



BMJ Open Cohort profile: molecular signature in pregnancy (MSP): longitudinal high-frequency sampling to characterise cross-omic trajectories in pregnancy in a resource-constrained setting

Tobias Brummaier ^{1,2,3,4}, Basirudeen Syed Ahamed Kabeer,⁵ Pornpimon Wilaisrisak,¹ Mupawjay Pimanpanarak,¹ Aye Kyi Win,¹ Sasithon Pukrittayakamee,^{6,7} Alexandra K Marr,⁵ Tomoshige Kino,⁵ Souhaila Al Khodor,⁵ Annalisa Terranegra,⁵ Verena I Carrara,^{1,2} Francois Nosten ^{1,2}, Jürg Utzinger,^{3,4} Damien Chaussabel,⁵ Daniel H Paris,^{3,4} Rose McGready^{1,2}

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For numbered affiliations see end of article.

Correspondence to

Dr Tobias Brummaier;
tobias.brummaier@gmx.at

ABSTRACT

Purpose A successful pregnancy relies on the interplay of various biological systems. Deviations from the norm within a system or intersystemic interactions may result in pregnancy-associated complications and adverse pregnancy outcomes. Systems biology approaches provide an avenue of unbiased, in-depth phenotyping in health and disease. The molecular signature in pregnancy (MSP) cohort was established to characterise longitudinal, cross-omic trajectories in pregnant women from a resource constrained setting. Downstream analysis will focus on characterising physiological perturbations in uneventful pregnancies, pregnancy-associated complications and adverse outcomes.

Participants First trimester pregnant women of Karen or Burman ethnicity were followed prospectively throughout pregnancy, at delivery and until 3 months post partum. Serial high-frequency sampling to assess whole blood transcriptomics and microbiome composition of the gut, vagina and oral cavity, in conjunction with assessment of gene expression and microbial colonisation of gestational tissue, was done for all cohort participants.

Findings to date 381 women with live born singletons averaged 16 (IQR 15–18) antenatal visits (13 094 biological samples were collected). At 5% (19/381) the preterm birth rate was low. Other adverse events such as maternal febrile illness 7.1% (27/381), gestational diabetes 13.1% (50/381), maternal anaemia 16.3% (62/381), maternal underweight 19.2% (73/381) and a neonate born small for gestational age 20.2% (77/381) were more often observed than preterm birth.

Future plans Results from the MSP cohort will enable in-depth characterisation of cross-omic molecular trajectories in pregnancies from a population in a resource-constrained setting. Moreover, pregnancy-associated complications and unfavourable pregnancy outcomes will be investigated at the same granular level, with a particular focus on population relevant needs such as effect of tropical infections on pregnancy. More detailed

Strengths and limitations of this study

- The major strength is the prospective nature of the study and frequent follow-up, coupled with high-frequency sampling and thus, availability of detailed clinical information and a considerable number of biological samples.
- High-throughput analysis, in combination with clinical data, will enable investigation of a number of pregnancy-related physiological and pathological changes.
- Frequently populations from low-resource settings are disproportionately burdened by adverse birth outcomes that may be based on exposure to different communicable diseases; hence, including them in high-end clinical research, addresses a significant research gap and may result in improvements of limited relevance to high-income countries.
- Low numbers for some phenotypes (eg, preterm birth) may prove to be detrimental for the validity of observed differences; although the power will depend on the magnitude of observed differences in molecular signatures.
- In low-resource settings, complete biological sample sets are often difficult to obtain, which may downsize the richness of the data.

knowledge on multiomic perturbations will ideally result in the development of diagnostic tools and ultimately lead to targeted interventions that may disproportionately benefit pregnant women from this resource-limited population.

Trial registration number NCT02797327.

INTRODUCTION

A successful pregnancy relies on well-timed adaptations and the interplay of multiple maternal biological systems. These



interactions and temporal changes affect various organ systems, such as the cardiovascular, respiratory, endocrine system or metabolic systems, and, more recently, pivotal immunological adaptations and changes in the human microbiome became evident.¹⁻⁵ Physiological adaptations of the immune system during pregnancy play a central role in implantation and placentation, promotion of fetal growth and initiation of labour and delivery.² Deviations from the norm of this fine-tuned immune clock may lead to dysregulation in biological networks and cause various pregnancy-associated complications with their immediate consequences for the mother and fetus.⁶

With a growing body of evidence of the human microbiome's significance in health and disease, investigating its role in reproductive medicine has opened up an avenue to a deeper understanding of adverse pregnancy outcomes.⁷ The placenta represents the fetomaternal link. Its crucial role during pregnancy is underlined by the fact that failure in its development and function is associated with a variety of pregnancy-associated complications (eg, pre-eclampsia, intrauterine growth restriction and preterm birth, PTB).⁸

In recent years, investigation of these biological systems has greatly improved our understanding of their role in healthy pregnancies and in pregnancies resulting in unfavourable outcomes. However, there is a need for longitudinal, multiomics profiling studies that may further contribute to the understanding of physiological adaptations, the interconnections of various biological systems and their significance in pregnancy-associated complications.³

Research, in particular high-end research, is often biased towards high-income countries.⁹ This imbalance is aggravated by the fact that, due to population based differences, results are often not generalisable,¹⁰ populations living in resource-constrained settings are more affected by the consequences of pregnancy-associated complications (eg, inadequate access to safe iatrogenic birth or lack of advanced neonatal care) and have different epidemiological patterns of communicable diseases. Consequently, pregnant women in low-income settings would benefit disproportionately from early identification of pregnancy-associated complications and targeted interventions.

Thus, the aim of establishing this prospective molecular signature in pregnancy (MSP) cohort was to characterise cross-omic trajectories in pregnant women from a resource-limited setting and describe pregnancy-associated complications (eg, PTB, gestational diabetes mellitus (GDM), anaemia, underweight and born too small for gestational age (SGA)). The term 'molecular signature' was chosen, as it refers to molecular markers that can be used for in-depth description of a particular phenotype.¹¹

Perturbations related to the immune response will be investigated by measuring the abundance of RNAs in circulating nucleated cells (ie, leucocytes) via capillary blood sampling at multiple time points.¹² Microbiome

profiling of the intestinal and vaginal niche in pregnancy, at delivery and post partum will be complemented by assessment of the oral microbiome. Lastly, placental tissue will be investigated for the presence of bacterial colonisation as well as for the relationship between the genome structure and changes in global patterns of gene expression. The generated molecular data will then be analysed in the context of clinical and patient related data. As described in the study protocol,¹³ an immediate focus was the investigation of PTB.

The MSP cohort profile represents the link between development of the study protocol and the results. Accordingly, cohort characteristics, including the recruitment and follow-up process, demographics, pregnancy outcomes, biological samples available for analysis and preliminary results, are presented.

COHORT DESCRIPTION

Setting and participants

Women with an unremarkable medical and obstetric history attending the antenatal care (ANC) facilities of Shoklo Malaria Research Unit (SMRU), a field station of the Faculty of Tropical Medicine at Mahidol University (Bangkok, Thailand) that is part of the Mahidol-Oxford Research Unit, were invited to participate. SMRU operates free-of-charge walk-in services for marginalised migrant populations in clinics at the frontier of Thailand with Myanmar (figure 1). This mobile population mostly resides in small, rural villages. The major source of income in this community comes from work in the agricultural sector or as daily labourers, while about one in four women stay at home to look after the household.¹⁴ As most of these daily employments provide only minimum wages coupled with the often undocumented legal status, the living conditions of most pregnant women in this community are very basic.¹⁵ The official minimal wages in Myanmar are around MMK4800 (US\$3) per day, and in Thailand around THB300 (US\$9) per day.

Between September 2016 and July 2018, trained counsellors from the community provided general information about the trial to all pregnant women who registered at SMRU ANC. Detailed information, including visual aids, was used to illustrate the purpose, procedures and timing of follow-up. Potential risks and benefits of the study were explained to women meeting the following inclusion criteria: (1) pregnant woman willing and able to give written informed consent; (2) Karen or Burman ethnicity; (3) age 18–49 years; (4) healthy, with viable singleton first trimester (8+0 to <14+0 weeks) pregnancy; (5) planning to deliver at the SMRU birth unit and (6) able and willing to comply with study requirements. If these criteria were met and appropriate consent was provided, women were followed from the first trimester, throughout pregnancy, at delivery and until 3 months post partum.

It was estimated that approximately 400 women could be enrolled to build the cohort from this low-resource environment when considering constraints of clinical capacity

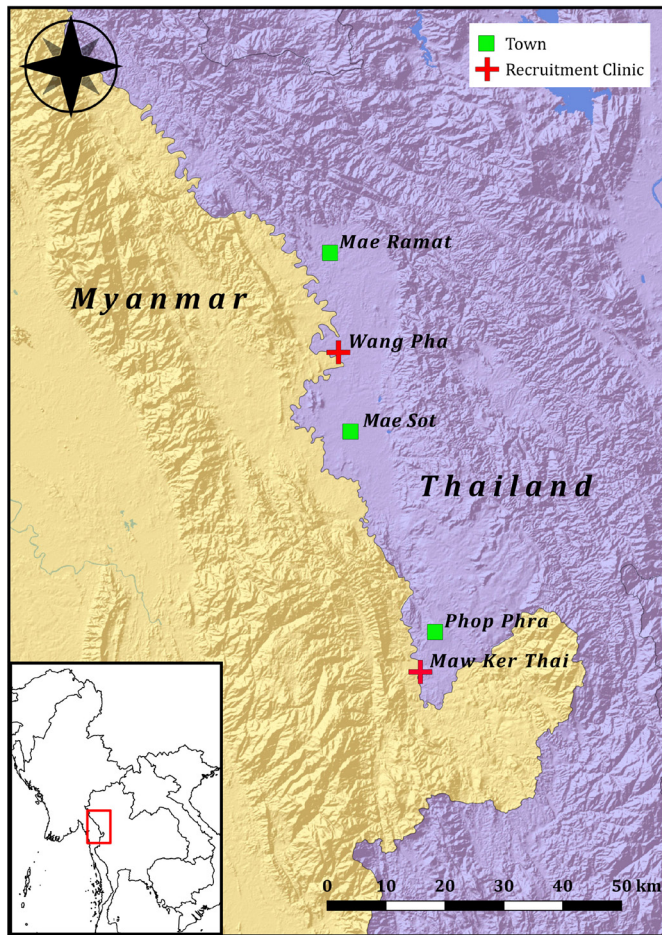


Figure 1 Setting and location of recruitment clinics.

and budget availability. With this sample size, events such as PTB (estimated at 8%, that is, 30 cases expected) would be feasible but also provide scope to engage with other pregnancy-associated complications, tropical infections (eg, small liver fluke infections estimated at 5%, that is, 20 cases expected) and febrile illness (estimated at 10%, that is, an expectation of 40 cases) with a sufficient number of uneventful pregnancies remaining to act as controls.

Figure 2 provides an overview of the number of participants recruited and followed. During the active recruitment period, 4292 pregnant women registered to ANC. Seventy per cent (2544/3609) were ineligible due to late presentation to ANC. There were two local conditions that limited recruitment. The first issue pertained to access; pregnant women following SMRU ANC come from villages scattered along the Thailand-Myanmar border (figure 1) with transportation constraints affecting their ability to attend, further aggravated in the monsoon season as roads become impassable. The second issue related to staffing. As only one ultrasound machine was available at each site, there was an upper limit to the number of women who could have study related fetal growth scans done on a single day. Challenges in access and staffing issues led to the non-consideration of 178 and 475 women, respectively.

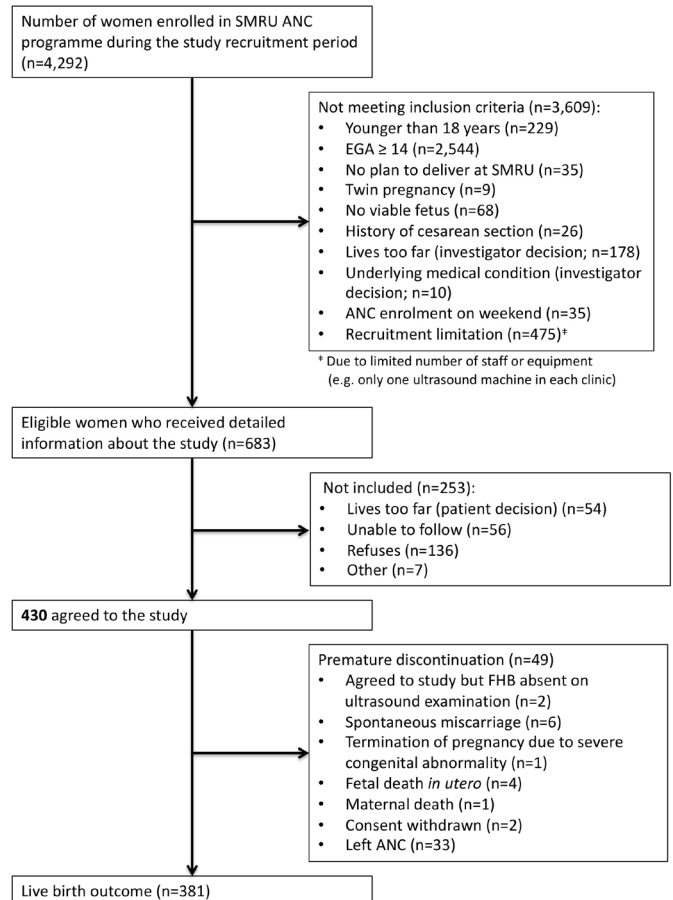


Figure 2 Screening, enrolment and outcome flow chart. ANC, antenatal care; EGA, estimated gestational age; FHB, fetal heartbeat; SMRU, Shoklo Malaria Research Unit.

Overall, 683 women received full information about the purpose and procedures of the study. Of these, 430 agreed to participate (response rate: 63%) and consented by providing a signature or thumbprint if the event of an illiterate participant. Thirty participants were recruited in the cohort to replace women who were lost before delivery, while the study was still open to recruitment. Two participants were excluded as the fetal heartbeat (FHB) was absent after they had already agreed to the study (figure 2).

Routine ANC procedures at SMRU are described in the study protocol.¹³ Demographic characteristics of 381 enrolled women with a live birth outcome are summarised (table 1). To demonstrate the scope of the cohort, selected subgroups are summarised alongside the characteristics for all 381 women.

Participant follow-up

Women were followed every 2 weeks during pregnancy, at delivery and monthly in the first 3 months after delivery. Trial participants were encouraged to report to the study clinic if they experienced an adverse event, with special consideration for febrile episodes to maximise information on the nature of the experienced morbidity and its effect on the pregnancy.¹³ Overall, 6443 study visits

**Table 1** Basic characteristics of women with a live birth outcome (n=381) and subgroups of selected pregnancy-associated complications in the molecular signature in pregnancy cohort

| | Overall (n=381) | Preterm (n=19) | GDM (n=50) | Anaemia (n=62) | Underweight (n=73) | SGA (n=77) |
|--------------------------------------|--------------------|-------------------|---------------|-------------------|-----------------------|---------------|
| Inclusion EGA (days); median (range) | 82 (56–97) | 83 (60–94) | 82.5 (56–97) | 81 (60–94) | 81 (57–95) | 81 (62–97) |
| Maternal age groups (years) | | | | | | |
| 18–24 | 185 (48.6) | 14 (73.7) | 25 (50.0) | 28 (45.2) | 43 (58.9) | 38 (49.4) |
| 25–29 | 95 (24.9) | 2 (10.5) | 12 (24.0) | 14 (22.6) | 18 (24.7) | 18 (23.4) |
| 30–34 | 64 (16.8) | 3 (15.8) | 8 (16.0) | 12 (19.4) | 10 (13.7) | 13 (16.9) |
| 35–39 | 25 (6.6) | 0 | 4 (8.0) | 5 (8.1) | 2 (2.7) | 6 (7.8) |
| 40–49 | 12 (3.1) | 0 | 1 (2.0) | 3 (4.8) | 0 | 2 (2.6) |
| Residence | | | | | | |
| Myanmar | 263 (69.0) | 10 (52.6) | 33 (66.0) | 42 (67.7) | 43 (58.9) | 52 (67.5) |
| Thailand | 118 (31.0) | 9 (47.4) | 17 (34.0) | 20 (32.3) | 18 (24.7) | 25 (32.5) |
| Gravidity | | | | | | |
| Primigravida | 103 (27.0) | 8 (42.1) | 12 (24.0) | 17 (27.4) | 32 (43.8) | 26 (33.8) |
| 2 | 111 (29.1) | 8 (42.1) | 16 (32.0) | 21 (33.9) | 21 (28.8) | 18 (23.4) |
| 3 | 79 (20.7) | 2 (10.5) | 12 (24.0) | 14 (22.6) | 9 (12.3) | 13 (16.9) |
| 4 | 47 (12.3) | 1 (5.3) | 7 (14.0) | 4 (6.5) | 8 (11.0) | 11 (14.3) |
| ≥5 | 41 (10.8) | 0 | 3 (6.0) | 6 (9.7) | 3 (4.1) | 9 (11.7) |
| Parity | | | | | | |
| 0 (nullipara) | 127 (33.3) | 9 (47.4) | 16 (32.0) | 23 (37.1) | 37 (50.7) | 32 (41.6) |
| 1 | 117 (30.7) | 8 (42.1) | 15 (30.0) | 18 (29.0) | 18 (24.7) | 16 (20.8) |
| 2 | 70 (18.4) | 1 (5.3) | 11 (22.0) | 14 (22.6) | 11 (15.1) | 13 (16.9) |
| 3 | 39 (10.2) | 1 (5.3) | 7 (14.0) | 2 (3.2) | 4 (5.5) | 11 (14.3) |
| ≥4 | 28 (7.3) | 0 | 1 (2.0) | 5 (8.1) | 3 (4.1) | 5 (6.5) |
| Literate | 244 (64.0) | 13 (68.4) | 29 (58.0) | 43 (69.4) | 53 (72.6) | 51 (66.2) |
| Smoking during in pregnancy | 29 (7.6) | 0 | 6 (12.0) | 5 (8.1) | 4 (5.5) | 10 (13.0) |
| Obstetric history* | | | | | | |
| Miscarriage | 94 (33.8) | 2 (18.2) | 11 (28.9) | 15 (33.3) | 13 (31.7) | 19 (37.3) |
| Stillbirth | 4 (1.4) | 0 | 0 | 1 (2.2) | 0 | 0 |
| PIH | 2 (0.7) | 0 | 0 | 1 (2.2) | 0 | 0 |
| Preterm rupture of membranes | 8 (2.9) | 2 (18.2) | 0 | 3 (6.7) | 1 (2.4) | 1 (2.0) |
| Preterm labour | 23 (8.3) | 6 (54.5) | 0 | 9 (20.0) | 3 (7.3) | 3 (5.9) |
| Vacuum delivery | 3 (1.1) | 0 | 0 | 1 (2.2) | 1 (2.4) | 0 |
| Postpartum haemorrhage | 9 (3.2) | 0 | 0 | 3 (6.7) | 2 (4.9) | 2 (3.9) |
| Retained placenta | 1 (0.4) | 0 | 0 | 0 | 0 | 0 |
| Previous neonatal death | 9 (3.2) | 3 (27.3) | 0 | 3 (6.7) | 0 | 1 (2.0) |
| History of GDM | 2 (0.7) | 0 | 2 (5.3) | 0 | 0 | 0 |
| Macrosomia | 2 (0.7) | 0 | 1 (2.6) | 0 | 0 | 0 |

Basic characteristics of pregnant women who completed per protocol follow-up with a live birth outcome, compared between various pregnancy-associated complications and adverse pregnancy outcomes. One study participant may be represented in multiple subgroups.

Data presented as proportion n (%) or median (range).

*Proportions calculated based on multigravida (n=278) as denominator.

EGA, estimated gestational age; GDM, gestational diabetes; PIH, pregnancy induced hypertension; SGA, born too small for gestational age.

during pregnancy were recorded, of which 6140 were from women with a live birth outcome. The median number of ANC visits for women with a live birth was 16

(IQR 15–18). In the uncommon event of cohort participants attending other health facilities, diagnosis and treatment was recorded at the next visit. All participants

were encouraged to deliver at SMRU under the care of a skilled birth attendant. Women from the cohort achieved a low rate of home birth 6.6% (25/381) in contrast to the estimated 7 in 10 births that take place at home in rural Myanmar.¹⁶

As an estimator for adherence to study protocol, the number of expected and actual samples provided by women with a known pregnancy outcome were calculated. No significant difference was noted when comparing the proportion of expected and actual samples between capillary blood, stool and vaginal swab samples ($p=0.070$, $p=0.767$ and $p=0.919$, respectively). Missed follow-ups were the main reason for less than expected samples being provided. Additional samples were provided from participants who experienced a febrile illness, and on a few occasions, samples were erroneously taken as they were not required by the study protocol.

Early termination from the study occurred in 11.0% (47/428) of pregnancies, most of whom left ANC before birth 70.2% (33/47) due to migration. Adverse pregnancy outcomes were recorded in 25.5% (12/47) cases and two (4.3%) participants withdrew consent, as they no longer wanted to provide vaginal swab samples (figure 2).

Data collection

Data covering three major aspects were collected, namely (1) clinical data; (2) genome-wide transcript abundance and (3) analysis of microbial composition in various anatomical sites. Table 2 summarises all study relevant procedures.

Routine ANC procedures included physical and obstetric examination, recording of concomitant medications and 6-weekly ultrasound scans to monitor fetal growth. Clinical data were collected, so that trajectories of maternal and fetal physical change would be available by the study endpoint. Two-weekly gestational weight, fetal growth and haematocrit were done as malnutrition and anaemia (and formerly malaria) are prevalent pregnancy-associated morbidities.^{17–19}

Ultrasound scans to monitor fetal growth were performed 6-weekly. Throughout the study period, 2850 scans were done; 2693 of these were scans in women with a known, viable birth outcome (157 scans accounted for other pregnancy outcomes).

Additional details describing the study conduct and routine ANC procedures are available elsewhere.¹³ Briefly, capillary blood, faecal and vaginal swab samples

Table 2 Study procedures and timing of the molecular signature in pregnancy cohort¹³

| | Screening | Baseline | Follow-up | Unwell episode | Birth | Post partum |
|--|-----------|----------|-----------|----------------|-------|-------------|
| Viable singletons pregnancy | X | | | | | |
| Obstetric ultrasound* | X | X | X | | | |
| Eligibility assessment | X | | | | | |
| Written informed consent | | X | | | | |
| Demographics | | X | | | | |
| Medical and obstetric history | | X | | | | |
| Concomitant medications | | X | X | X | X | X |
| Physical examination | | X | X | X | X | X |
| Universal pregnancy screening, for example, thick and thin blood film for malaria diagnosis, CBC and OGTT† | | X | | | | (X)† |
| Sample maternal 100 µL capillary blood‡ | | X | X§ | X¶ | X** | X†† |
| Sample vaginal swab, stool specimen and 24 hours food recall | | X‡‡ | X‡‡ | X | X | X§§ |
| Acceptability survey | | X | | | | X |
| Sample saliva | | | X¶¶ | | X | |
| Sample placenta, cord blood and maternal venous blood | | | | | X | |

*Fetal growth scans on a 6-weekly basis.

†OGTT at 24–26 weeks of gestation; repeated at 12 weeks post partum if positive during pregnancy.

‡50 µL for whole blood transcriptome analysis and 50 µL for haematocrit.

§2-weekly; if the woman attended all expected 15 visits total blood is 1.5 mL.

¶If the woman attended for an unwell visit, an additional 100 µL of blood were drawn.

**If delivery at SMRU clinic, then an additional 100 µL of blood were drawn.

††At 1, 2 and 3 months postpartum, including maternal haematocrit.

‡‡In each trimester of pregnancy: 8–14, 20–22 and 34–35 weeks.

§§Vaginal swab samples at 4–6 weeks and at 3 months.

¶¶At 24–26 weeks of gestation.

CBC, complete blood count; OGTT, oral glucose tolerance test; SMRU, Shoklo Malaria Research Unit.



were taken during pregnancy, at delivery and in the post-partum period. To assess the influence of nutrition on the faecal microbiome, each faecal sample was coupled with a 24-hour food recall (table 2). In the event of a febrile episode, an additional set of these samples were taken together with a standardised fever screening battery. Oral microbiome was assessed in the second trimester and at delivery. Placental tissue samples were taken at delivery along with umbilical cord blood serum and maternal serum.

As SMRU birthing units do not have capacity for caesarean sections, participants were referred to the closest public hospitals when indicated, and hence, delivery samples were not available, in common with births at home or in other health facilities. Even though gestational tissue samples were not available for these participants, outcome measures and neonatal anthropometry were mostly available.

Lastly, in an effort to understand the acceptance of the high-frequency sampling in combination with the dense follow-up schedule and the perception of invasiveness of sampling procedures, an acceptability survey at study enrolment and completion was conducted.

Biological samples

Standard operating procedures (SOPs) focused on detailed description of the sample collection procedures were drafted before study commencement and are available on request. To accommodate the high number of samples, a unique barcoded sample sticker was placed on each tube, containing the participants study code that was assigned at enrolment, as well as date and time when samples were collected. All samples were transferred from the study sites to SMRU's central laboratory daily and stored according to SOPs. Freezerworks (Dataworks Development, Mountlake Terrace, Washington, USA), a biorepository software was used for sample management and tracking.

Including women lost to follow-up and pregnancies that resulted in a miscarriage or stillbirth, 13 536 biological samples were taken between study enrolment and 3-month post partum. Considering multiple aliquots for vaginal swab samples (four aliquots at each sampling time point), saliva samples (n=4) and placenta samples (n=12), the total number of samples available for testing is 25 816.

Table 3 Overview of available biological samples for women with a live birth outcome (n=381) and selected subgroups of the molecular signature in pregnancy cohort

| | Overall (n=381) | Preterm (n=19) | GDM (n=50) | Anaemia (n=62) | Underweight (n=73) | SGA (n=77) |
|-------------------------------|-----------------|----------------|------------|----------------|--------------------|------------|
| Capillary blood | | | | | | |
| Pregnancy | 5263 | 226 | 672 | 871 | 1000 | 1070 |
| Delivery | 346 | 19 | 44 | 57 | 68 | 70 |
| Post partum | 1037 | 48 | 132 | 157 | 196 | 210 |
| Stool | | | | | | |
| Pregnancy | 1159 | 52 | 149 | 189 | 220 | 233 |
| Delivery | 299 | 18 | 43 | 47 | 58 | 59 |
| Post partum | 877 | 34 | 115 | 129 | 147 | 166 |
| Vaginal swab* | | | | | | |
| Pregnancy | 1175 | 55 | 151 | 191 | 223 | 236 |
| Delivery | 329 | 19 | 45 | 55 | 67 | 65 |
| Post partum | 686 | 30 | 84 | 105 | 128 | 136 |
| Saliva | | | | | | |
| Pregnancy | 371 | 19 | 49 | 62 | 71 | 74 |
| Delivery | 336 | 19 | 44 | 57 | 66 | 67 |
| Placenta | 301 | 16 | 41 | 50 | 63 | 63 |
| Maternal serum | 322 | 17 | 43 | 55 | 66 | 66 |
| Cord blood serum | 299 | 15 | 41 | 48 | 63 | 64 |
| Cord blood EDTA† | 294 | 14 | 41 | 49 | 59 | 60 |
| Malaria tests in pregnancy | 5662 | 226 | 819 | 988 | 1112 | 1240 |
| HCT tests in pregnancy | 5674 | 227 | 817 | 989 | 1115 | 1242 |
| Stool microscopy in pregnancy | 620 | 29 | 84 | 107 | 99 | 122 |

*Number refers to sampling time points; four swabs were taken at each time point.

†Less samples as amended to the protocol at a later stage.

EDTA, ethylenediaminetetraacetic acid; GDM, gestational diabetes mellitus; HCT, haematocrit; SGA, born too small for gestational age.

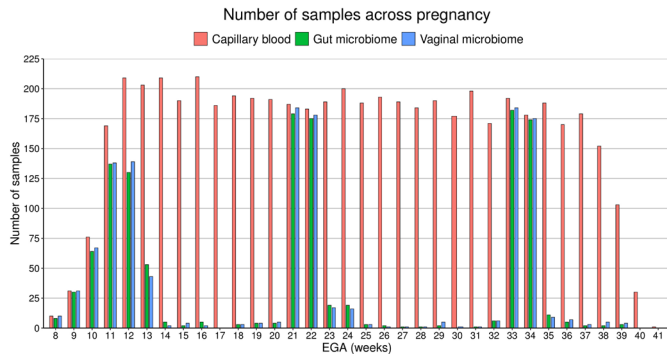


Figure 3 Number of samples over the course of pregnancy by week of gestation. EGA, estimated gestational age.

Table 3 provides an overview of biological samples available for analysis and **figure 3** depicts the distribution of capillary blood, faecal and vaginal swab samples in relation to the estimated gestation of pregnancy.

All biological samples will be analysed and interpreted in the context of clinical data. To investigate physiological trajectories and deviations from these in the event of pregnancy-associated complications, a nested case–control approach will be applied, and potential confounding factors will be adjusted for. Results will be published in original research articles alongside more detailed background information pertaining to the respective content knowledge, precise methods of sampling procedures and analytical steps of respective laboratory-based methods to enable the scientific community to draw their conclusions on appropriateness. The MSP cohort profile and the previously published study protocol¹³ will serve as a cross-reference for all results.

The molecular signatures pertaining to biological samples will be analysed by collaborators at Sidra Medicine (Doha, Qatar). To assure appropriate sample handling, shipment is delegated to World Courier (AmerisourceBergen; Chesterbrook, Pennsylvania, USA), a leading, global specialty and logistics provider. All samples are kept on dry ice and shipped in freezer boxes with thick styrofoam walls that are equipped with temperature monitors. At the time of writing, 16916 biological samples have been successfully transferred.

Participant involvement

Study participants were not directly involved in the study design, the elaboration of research questions and outcome measures or the recruitment and conduct of the study. However, the protocol was reviewed and approved by a local community advisory board whose members are representatives of the local community and act as a bridge between researchers and the local population.²⁰ Their role was to advise on ethical and operational aspects of the study and they will provide a channel to inform the community of the results of this study.

FINDINGS TO DATE

Demographic data, findings during pregnancy and relevant outcome data for the overall cohort population with a live birth are presented in **tables 1 and 4**, respectively. According to study objectives, data of commonly observed

pregnancy-associated complications (GDM, anaemia and underweight) and adverse pregnancy outcomes (PTB and SGA) are presented alongside the overall cohort.

At a rate of 5.0% (19/381), PTB (defined as delivery before 37 weeks of gestation), was considerably less than the worldwide estimate of approximately 10.6% (uncertainty interval 9.0%–12.0%) of live-born infants.²¹ When considering all pregnancies followed at SMRU during the study period, the observed proportion of PTB was roughly 6.8%, a number still below the worldwide average. The estimated gestational age (EGA) at delivery of PTB cases ranged from 30⁺² to 36⁺⁶ (weeks^{+days}) with the majority of cases (89.5%) being moderate/late PTB (i.e. ≥ 32 and < 37 weeks of gestation). Of the 19 PTB cases, one was iatrogenic with induction for severe preeclampsia at an EGA of 36⁺³, while the remainder were spontaneous, without any discernible causative factor. Prioritising healthy women at the outset and frequent visits with healthcare providers might have contributed to earlier identification of health issues and timely intervention. Population-based factors that might be associated with the low PTB rate are the low rate of sexually transmitted diseases, and preliminary data suggest that prevalence of *Gardnerella vaginalis*, a facultative anaerobic coccobacillus that is associated with PTB, is low in this population. This low PTB rate is unique in itself and will be thoroughly assessed by a holistic investigation of available demographic, clinical and cross-omic data.

There were six spontaneous miscarriages (median EGA 16⁺³ (IQR 16⁺¹–17⁺²)) and one pregnancy was terminated due to a congenital abnormality (severe hydrops fetalis) at an EGA of 20⁺¹. There were four stillbirths; all were identified antepartum as intrauterine fetal deaths before completion of 37 weeks of gestation. One was unexplained with an absent FHB at 28⁺¹ weeks, one was associated with pre-eclampsia (absent FHB at 31⁺¹), one had a tight cord around the neck at birth (absent FHB at 35⁺³), and in one case the mother ingested organophosphate with suicidal intent (absent FHB at 36⁺³). One unexpected neonatal death was recorded in a term neonate born to a mother with an unremarkable pregnancy and delivery.

Seven of 381 (1.8%) women experienced *Plasmodium vivax* malaria during pregnancy and one woman was diagnosed with *P. vivax* malaria at delivery. Soil-transmitted helminth infections are common in this population and were detected in 21.3% (81/381) of pregnant women with a live birth outcome. Hookworm was detected in 15.0% (57/381) cases, *Trichuris trichiura* in 6.3% (24/381) and *Ascaris lumbricoides* in 4.2% (16/381). Fourteen women had more than one soil-transmitted helminth infection concurrently. Food-borne trematode infections (eg, small liver flukes) were detected in 6.8% (26/381) of the participants. The immunological fingerprint of helminthic infections and their effect on pregnancy is poorly understood. Hence, effects of helminth infections on the immune system or the intestinal microbiome in pregnancy will be studied with data from this cohort.

Table 4 Pregnancy outcome and neonatal data of women with a life birth outcome (n=381) and selected subgroups in the molecular signature in pregnancy cohort

| | Overall (n=381) | Preterm (n=19) | GDM (n=50) | Anaemia (n=62) | Underweight (n=73) | SGA (n=77) |
|--|------------------|------------------|------------------|------------------|--------------------|------------------|
| Outcome EGA (days); median (IQR) | 277 (271–282) | 253 (244–255) | 275 (268–279) | 275 (268–283) | 277 (271–281) | 279 (274–283) |
| Preterm categories | | | | | | |
| Term (≥37 weeks) | 362 (95.0) | 0 | 49 (98.0) | 57 (91.9) | 68 (93.2) | 73 (94.8) |
| Moderate/late PTB (≥32) | 17 (4.5) | 17 (89.5) | 1 (2.0) | 3 (4.8) | 5 (6.8) | 4 (5.2) |
| Very PTB (28–<32) | 2 (0.5) | 2 (10.5) | 0 | 2 (3.2) | 0 | 0 |
| Extremely PTB (<28) | 0 | 0 | 0 | 0 | 0 | 0 |
| Infant sex (male) | 185 (48.6) | 11 (57.9) | 26 (52.0) | 18 (29.0) | 33 (45.2) | 25 (32.5) |
| Apgar after 1 min; median (IQR) | 9 (9–9) | 9 (9–9) | 9 (9–9) | 9 (9–9) | 9 (9–9) | 9 (9–9) |
| Apgar after 5 min; median (IQR) | 10 (10–10) | 10 (10–10) | 10 (10–10) | 10 (10–10) | 10 (10–10) | 10 (10–10) |
| Resuscitation | | | | | | |
| Yes | 8 (2.1) | 1 (5.3) | 0 | 2 (3.2) | 1 (1.4) | 5 (6.5) |
| No | 359 (94.2) | 17 (89.5) | 49 (98.0) | 59 (95.2) | 71 (97.3) | 70 (90.9) |
| Unknown | 14 (3.7) | 1 (5.3) | 1 (2.0) | 1 (1.6) | 1 (1.4) | 2 (2.6) |
| Abnormal newborn exam* | 5 (1.3) | 1 (5.3) | 0 | 2 (3.2) | 1 (1.4) | 0 |
| Birth weight (g); median (IQR)† | 2955 (2740–3200) | 2273 (2025–2440) | 3010 (2840–3375) | 2870 (2620–3220) | 2823 (2611–2965) | 2600 (2400–2740) |
| Birth weight categories‡ | | | | | | |
| Small for GA | 77 (21.8) | 4 (22.2) | 7 (14.3) | 14 (23.0) | 23 (33.8) | 77 (100.0) |
| Appropriate for GA | 269 (76.2) | 14 (77.8) | 39 (79.6) | 47 (77.0) | 45 (66.2) | 0 |
| Large for GA | 7 (2.0) | 0 | 3 (6.1) | 0 | 0 | 0 |
| Infant length (cm); median (IQR)‡ | 48.2 (47.0–49.4) | 45.7 (44.1–46.6) | 48.4 (47.4–49.4) | 48.1 (46.5–49.0) | 47.6 (46.5–48.5) | 46.7 (45.6–48.0) |
| Length categories‡ | | | | | | |
| Short for GA | 70 (19.9) | 2 (11.1) | 7 (14.3) | 14 (23.0) | 23 (33.8) | 44 (57.1) |
| Appropriate for GA | 266 (75.6) | 16 (88.9) | 37 (75.5) | 44 (72.1) | 43 (63.2) | 32 (41.6) |
| Tall for GA | 16 (4.5) | 0 | 5 (10.2) | 3 (4.9) | 2 (2.9) | 0 |
| Head circumference at birth; median (IQR)‡ | 32.9 (32.0–33.6) | 31.0 (30.1–31.6) | 33.1 (32.5–34.2) | 32.4 (31.6–33.6) | 32.4 (31.5–33.3) | 32.0 (31.4–32.8) |
| Head circumference categories‡ | | | | | | |
| Small HC for GA | 106 (30.1) | 4 (22.2) | 8 (16.3) | 21 (34.4) | 30 (44.1) | 48 (63.2) |
| Appropriate HC for GA | 240 (68.2) | 14 (77.8) | 37 (75.5) | 39 (63.9) | 37 (54.4) | 28 (36.8) |
| Large HC for GA | 6 (1.7) | 0 | 4 (8.2) | 1 (1.6) | 1 (1.5) | 0 |
| Caesarean section | 22 (5.8) | 0 | 1 (2.0) | 5 (8.1) | 2 (2.7) | 5 (6.5) |
| Breech delivery | 5 (1.3) | 1 (5.3) | 0 | 0 | 1 (1.4) | 3 (3.9) |
| Vacuum delivery | 11 (2.9) | 0 | 1 (2.0) | 4 (6.5) | 3 (4.1) | 3 (3.9) |
| Delivered by | | | | | | |

Continued

Table 4 Continued

| | Overall (n=381) | Preterm (n=19) | GDM (n=50) | Anaemia (n=62) | Underweight (n=73) | SGA (n=77) |
|---------------------------|-----------------|----------------|---------------|----------------|--------------------|---------------|
| Midwife | 328 (86.1) | 17 (89.5) | 47 (94.0) | 52 (83.9) | 66 (90.4) | 70 (90.9) |
| TBA | 25 (6.6) | 2 (10.5) | 2 (4.0) | 3 (4.8) | 5 (6.8) | 1 (1.3) |
| Doctor | 24 (6.3) | 0 | 1 (2.0) | 6 (9.7) | 2 (2.7) | 5 (6.5) |
| Other§ | 4 (1.0) | 0 | 0 | 1 (1.6) | 0 | 1 (1.3) |
| Place of delivery | | | | | | |
| SMRU clinic | 304 (79.8) | 16 (84.2) | 43 (86.0) | 51 (82.3) | 63 (86.3) | 63 (81.8) |
| Home | 29 (7.6) | 2 (10.5) | 2 (4.0) | 4 (6.5) | 5 (6.8) | 2 (2.6) |
| Thai Hospital | 35 (9.2) | 0 | 4 (8.0) | 7 (11.3) | 3 (4.1) | 10 (13.0) |
| Myanmar Hospital | 4 (1.0) | 0 | 0 | 0 | 1 (1.4) | 0 |
| Other¶ | 9 (2.4) | 1 (5.3) | 1 (2.0) | 0 | 1 (1.4) | 2 (2.6) |
| Induction of labour | 23 (6.0) | 1 (5.3) | 3 (6.0) | 4 (6.5) | 4 (5.5) | 4 (5.2) |
| Augmentation of labour | 36 (9.4) | 0 | 5 (10.0) | 5 (8.1) | 9 (12.3) | 6 (7.8) |
| Postpartum haemorrhage | 19 (5.0) | 0 | 1 (2.0) | 2 (3.2) | 4 (5.5) | 2 (2.6) |
| Estimated blood loss (mL) | 150 (100–240) | 100 (100–150) | 115 (100–200) | 150 (100–200) | 140 (100–200) | 138 (100–200) |
| Neonatal death | 1 (0.3) | 0 | 0 | 0 | 0 | 0 |

One study participant may be represented in multiple subgroups.

Data presented as proportion n (%) or median (IQR).

*Spina bifida (n=2), cleft lip (n=1). Down's syndrome (n=1), congenital heart disease (n=2).

†28 cases with missing information (eg, home delivery).

‡29 cases with missing information.

§Delivered by other: spontaneous self-delivery (n=2), husband (n=1), village health worker (n=1).

¶Place of delivery other: delivery on the way to the SMRU clinics (n=6), in another clinic (n=3).

EGA, estimated gestational age; GA, gestational age; GDM, gestational diabetes mellitus; HC, head circumference; PTB, preterm birth; SGA, born too small for gestational age; SMRU, Shoklo Malaria Research Unit; TBA, traditional birth attendant.

Generally, communicable diseases are often associated with adverse pregnancy outcomes. Fever, which is often the cardinal symptom of an infectious disease, was recorded in 7.1% (27/381) of pregnant women. Whether longitudinal, high-frequency sampling has potential for early detection, or detection prior to the onset of symptoms, of communicable diseases in pregnancy, will be determined. Availability of data pertaining to communicable disease will also allow appropriate adjustment for investigation of other objectives (eg, PTB).

Overall, only four cases (1.0%) of preeclampsia and one case (0.3%) of eclampsia were recorded which is also at the lower end of the epidemiological range (0.2–9.2).²² A common pregnancy-associated complication was GDM, which affected 13.1% (50/381) pregnancies with a live birth. GDM is the most common metabolic disorder in pregnancy and is of increasing concern in high-income settings around the globe but also in this resource-limited population.^{23 24} While the proportion of pregnant women diagnosed with underweight in this population is decreasing, 19.2% (73/381) of pregnancies were still complicated by a diagnosis of underweight.¹⁹ Maternal anaemia defined as haematocrit of less than 30% complicated 16.3% (62/381) of pregnancies and SGA (20.2% (77/381)) according to standards as published by the Intergrowth-21st consortium²⁵ was also commonly observed and remains a solicitude in this pregnant population. As shown in figure 3, cohort enrolment in early pregnancy, coupled with the high-frequency sampling provides a plethora of cross-omic data. This will enable to identify signatures preceding the development of these complications, their impact on pregnancy outcome and to characterise how they plateau in the postpartum period.

Ultralow capillary blood samples are safe and a reliable method to monitor longitudinal gene expression trajectories. Samples acquired from this MSP cohort were used to develop a modified SOP for extraction of RNA from small volume whole blood samples preserved in Tempus

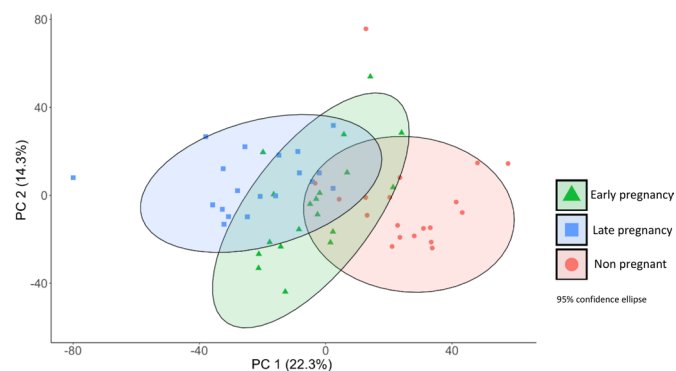


Figure 4 Principal component (PC) analysis of whole blood gene expression data (RNAseq) of 19 uneventful, term pregnancies of the MSP cohort compared between first trimester (early pregnancy), third trimester (late pregnancy) and 3-month post partum (non-pregnant). MSP, molecular signature in pregnancy.

solution²⁶ and the performance of ultralow blood samples was benchmarked previously.²⁷ Figure 4 shows a comparison between different stages in pregnancy (early pregnancy and late pregnancy), and the non-pregnant control (3-month post partum) by exploratory analysis of gene expression patterns (RNASeq) in 19 MSP women with uneventful, term pregnancies. The preliminary finding of clustering of different timepoints in pregnancy and post partum, respectively, follows patterns reported by Ngo *et al* who were able to predict EGA by analysing cell free RNA transcripts in maternal blood.²⁸

STRENGTHS AND LIMITATIONS

The major strength of this cohort is the prospective and the frequent follow-up along the trajectory from first trimester to 3 months post partum. Coupled with the availability of a large number of well characterised biological samples, the high-throughput analysis, in combination with clinical data, we anticipate that a number of pregnancy-related physiological and pathological changes can be investigated and described in detail. Systematic approaches enable the researchers to assess the entirety of a biological system and are thought to minimise the bias introduced through selective parameters.

Populations originating from low-resource settings do not often have the same level of access to advanced research when compared with high-income settings. While some data on intestinal microbiome are available,²⁹ data generated from the MSP cohort will characterise composition and perturbations of the vaginal microbiome for the first time in this population.

The small number of PTB was not anticipated and is lower than rates reported internationally. While a larger figure would be desirable from an analytical point of view, comparison of molecular markers and associated population-based factors to populations with a higher risk of PTB will provide novel insight for this low observed proportion. Generally, a selection and exclusion bias at participant enrolment might have contributed to the low number of PTB, as women with an unremarkable medical and obstetric history were given priority to minimise the risk of potential lost to follow-up and subsequent sample loss. Hence, inference on the power of the result cannot be made at this point, as the power will depend on the magnitude of observed differences in molecular signatures between term and preterm pregnancies.

More faecal samples were missed compared with capillary blood or vaginal swab samples. In an effort to reduce sample loss for potential future research, a nested project was conceived to assess whether collection and transfer of fresh faecal samples in the long-term storage tube at home is feasible, sample quality is affected, and acceptability of self-collection is favourable.

Larger cohorts would be preferable to address critical research questions such as PTB. However, with the high-frequency sampling and the overall cost of the study conduct and sample analysis, the researchers enrolled

not more participants than deemed to be necessary to address the objectives of this project.

COLLABORATION

Proposals for collaborations and other ideas to enhance scientific output from data generated in this cohort are welcome. SMRU is part of the Mahidol Oxford Tropical Medicine Research Unit and follows data sharing policies, as published by the Bioethics and Engagement Department. Data from the MSP cohort will be made available through the Data Access Committee at Mahidol Oxford Tropical Medicine Research Unit. Data sharing policies are available at: <http://www.tropmedres.ac/data-sharing>. An application form can be downloaded under this link. Gene expression data will be deposited on the NCBI Gene Expression Omnibus and data from 16S rRNA sequencing will be stored as a bioproject in NCBI for public Sequence Read archives.

Author affiliations

¹Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Mae Sot, Tak, Thailand

²Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK

³Swiss Tropical and Public Health Institute, Basel, Switzerland

⁴University of Basel, Basel, Switzerland

⁵Sidra Medicine, Doha, Qatar

⁶Mahidol Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

⁷Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

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Data availability statement Data are available on reasonable request. All data relevant to the study are included in the article. Deidentified data from the MSP cohort will be accessible through the Data Access Committee at Mahidol Oxford

Tropical Medicine Research Unit. Gene expression data and data from 16S rRNA sequencing will be deposited on public platforms.

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ORCID iDs

Tobias Brummaier <http://orcid.org/0000-0002-9739-2084>

Francois Nosten <http://orcid.org/0000-0002-7951-0745>

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