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The single-cell immune profile throughout gestation and its potential value for identifying women at risk for spontaneous preterm birth

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ABSTRACT

Precisely timed immune adaptations, observed in the maternal circulation, underpin the notion of an immune clock of human pregnancy that supports its successful progression and completion at delivery. This immune clock is divided into three immunological phases, with the first phase starting at the time of conception and implantation, shifting into the second phase that supports homeostasis and tolerance throughout pregnancy, and culminating in the last phase of labor and parturition. Disruptions of this immune clock are reported in pregnancy complications such as spontaneous preterm birth. However, our understanding of the immune clock preceding spontaneous preterm birth remains scattered. In this review, we describe the chronology of maternal immune cell adaptations during healthy pregnancies and highlight its disruption in spontaneous preterm birth. With a focus on single-cell cytometric, proteomic and transcriptomic approaches, we review recent studies of term and spontaneous preterm pregnancies and discuss the need for future prospective studies aimed at tracking pregnancies longitudinally on a multi-omic scale. Such studies will be critical in determining whether spontaneous preterm pregnancies progress at an accelerated pace or follow a preterm-intrinsic pattern when compared to those delivered at term.

Introduction

Premature delivery, a condition impacting 10 % of all pregnancies world-wide, is associated with severe adverse outcomes that affect neonatal and long-term health [1–3]. The majority of preterm birth cases (60–70 %) occurs after the spontaneous onset of premature labor with intact membranes or preterm prelabor rupture of membranes (PPROM), which are together considered as spontaneous preterm births (sPTB) [4]. The remaining 30–40 % are medically indicated preterm deliveries (i.e., iatrogenic) [4]. Spontaneous preterm birth is typically associated with intra-amniotic infection, sterile intra-amniotic inflammation, and a breakdown of maternal-fetal homeostasis [5]. However, for a significant subset of sPTB (20–30 %), the cause remains unidentified (*i.e.,* idiopathic) [1,4–6]. Hence, sPTB remains largely unpredictable due to a lack of reliable biomarkers and an incomplete understanding of the underlying pathobiological mechanisms [7].

In healthy pregnancies, precisely timed cellular immune dynamics can be observed in the peripheral blood over the course of pregnancy and have been proposed as a target for monitoring healthy pregnancy progression, including the onset of physiologic labor [8,9]. The term "immune clock of pregnancy" encompasses such systemic immune dynamics [8]. However, how the immune clock deviates in sPTB and to

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what extent immune dysregulation precedes sPTB remain areas that must be elucidated for the immune clock to be employed in predicting and monitoring sPTB. Deviations from healthy immune dynamics may manifest as a cascade of events that simply occurs earlier in pregnancies that will culminate in sPTB, *i.e.,* as an accelerated immune clock. Alternatively, these immune dysfunctions may follow a preterm-intrinsic pattern that deviates from term gestation.

The goal of this review is to summarize the current understanding of how the peripheral immune clock deviates in spontaneous preterm birth compared to uncomplicated term pregnancies. The contents of this review are systematically approached according to the three primary immunological phases - early pregnancy (first trimester), mid-pregnancy (from the second trimester until the early third trimester), and labor and delivery - to begin to define the immune clock of sPTB. We define the maternal immune clock here as measurements that assess the immune system directly through transcriptomic or cytometry studies of maternal immune cells, and indirectly via plasma/serum measurement of miRNA, proteins, and metabolites.

The maternal peripheral immune clock of pregnancy

To gain an understanding of immune dysfunctions associated with sPTB, we first describe the changes occurring in the maternal peripheral immune system during an uncomplicated, term pregnancy. Pregnancy is a defined period of dramatic physiological, hormonal, and metabolic alterations, which are paralleled by shifts in the maternal immune system [8,10–19]. The maternal-fetal interface dynamically alters its immune cell composition and function over the course of pregnancy (as reviewed extensively by PrabhuDas et al., Yang et al., Gomez-Lopez et al., and Green et al. [20–23]) to establish a local microenvironment conducive to decidualization, implantation, and placentation in early pregnancy, and to support the immunomodulatory processes required for maternal-fetal homeostasis and ultimately parturition [20,21,23–27]. The systemic immune clock of pregnancy is initiated by hormonal changes occurring during implantation and placentation, and is reinforced when spiral artery remodeling occurs in the human decidua at eight to ten weeks of gestation [28], allowing passage of fetal antigens and placental material, including cell-free fetal DNA and RNA, to the maternal circulation [29–34]. This physiologic change also coincides with an exponential increase in pregnancy-associated hormone concentrations in the circulation [35], which significantly contribute to functional immune adaptations [16,36]. Echoing the immune dynamics at the maternal-fetal interface, the peripheral immune clock of pregnancy can be divided into three immunological phases. The early phase of pregnancy, reflecting the first trimester [$< 13 + 6$ weeks gestational age (GA)], coincides with conception, implantation, and placentation. The second phase during mid-pregnancy (14–34 weeks GA) reflects a state of pregnancy homeostasis and tolerance. At the latter part of the mid-pregnancy phase, a shift towards preparation for parturition can be observed. The very last phase represents the acute process of labor and delivery. These dynamics, measured in the maternal blood, are well established and have been used to inform computational models to predict GA and time to the onset of labor [8–10,12,14,17,18,37,38].

Numerous studies have reported the specific modulation of individual cell types throughout gestation. For instance, pregnancy is a state of relative neutrophilia [39–41], likely exacerbated throughout gestation by a progressive delay in spontaneous neutrophil apoptosis [42]. Immature-like neutrophils progressively accumulate in the maternal circulation, peaking in the third trimester [43]. Moreover, neutrophils display an altered state of activation during pregnancy [40,44,45], which has been compared to that observed in patients with sepsis [46,47]. Monocytes, like neutrophils, increase in number and proportion throughout gestation [10], and their subpopulations shift towards increased proportions of the intermediate phenotype [40,48,49]. Type I interferon gene expression in monocytes spikes in very early pregnancy (approximately six to nine weeks), coinciding with the initial release of fetal material into the maternal circulation, before this signature disappears in the later part of the first trimester [10]. Circulating monocytes display increased expression of genes involved in inflammatory processes, migration, and aging from early to mid and mid to late gestation [50]. Circulating Natural Killer (NK) cells, another type of innate immune cell, progressively increase their expression of genes involved in responses to IFN and viral infection across pregnancy [10]. However, in the last 100 days of pregnancy, NK cell responses to IFNα stimulation are dampened with approaching labor [9]. Further, the proportion of innate-lymphoid cell (ILC) subtypes in the circulation changes throughout pregnancy, with ILC2 becoming the dominant subtype in late gestation [51].

Adaptive immune cell subsets demonstrate pronounced changes with progressing pregnancy. Transitional and B-1 cell frequencies decrease throughout pregnancy while plasmablasts increase [10,37,52]. Functionally, T and B cells overexpress alternative RNA splicing processes and downregulate canonical pathways of adaptive immunity [10]. Signatures of lymphocyte-mediated cytotoxicity gradually decrease over the course of pregnancy [50]. A classic, yet debated, T-cell paradigm describes pregnancy as a state of CD4 Th2 dominance over Th1 and Th17 responses [37,53]; this may be partially due to the enhanced activity of homeostatic cells, such as regulatory T-cells (Tregs) and macrophages, throughout the second phase of pregnancy [5]. Indeed, regulatory and antigen-specific T-cells increase in the maternal circulation as pregnancy progresses [54–56], and tolerogenic adaptations, such as enhanced IL-2-associated STAT5 phosphorylation in $CD4^+$ T-cell subsets, peak in the third trimester [8]. Yet, as labor approaches, the systemic T-cell signature becomes increasingly reflective of an inflammatory state, which is in line with the role of immune activation in this pregnancy phase [12,17]. Immune dynamics of term, and also preterm, labor culminate in an efflux of activated immune cells into the maternal-fetal interface [5,14,26].

Together, the above studies describing the immune clock of healthy pregnancy support the notion that tracking precisely timed, gestational age-dependent, and labor-specific immune dynamics in the maternal circulation may be used for monitoring of pregnancy progression to identify imbalanced or accelerated immune mechanisms indicative of a risk for sPTB.

Deviations in the maternal peripheral immune clock associated with spontaneous preterm birth

Deviations in the maternal immune clock that may drive sPTB could occur during any phase of pregnancy. During early pregnancy, such deviations could indicate a predisposition to undergo sPTB later in gestation, whereas deviations occurring during mid-pregnancy could indicate an earlier or accelerated parturition process. While early- and mid-pregnancy signatures would indicate immune deviations in individuals who are still asymptomatic, the observation of such signatures near or during active labor might indicate a distinct labor-specific pathology. Below, we describe the sPTB-associated immune deviations that have been identified so far during the different maternal immune clock phases (Fig. 1).

Peripheral immune profiles during spontaneous preterm labor

The immune profile of sPTB in the maternal circulation has been most extensively studied during the acute process of labor. In both women with term or preterm labor, the absolute numbers of peripheral monocytes and neutrophils are increased as assessed by clinical hematological analyses [57]. Circulating innate immune cell activation [8,9,12] and infiltration into local tissues [58–62] is a hallmark of both term and preterm labor. However, it is important to note that spontaneous preterm labor (sPTL) is not simply labor that begins prematurely, but likely represents a distinct pathological process that shares a common pathway with labor at term [12,14]. For example, sPTL involves a

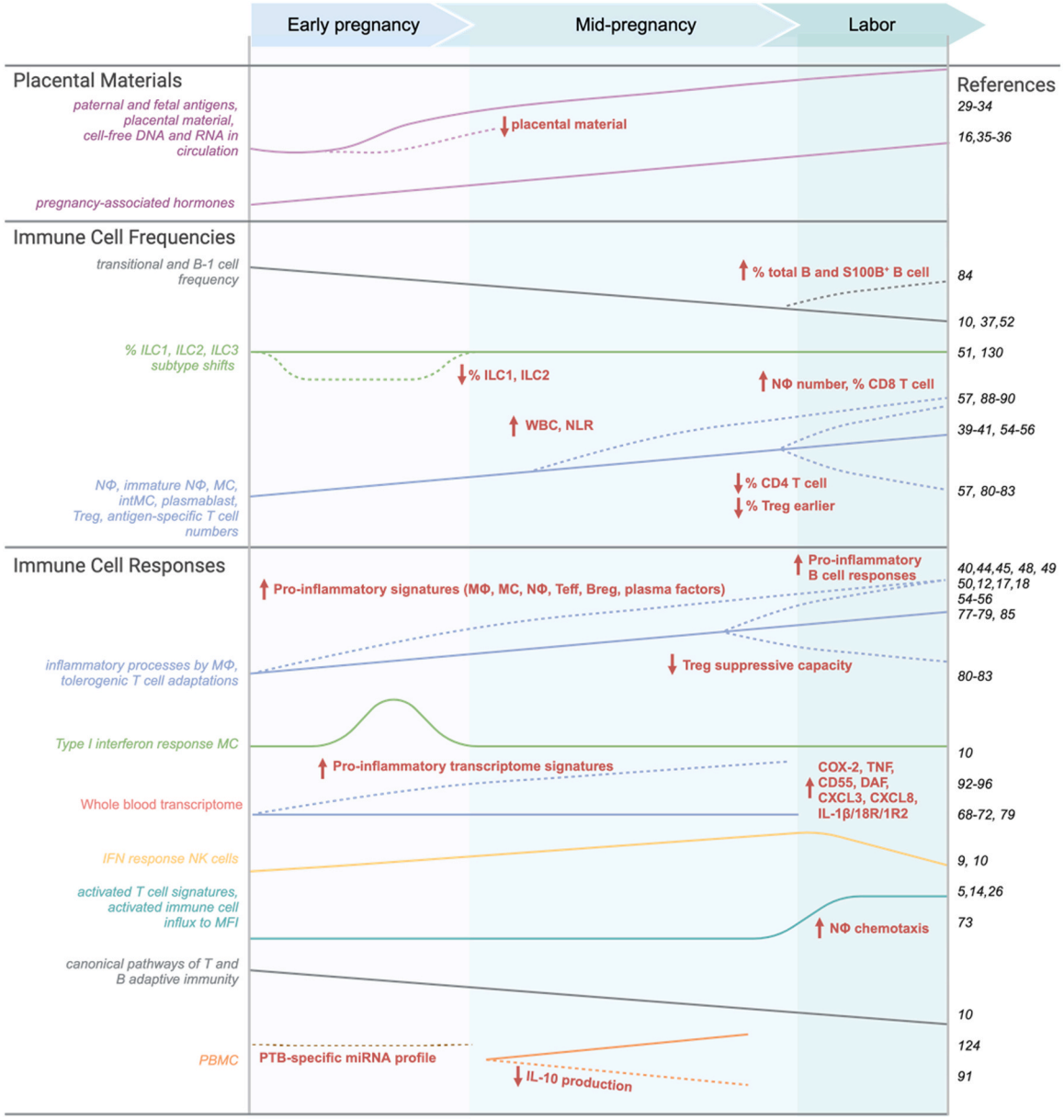

Fig. 1. The immune clock of human pregnancy in uncomplicated and spontaneous preterm birth pregnancies. Solid lines reflect immune dynamics observed in uncomplicated pregnancy, while bold text in red and dotted lines indicate observations made in pregnancies affected by spontaneous preterm birth. Scarcity of studies on single-cell immune profiles throughout preterm pregnancies, as displayed in this overview, currently limit the opportunity to leverage these dynamics for sPTB prediction. *ILC: innate lymphoid cell, Breg: regulatory B cell, WBC: white blood cell, NLR: neutrophil-to-lymphocyte ratio, NΦ: neutrophil, MΦ: macrophage, MC: monocyte, intMC: intermediate monocyte, Treg: $CD4^+$ regulatory T cell, Teff: T effector cell, NK: Natural Killer cell, MFI: maternal-fetal interface, PBMC: peripheral blood mononuclear cell, IL: interleukin.*

breakdown of maternal-fetal tolerance mediated by enhanced pro-inflammatory effector T-cell and macrophage responses that can be distinct from those observed in term labor [5,26,63–66]. Moreover, these local processes of sPTL are reflected in the peripheral maternal blood immune profile [17,18,67–69].

In immune profiling studies, sPTL has been compared to: (1) threatened PTL (regular premature uterine contractions); (2) PPROM; (3) immune profiles of healthy pregnancies carrying to term matched for preterm GA at time of sampling; (4) immune profiles at term in labor (TL); or (5) at term not in labor (TNL, *i.e.,* elective C-section). One study carefully curated cases in four conditions (sPTL, preterm no labor [PTNL/mainly iatrogenic non-labor PTB], TL, and TNL) and performed gene expression profiling of five tissues (chorion, amnion, placenta/villi, decidua, myometrium) as well as maternal and fetal blood [69]. A unique local uterine signature of immune activation, mainly associated with infection and inflammation, was overrepresented in PTL and not found in any of the other conditions shortly before delivery [69]. The maternal blood reflected these transcriptomic changes occurring with delivery although it showed the lowest proportion of substantial transcript changes (>1 or 2 standard deviations) when compared with local tissues [*e.g.,* decidua, chorion, and amnion (Bukowski et al. [69], meta-analyzed in Vora et al. [68]].

It is important to consider the different comparators and confounding variables across studies when synthesizing a peripheral immune profile of sPTL. sPTL cases are routinely compared either to spontaneous labor at term (sTL) or to non-laboring gestational-age matched samples. The comparison between sPTL and sTL is confounded by the difference in GA. The comparison of sPTL to any non-laboring gestational-age

matched sample is confounded by the absence of labor. As such, an optimal control group for sPTL does not exist. Below, we summarize evidence of preterm signatures in the major immune cell subsets according to the available comparator groups that have been employed in different studies.

Compared with term labor cases, studies report an exaggerated inflammatory response accompanying PTL, in which the immune balance of PTL is shifted towards an innate-dominant transcriptomic blood signature with corresponding dampened adaptive immunity [68]. Maternal whole blood Cyclooxygenase (COX)-2 and tumor necrosis factor (TNF) mRNA expression is elevated in sPTL compared to TL [70]. Moreover, peripheral blood leukocytes express higher mRNA levels of CD55, an activation marker and negative regulator of membrane complex formation, in idiopathic or infection-associated PTL relative to non-PTL controls, although it was not reported whether controls were GA-matched or term labor cases [71].

Compared with preterm cases not in labor, women with late PTL (34–37 weeks GA) show increased gene expression of Decay-Accelerating Factor (DAF), an inhibitor of the complement pathway, among their peripheral leukocytes, suggesting dampening of this inflammatory pathway [72]. Peripheral blood neutrophils of women with PTL exhibit enhanced migratory capacity towards a fetal membrane substrate compared with neutrophils taken prior to the active process of labor from women with PPROM or TNL deliveries [73]. Moreover, PTL-derived neutrophils show enhanced activation and adhesion marker expression, Toll Like Receptor (TLR)-4 expression, and increased capacity for oxidative burst following stimulation compared to GA-matched healthy cases [74,75]. Neutrophil numbers have been proposed as a prognostic marker for PTB through calculation of the neutrophil-lymphocyte ratio (NLR), which is significantly elevated during mid-to-late gestation in pregnancies culminating in sPTL and PTB [76].

Peripheral blood monocytes from PTL cases are potent reactive oxygen species (ROS) producers when stimulated compared with monocytes from preterm non-laboring women [57,74]. More $CD14^+$ monocytes expressed TLR4 and at a higher abundance per cell in PTL cases compared with GA-matched non-laboring controls [77]. The monocyte gene expression profile in sPTL compared with GA-matched non-laboring pregnancies revealed an enrichment of miRNA target genes involved in 'positive regulation of Interleukin (IL)-2 production', 'positive regulation of mononuclear cell proliferation', and 'negative regulation of protein kinase activity' [78]. Moreover, the RNA expression of C-X-C-motif ligand 3 (CXCL3), CXCL8, Interleukin (IL)-1β, IL-18R, and IL-1R2 in both whole peripheral blood and peripheral monocytes was positively correlated with PTL [79]. Such observations are reaffirmed by the reported enrichment of gene expression signatures from macrophages and monocytes in sPTL compared with other immune subsets [12].

Among the adaptive immune cell subsets, absolute lymphocyte numbers remain stable, however, their proportions are decreased relative to increased neutrophil and monocyte numbers in labor cases compared to cases not in labor, regardless of gestational age at birth [57]. Within the T-cell compartment, one study reported a PTL-specific shift towards increased $CD8^+$ and decreased $CD4^+$ circulating T-cell frequencies [57]. Despite altered frequencies, an activated T-cell gene expression signature is enhanced in PTL compared to GA-matched non-laboring pregnancies [12]. Idiopathic or chorioamnionitis-associated PTL at 28–36 weeks of gestation is associated with decreased proportions of $CD4^+CD25^+FoxP3^+$ Tregs in the circulation compared to GA-matched controls or threatened PTL cases (regular uterine contractions) [80]. However, Treg proportions from sPTL cases resemble those from TL cases, supporting the notion of an immune regulatory shift that occurs as part of the common pathway of parturition [80]. This observation is further reinforced by the correlation of decreasing $CD4^+CD25^{int/hi}CD127^{lo}$ Treg proportions with a shorter time to delivery interval in patients with cervical insufficiency and/or sPTL [81]. Phenotypically, $Foxp3^+$ Tregs from PTL cases (occurring between 24 and 36 GA) show decreased HLA-DR expression and suppressive capacity relative to cases prior to active labor (PPROM) and GA-matched term controls [82,83], potentially indicating altered function. Together, this indicates an association of Treg phenotype and function with the state of labor.

Fewer studies on circulating B cells in sPTL are available. Significantly increased total B cell and $S100B^+$ B cell frequencies can be observed in sPTB cases (preterm, laboring) compared to TNL (term, non-laboring) controls [84]. A significantly higher maternal anti-HLA class I seropositivity was noted in sPTL at 34 weeks GA compared with PPROM cases and normal term deliveries with or without labor [85].

While changes in the profile of circulating NK cells are associated with pregnancy complications such as preeclampsia, recurrent pregnancy loss, and implantation failure [86], their role in PTB is less clear. Peripheral NK cell proportions increase in both preterm or term labor compared to cases without labor [57], and, together with other leukocytes, NK cells are recruited to the human chorioamniotic membranes in term labor [62]. Consistent with these observations, an NK cell signature was reported to diminish in mid-gestation and then peak as the end of pregnancy approaches [12]. Moreover, this signature was significantly increased in TL cases compared to TNL; however, such a change was not reported in sPTL cases [12]. A definitive role for NK cells in preterm birth remains to be fully demonstrated.

Taken together, these studies of the immune signature of sPTB at time of labor and delivery indicate a general loss of immune regulation, including in innate immune cells, resulting in an enhanced pro-inflammatory state.

Peripheral immune profiles preceding spontaneous preterm birth during mid-pregnancy (2nd and first half of 3rd trimester)

To computationally identify biomarker profiles of preterm birth in individuals without any sPTB symptoms during mid-pregnancy, peripheral maternal blood immune profiles of sPTB obtained at the time of diagnosis [17,18,67–69] have been successfully mapped onto maternal blood obtained during the second trimester [12,14,19,68]. This suggests that mechanisms of labor may be detectable in the maternal blood before the onset of preterm labor symptoms. In other words, there is computational support for the notion that in mid-pregnancy, sPTB immune profiles can be expected to be distinguishable from healthy mid-pregnancy term pregnancies. Indeed, Stelzer et al. have shown that maternal proteome, metabolome, and immune signatures assessed during the last 100 days of pregnancy are able to predict the time to onset of labor in both term and a small number of preterm labor cases [9]. Similarly, another study found mid-trimester proteomic biomarkers in serum to be correlated with time to delivery in sPTB cases [87]. These studies suggest that regulation of the maternal immune clock, particularly the shift from increased immune activation to dampened immune responsiveness in innate immune populations four weeks before the onset of labor, is at least partially shared between term and preterm labor [9]. As such, measurements during the second phase of pregnancy can uncover an earlier or accelerated parturition process in asymptomatic pregnant individuals who end up delivering preterm. For instance, significantly increased white blood cell (WBC) counts and neutrophil-to-lymphocyte ratio (NLR) together with lower whole blood lymphocyte counts and lymphocyte-to-monocyte ratio (LMR) can be detected during the second to third trimester (17–32 weeks GA) in asymptomatic pregnant individuals who will deliver preterm compared to pregnant individuals who deliver at term [88–90]. This indicates a shift towards increased innate immunity and systemic inflammation during mid-pregnancy. These changes in maternal blood cell counts were detected on average 70 days prior to hospitalization for the study by Ma et al. and four weeks for Park et al., and were able to predict the risk of sPTB. In parallel, PBMC-derived IL-10, induced by *in vitro* LPS stimulation, decreased from 16 to 22 weeks GA to 25–28 weeks GA in

pregnant individuals who delivered before 35 weeks GA, while IL-10 production increased in those who delivered at term, indicating a potential dysfunction of homeostatic processes in preterm cases [91].

The mid-pregnancy shift towards inflammation in asymptomatic PTB cases has also been reported by transcriptomic studies. Maternal whole blood transcriptome signals, collected between 14 and 36 weeks of gestation and binned by GA, show that inflammatory transcripts are upregulated in asymptomatic individuals who will undergo sPTB compared to term delivery [92,93]. Gene set enrichment of differentially expressed genes from sPTB vs. term delivery identified an immune signature (PPARG1-FOXP3 gene set) associated with sPTB at 20 weeks GA [94]. Another transcriptomic study, reanalyzing Heng et al., 2016 data, tested whether genetic variants previously associated with GA and PTB were different in terms of their peripheral blood expression [95]. They found that early B cell factor 1 (EBF1) and selenocysteine-tRNA-specific eukaryotic elongation factor (EEFSEC) were downregulated in sPTB pregnancies collected between 17 and 23 weeks' GA. Similarly, the expression of genes involved in the inflammatory response (e.g., B2M, RUNX3, TLR4, CXCR3 TLR2, and IL10) measured before 28 weeks GA contributed to the prediction sPTB (<34 weeks) and correlated with their potential miRNA regulators [96].

The maternal soluble profile in mid-pregnancy, i.e., serum/plasma analytes during the second trimester, has received much attention when searching for predictive biomarkers. Across these studies, the general theme of an increased inflammatory environment before the onset of symptoms culminating in sPTB has been repeatedly demonstrated. This environment is characterized by elevated levels of cytokines [97,98], growth factors and enzymes (e.g., TGFβ, G-CSF, MMP-9, ITIH4) [99–102], and complement factors (e.g., C5a, thrombin antithrombin) [87,103]. G-CSF and thrombin-antithrombin remain associated with sPTB at 28 weeks gestation, i.e., the beginning of the third trimester [104,105]. C-reactive protein (CRP) levels are increased in the first trimester and remain elevated in the second trimester in sPTB cases [106–109]. However, the inflammatory profile is accompanied by changes in other systems such as serum eicosanoid lipid profiles [109], alkane metabolites such as undecane [110], and proteins involved in metabolism and endocrine function, such as insulin-like growth factor-binding protein 4 (IBP4), sex hormone-binding globulin (SHBG), and corticotropin-releasing hormone (CRH) [87,101]. CRH levels, suggested as an indicator of the placental clock of pregnancy [111] and enabling the neuroendocrine communication between placenta and brain, have been tested in multiple studies and found to be associated with sPTB in the first half of the second trimester [112,113] and in the early third trimester [114]. Yet, CRH failed to predict sPTB in the second trimester in high-risk recurrent sPTB patients [115]. Standard blood lipid measurements show LDL, HDL, and cholesterol concentrations to be associated with sPTB, pointing towards metabolic imbalances in mid-pregnancy [7].

Plasma miRNA profiles at 20–24 weeks GA in sPTB cases are profoundly different from term pregnancies, with specific miRNAs being completely absent in sPTB [116] or clustering in a PTB- and chromosome-specific profile, suggesting co-regulated expression [117]. More specifically, slight differences in miRNA cargo were observed in placental exosomes at 15–18 weeks GA in sPTB cases, as compared to term pregnancies [118]. These results are linked to the general dysregulation of miRNA profiles analyzed over the course of preterm pregnancies [119].

Last, integrating known clinical risk factors and local cervical indicators of PTB risk improves the identification of sPTB cases: measuring cervicovaginal IL-6 and serum ferritin, TNF, α-fetoprotein, alkaline phosphatase, or G-CSF together with cervical length or fetal fibronectin enhances the clinical ability to predict sPTB [120–123].

Overall, the involvement of multiple biological modalities that interact with immune cell function, as measurable in the maternal circulation, imply that preterm birth is preceded by differential adaptations to pregnancy that extend beyond immune dysregulation.

Peripheral immune profiles preceding spontaneous preterm birth in early pregnancy (first trimester)

Measurements of the immune system obtained at the start of pregnancy offer the promise of identifying early and actionable biomarkers for estimating the risk for sPTB. Most first trimester PTB studies to date have identified plasma/serum markers to screen for preterm birth signatures, while three studies have measured cellular attributes in the first trimester maternal blood [7]. First trimester miRNA profiles expressed in PBMC proved to be predictive of sPTB before 35 weeks GA [124]. These miRNAs regulate genes that are enriched in pathways related to transcription, membrane trafficking, the circadian clock, and estrogen signaling - all basic processes of cellular functionality. Further, whole genome RNA expression analyses of blood via microarray were most predictive of sPTB when conducted in the first trimester compared to the second or third. Such prediction was based on an enhanced IL-6 gene expression signature, suggesting an inflammation-skewed environment present from the outset of pregnancy [93]. This is in line with IL-6 being the most potent biomarker of intra-amniotic inflammation associated with preterm labor when measured in the amniotic fluid [125–128], while a similar correlation has been reported between intra-amniotic inflammation and maternal plasma IL-6 [129]. Lastly, decreased frequencies of all three types of innate lymphoid cells (ILC) were observed in the circulation of women in the first trimester who will experience sPTB [130], suggesting an increased recruitment to the maternal-fetal interface where they might be contributing to pathological processes, culminating in the increased ILC frequencies that have been reported at time of delivery [131].

To broaden the context around these isolated cellular measurements, it is insightful to look at the soluble mediator profile of sPTB, which has been found to be different from term pregnancies. Immune activating and inflammatory markers in first trimester serum are associated with PTB [132] together with complement cascade components [133–135], phospho- and glycoproteome components [136], and specifically G-CSF, MIF, IL-1β, IL-6, IL-10, MMP-9, and VCAM-1 [105,137–140]. However, other studies do not report a specific cytokine or progesterone-induced blocking factor (PIBF) dysregulation early in pregnancy [108, 141–143] or found them to only be present in the context of extreme maternal body-mass-indices (BMI) [98]. Increased levels of plasma CRP, a circulating indicator of inflammation, during early gestation were associated with sPTB by some studies [137,144], while others found no difference [108,141,145].

The amount of cell-free maternal/fetal DNA in the first trimester maternal circulation was not different between healthy and pathologic pregnancies [146]; however, the profile of miRNA expression in plasma exosomes during early pregnancy was drastically different between sPTB and term pregnancies and continued with dynamically different trajectories until delivery, with TGFβ, p53, and glucocorticoid receptor signaling among the differentially expressed processes compared to term pregnancies [119]. Furthermore, circulating metabolites found to be different in early sPTB pregnancies belonged to pathways of inflammation-related tryptophan metabolism [142] and Treg-mediated modulation of antigen-presenting cell functions [147]. Lastly, circulating markers released by the placenta but not linked with inflammation per se, such as pregnancy-associated plasma protein-A (PAPP-A), and markers pointing towards imbalances in immune-modulating metabolism, such as lipid markers linked to insulin, have been associated with preterm birth when measured during the first trimester [7].

Thus, despite numerous advances in characterizing the systemic signature of sPTB in the first trimester of pregnancy, there remains a need for comprehensive, high-dimensional approaches that prospectively follow pregnancies until delivery to identify reliable biomarkers that can then be monitored during early pregnancy to evaluate sPTB risk.

Conclusion and considerations for future research assessments

Our understanding of peripheral immune clock deviations tracking with spontaneous preterm birth is incomplete. Current knowledge is insufficient for delineating the precise timeline of immunological events preceding sPTB, given that: (1) systemic studies at the cellular level are still few; (2) the majority of current studies perform cross-sectional analyses of data collected over relatively short or longer (masking temporal dynamics) time frames within a trimester; and (3) longitudinal data (or analyses of such) are critically lacking. Thus, whether spontaneous preterm pregnancies progress at an accelerated pace or follow a preterm-intrinsic pattern when compared to term pregnancies cannot be discerned based on currently existing data. Future prospective studies aimed at tracking pregnancies longitudinally will be critical to identifying early and actionable biomarkers and therapeutic candidates for sPTB risk assessment and treatment.

Inflammation that is distinct from the inflammatory signatures of term delivery is associated with spontaneous preterm labor at delivery. Yet, it remains unclear whether a canonical inflammatory profile occurs in all phenotypes of this syndrome before the onset of symptoms, as the changes in lipid metabolism, endocrine factors, and placental miRNA cargo described above all suggest multifocal involvement in the pathophysiology of preterm birth. It is imperative to consider that preterm birth is a syndrome with a range of underlying pathophysiology. Available studies do not always stratify between known etiologies of sPTB, which may include intraamniotic infection, sterile inflammation, or fetal immune activation [1,5]. This limitation may explain discrepancies of sPTB-associated markers between studies, hindering the description of an immune profile preceding sPTB and identification of reliable biomarkers. One example where the peripheral immune clock profile is impacted by preterm birth etiologies are cases with acute histologic chorioamnionitis that present with a more pronounced inflammatory phenotype compared to cases without, which is also dependent on factors such as GA or maternal weight [97,106]. Another example from a recent single-cell study suggests that placental signatures detectable in the maternal circulation of pregnancies who ultimately undergo sPTL or PPROM involve both shared and distinct signatures, depending on the sPTB subset [14]. These examples highlight the necessity for reporting and distinguishing sPTB subsets, whose specific underlying mechanisms may precipitate in differing immune profiles. In addition, while maternal blood is an easily accessible sample, monitoring local processes, such as the cervicovaginal environment including cervical parameters, can yield additional puzzle pieces to elucidate biomarkers of the immune clock of preterm pregnancy. This strategy promises to lead to clinical improvements [120–123]. Similarly, longitudinal studies which track not just clinical outcome(s), but also placental gross and histologic pathology in a systematic manner will yield significantly more insights into the underlying processes of sPTB at the maternal-fetal interface, potentially reflected and measurable in the maternal peripheral circulation [148–150]. Lastly, going forward, assessing the risk for sPTB and tracking its immune clock will require the consideration of other pre-pregnancy risk factors, such as BMI and nutritional status [151], stress perception [152], physical activity [153], microbiome [154], and (epi)genetics [155]. This will determine the extent to which the immune system can serve as the sole diagnostic or key therapeutic target in the early identification of asymptomatic pregnancies at risk of preterm birth.

CRediT authorship contribution statement

Diop Maïgane: Writing – review & editing, Visualization, Data curation. **Stelzer Ina:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Investigation, Data curation, Conceptualization. **Feyaerts Dorien:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Investigation, Data curation, Conceptualization. **Gomez-Lopez Nardhy:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Prins Jelmer R.:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Gyamfi-Bannerman Cynthia:** Writing – review & editing, Data curation. **Parast Mana:** Writing – review & editing, Data curation. **Winn Virginia D.:** Writing – review & editing, Data curation. **Diemert Anke:** Writing – review & editing, Data curation. **Arck Petra C.:** Writing – review & editing, Data curation. **Einhaus Jakob F.:** Writing – review & editing, Visualization, Data curation. **Galaz Jose:** Writing – review & editing, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that no conflicts of interest exist.

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