001 for PM+ and PM+ with intervillositis versus PM-       |
Table – Summary of all studied biomarkers (continued)

| Article              | Biomarker | Notes |
|----------------------|-----------|-------|
| Boeuf 2008           | HIF-1α    | ± Bonferroni Only significantly increased in syncytiotrophoblast layer by laser capture microdissection (p value 0.0005). |
| Moormann 1999        | IFN-γ     | NS Bonferroni p value <0.001 for PM+ and PM+ with intervillositis versus PM- |
| Muehlenbachs 2007    | IFN-γ     | ↑ UK Stratified for newborn genotype and only significantly increased in PM+ versus PM- in two out of three genotypes (p values <0.05 and <0.01). |
| Muehlenbachs 2008    | IFN-γ     | ± UK |
| Umbers 2011          | IGF1      | NS UK |
| Umbers 2011          | IGF2      | NS UK |
| Umbers 2011          | IGF1R     | NS UK |
| Umbers 2011          | IGF2R     | NS UK |
| Muehlenbachs 2007    | IGGH      | ± UK Increased, p value 0.007 for PM+, p value <0.001 for PM+ with intervillositis versus PM- |
| Article            | Biomarker | Peripheral levels in PM+ primigravid | Peripheral levels in PM+ secundigravid | Peripheral levels in PM+ multigravid | Peripheral levels in PM+ all gravidities | Placental levels in PM+ primigravid | Placental levels in PM+ secundigravid | Placental levels in PM+ multigravid | Placental levels in PM+ all gravidities | Correction for multiple comparisons |
|--------------------|-----------|--------------------------------------|----------------------------------------|--------------------------------------|------------------------------------------|--------------------------------------|----------------------------------------|--------------------------------------|------------------------------------------|----------------------------------------|
| Muehlenbachs 2008  | IGGH      | ±                                    |                                        |                                      |                                          |                                      |                                        |                                      |                                          |                                        |
| Muehlenbachs 2007  | IGH       | ±                                    |                                        |                                      |                                          |                                      |                                        |                                      |                                          |                                        |
| Moormann 1999      | IL-1α     | NS                                   |                                        |                                      |                                          | BS-ferroni                           | BS-ferroni                            | BS-ferroni                           | BS-ferroni                              | BS-ferroni                            |
| Moormann 1999      | IL-1β     | BS-ferroni                           |                                        | BS-ferroni                           | BS-ferroni                              | BS-ferroni                           | BS-ferroni                            | BS-ferroni                           | BS-ferroni                              | BS-ferroni                            |
| Moormann 1999      | IL-2      | NS                                   |                                        | BS-ferroni                           | BS-ferroni                              | BS-ferroni                           | BS-ferroni                            | BS-ferroni                           | BS-ferroni                              | BS-ferroni                            |
| Moormann 1999      | IL-4      | BS-ferroni                           |                                        | BS-ferroni                           | BS-ferroni                              | BS-ferroni                           | BS-ferroni                            | BS-ferroni                           | BS-ferroni                              | BS-ferroni                            |
| Moormann 1999      | IL-5      | BS-ferroni                           |                                        | BS-ferroni                           | BS-ferroni                              | BS-ferroni                           | BS-ferroni                            | BS-ferroni                           | BS-ferroni                              | BS-ferroni                            |
| Moormann 1999      | IL-6      | BS-ferroni                           |                                        | BS-ferroni                           | BS-ferroni                              | BS-ferroni                           | BS-ferroni                            | BS-ferroni                           | BS-ferroni                              | BS-ferroni                            |

Notes:
- Stratified for newborn genotype and only significantly increased in PM+ versus PM- in two out of three genotypes (p values <0.08, <0.05).
- Increased, p value 0.008 for PM+, p value <0.001 for PM+ with intervillositis versus PM-.
- Stratified for newborn genotype and only significantly increased in PM+ versus PM- in two out of three genotypes (p values <0.05).
- Borderline significantly increased in PM+.
- p value <0.001 for PM+ and PM+ with intervillositis versus PM-.
Table – Summary of all studied biomarkers (continued)

| Article            | Biomarker | Notes                        |
|--------------------|-----------|------------------------------|
| Moormann 1999      | IL-8      | NS Bonferroni                |
| Moormann 1999      | IL-10     | NS Bonferroni                |
|                     |           |                              |
| Sarr 2010          | IL-10     | ± UK                         |
| Moormann 1999      | IL-12     | NS Bonferroni                |
| Muehlenbachs 2007  | IL-18     | ↑ UK                         |
| Sarr 2010          | 15-LOX    | ± UK                         |
| Boeuf 2008         | PlGF      | NS Bonferroni                |

Significantly increased for active (p value 0.002), chronic (p value 0.04) and HRPl+/HIS- infections (p value 0.04) versus no placental infection. NS for past infection versus no infection.

p value <0.001 for PM+ and PM+ with intervillositis versus PM-

Significantly decreased for active (p value 0.001), past infections (p value 0.0006) and HRPl+/HIS- infections (p value 0.005) versus no placental infection. NS for chronic infections versus no placental infection.
| Article         | Biomarker | Peripheral levels in PM+ primigravid | Peripheral levels in PM+ secundigravid | Peripheral levels in PM+ all gravidities | Placental levels in PM+ primigravid | Placental levels in PM+ secundigravid | Placental levels in PM+ all gravidities | Notes                                                                 |
|----------------|-----------|--------------------------------------|----------------------------------------|------------------------------------------|-------------------------------------|----------------------------------------|------------------------------------------|--------------------------------------------------------------------------------|
| Boeuf 2013     | SNAT-1    | ±                                    |                                        |                                          |                                     |                                        |                                          | Only significantly increased for PM with intervillitis compared with uninfected placentas (in syncytiotrophoblast layer). |
| Boeuf 2013     | SNAT-2    | ±                                    |                                        |                                          |                                     |                                        |                                          | Only significantly increased for PM with intervillitis compared with uninfected placentas (in syncytiotrophoblast layer). |
| Moormann 1999 | TNF-α     | ↑                                    |                                        |                                          |                                     |                                        |                                          | Bonferroni                                                                              |
| Muehlenbachs 2007 | TNF       | ↑                                    |                                        |                                          |                                     |                                        |                                          | p value < 0.001 for PM+ and PM+ with intervillitis versus PM-                             |
| Moormann 1999 | TNF-β     | NS                                   |                                        |                                          |                                     |                                        |                                          | Bonferroni                                                                              |
| Moormann 1999 | TGF-β1    | ↓                                    |                                        |                                          |                                     |                                        |                                          | Bonferroni                                                                              |
| Boeuf 2008     | VEGF      | ±                                    |                                        |                                          |                                     |                                        |                                          | Significantly increased for PM+ without hypertension versus PM- without hypertension.   |
| Muehlenbachs 2006 | VEGF     | ±                                    |                                        |                                          |                                     |                                        |                                          |                                                                                       |
| Muehlenbachs 2007 | Many others |                                    |                                        |                                          |                                     |                                        |                                          |                                                                                       |
| Muehlenbachs 2007 | Many others |                                    |                                        |                                          |                                     |                                        |                                          |                                                                                       |