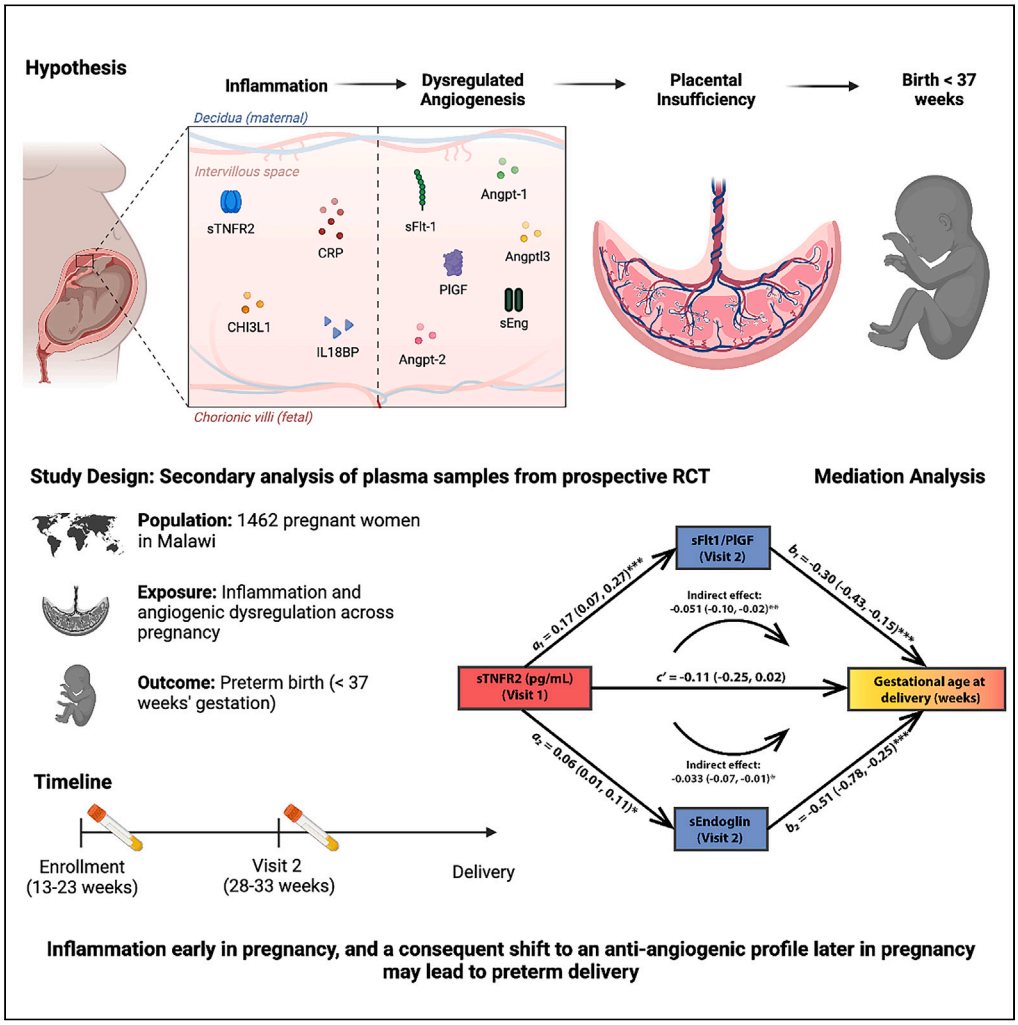
iScience

CellPress
OPEN ACCESS

Article

Sequential disruptions to inflammatory and angiogenic pathways and risk of spontaneous preterm birth in Malawian women



Andrea M. Weckman, Robyn E. Elphinstone, John M. Ssenkusu, ..., Feiko O. Ter Kuile, Chloe R. McDonald, Kevin C. Kain

kevin.kain@uhn.ca

Highlights

Inflammatory and angiogenic pathways were evaluated across pregnancy

Early inflammation and subsequent angiogenic dysregulation were linked to preterm birth

The pathobiology of preterm birth may be triggered early in pregnancy

Weckman et al., iScience 26, 106912
June 16, 2023 https://doi.org/10.1016/j.isci.2023.106912

iScience

CellPress
OPEN ACCESS

Article

Sequential disruptions to inflammatory and angiogenic pathways and risk of spontaneous preterm birth in Malawian women

Andrea M. Weckman,[1,2] Robyn E. Elphinstone,[2] John M. Ssenkusu,[3] Vanessa Tran,[1,2] Kathleen Zhong,[2] Mwayiwawo Madanitsa,[4] Carole Khairallah,[5] Linda Kalilani-Phiri,[4] Victor Mwapasa,[4] Andrea L. Conroy,[6] Feiko O. Ter Kuile,[5] Chloe R. McDonald,[2] and Kevin C. Kain[1,2,7,8,9,*]

SUMMARY

Preterm birth is a leading cause of death in children under five years of age. We hypothesized that sequential disruptions to inflammatory and angiogenic pathways during pregnancy increase the risk of placental insufficiency and spontaneous preterm labor and delivery. We conducted a secondary analysis of inflammatory and angiogenic analytes measured in plasma samples collected across pregnancy from 1462 Malawian women. Women with concentrations of the inflammatory markers sTNFR2, CHI3L1, and IL18BP in the highest quartile before 24 weeks gestation and women with anti-angiogenic factors sEndoglin and sFlt-1/PlGF ratio in the highest quartile at 28–33 weeks gestation had an increased relative risk of preterm birth. Mediation analysis further supported a potential causal link between early inflammation, subsequent angiogenic dysregulation detrimental to placental vascular development, and earlier gestational age at delivery. Interventions designed to reduce the burden of preterm birth may need to be implemented before 24 weeks of gestation.

INTRODUCTION

Preterm birth (PTB; <37 weeks gestation) and its complications are the most common cause of death in children under-five.[1] An estimated 14.9 million babies are born preterm each year, and almost one million die in the first 28 days of life from direct complications of PTB.[1,2] In those who survive their first month, PTB is associated with an increased risk of chronic conditions including visual, hearing, and respiratory impairments, neurodevelopmental disorders, and other chronic diseases (e.g. diabetes, chronic kidney disease, and cardiovascular disease).[3] PTB is associated with reduced nephron numbers at birth and places infants at increased risk of neonatal kidney injury and chronic kidney disease later in life.[4] Furthermore, females who are born preterm are at future risk of delivering preterm, contributing to an intergenerational risk of prematurity, hypertension, and kidney disease.[4] Even infants born near term (late preterm, 35–37 weeks' gestation) are at increased risk for short and long-term complications (e.g., respiratory distress, sepsis, infections, neurodevelopmental delays, and increased mortality), especially in low- and middle-income countries (LMIC) where resources for comprehensive postnatal care are most scarce.[5,6]

The global burden of PTB is not equally distributed. Approximately 80% of all PTB occurs in LMIC in Asia and sub-Saharan Africa.[7] Malawi has reported the highest rates of PTB worldwide, with an estimated 18.1% of all babies born preterm.[2] Many known risk factors for PTB are common in LMICs, including infectious diseases (e.g. HIV-1, sexually transmitted infections, and malaria), maternal malnutrition, lack of access to family planning and antenatal care, and undiagnosed clinical conditions;[8,9] however, a direct cause is not identified for up to 50% of all spontaneous PTB.[2] Even when risk factors are identified, it is poorly understood how they contribute to PTB.

A detailed understanding of the mechanistic pathways underlying spontaneous PTB is necessary to reduce prematurity, especially in resource-constrained settings where the burden of disease is the greatest. Healthy birth outcomes require appropriate regulation of inflammatory and angiogenic pathways and

[1]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada

[2]SAR Laboratories, Sandra Rotman Centre for Global Health, University Health Network-Toronto General Hospital, Toronto, ON, Canada

[3]Department of Epidemiology and Biostatistics, School of Public Health, Makerere University, Kampala, Uganda

[4]College of Medicine, University of Malawi, Blantyre, Malawi

[5]Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK

[6]Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, USA

[7]Tropical Disease Unit, Division of Infectious Diseases, Department of Medicine, University of Toronto, Toronto, ON, Canada

[8]Toronto General Research Institute, University Health Network-Toronto General Hospital, Toronto, ON, Canada

[9]Lead contact

*Correspondence: kevin.kain@uhn.ca

https://doi.org/10.1016/j.isci.2023.106912

several lines of evidence support a role for dysregulation of this axis in the pathobiology of PTB and other adverse birth outcomes.[10–15] The development of a robust placental vascular network is necessary to support the rapidly growing fetus. Dysregulation of mediators of angiogenesis (e.g. soluble endoglin [sEng], placental growth factor [PlGF], and soluble receptor soluble Fms-like tyrosine kinase-1 [sFlt-1]) has been associated with compromised placental vascular development and re-modeling and adverse birth outcomes including PTB.[10,14–20] The immune system modulates angiogenesis and vice versa, via crosstalk between components of each system.[21,22] In a case-control study of pregnant women in Malawi, structural equation modeling indicated a hierarchical relationship between immune activation, dysregulated angiogenesis, placental dysfunction, and adverse birth outcomes.[19] However, that study was cross-sectional at delivery and could not assess temporal relationships.[19] Collectively, these data support a mechanistic model of spontaneous PTB whereby maternal systemic inflammation alters closely regulated angiogenic pathways, leading to placental insufficiency and PTB.

Understanding the longitudinal kinetics of mediators involved in the pathobiology of PTB could facilitate both risk stratification of pregnant women and inform potential intervention strategies. Inflammation is a well-studied risk factor for PTB. Most studies have investigated pro-inflammatory responses in the induction of preterm labor (i.e., at delivery);[11,12] however, existing evidence suggests that inflammation could disrupt angiogenic mediators and placental vascular development earlier in gestation than typically studied.[14,19] We hypothesize that disruption of placental vascularization (via dysregulated angiogenesis) as a result of early pregnancy inflammation is a common pathway leading to PTB. Here, we use mediation analysis to examine the longitudinal dynamics of inflammatory and angiogenic mediators in a large cohort of Malawian women in a setting with a high burden of PTB.

RESULTS

Study population

Our study population consisted of 1462 pregnant women, with 286 (19.6%) preterm deliveries (Table 1; Figure 1). A total of 2392 plasma samples were analyzed (Figure 1). Of the preterm deliveries, 94.8% delivered moderate to late preterm (32 to <37 weeks gestation), and 5.2% delivered very preterm (<32 weeks gestation) (Table 1). Women who delivered preterm had a later gestational age at enrollment in the trial (21.3 weeks vs. 19.7 weeks, $p < 0.001$) and were more often primigravid (38.8% vs. 32.5%, $p = 0.050$) than those who delivered at term (Table 1). There was no difference in small-for-gestational age outcome between preterm and term babies (8.2% vs. 11.6%, $p = 0.132$); however, babies born preterm had a lower birth weight (2600 g vs. 3000 g, $p < 0.001$) than babies born at term in our cohort (Table 1). There was no difference in maternal age, BMI, hemoglobin, or blood pressure at enrollment, level of education, or socioeconomic status between term and preterm pregnancies.

Women who delivered preterm had altered levels of angiogenic and inflammatory factors at each visit

Compared to women who delivered at term, women who delivered preterm had elevated concentrations of PlGF, sTNFR2, and IL18BP at enrollment ($p < 0.001$); and higher circulating sEng and sFlt-1, and reduced PlGF at visit 2 ($p < 0.001$; Table 2). At enrollment, women who went on to deliver very preterm (<32 weeks) displayed different angiogenic and inflammatory profiles compared to women who delivered moderate to late PTB (32 to <37 weeks) or at term (Table S4). Women who delivered very preterm had a higher median concentration of Angpt-2 ($p = 0.05$; Table S4) and lower concentrations of Angpt-1 ($p < 0.05$; Table S4) at enrollment than women who delivered moderate to late preterm or at term. Angiogenic and inflammatory factors did not vary by treatment arm at enrollment or visit 2 (Table S5).

Inflammation early in pregnancy and an anti-angiogenic profile later in pregnancy are associated with an increased risk of PTB

We examined the association between angiogenic and inflammatory analytes and PTB by multivariate analysis at each gestational visit (Figure 2). At enrollment (13–23 weeks gestation), women in the highest quartile for pro-inflammatory markers had an increased relative risk of PTB compared to women in the lowest quartile for sTNFR2 [adjusted relative risk (aRR) = 1.60 (95% CI: 1.14, 2.27), $p = 0.007$], IL18BP [aRR = 1.58 (95% CI: 1.12, 2.25), $p = 0.010$], and CHI3L1 [aRR = 1.42 (95% CI: 1.05, 1.93), $p = 0.025$] (Figure 2). In early third trimester (28–33 weeks gestation), women with concentrations of anti-angiogenic mediators in the highest quartile had an increased relative risk of PTB for the ratio of sFlt-1/PlGF

Table 1. Clinical characteristics of cohort

	Birth Outcome[a,b]		
Variable	Term (n = 1176)	PTB (n = 286)	p value[a,b]
Treatment Group			>0.999
ISTp	582 (49.5)	142 (49.7)	
IPTp	594 (50.5)	144 (50.3)	
Gestational age at enrollment (weeks)	19.7 (18.0, 21.6)	21.3 (19.7, 22.4)	**<0.001**
Gestational age at delivery (weeks)	38.9 (38.0, 39.9)	36.0 (35.1, 36.4)	**<0.001**
Maternal age (years)	21 (19, 25)	21 (18, 26)	0.283
Body Mass Index (kg/m^2)	22.8 (21.2, 24.8)	22.6 (21.2, 24.4)	0.228
Hemoglobin at enrollment (g/dL)	11.2 (10.2, 12.1)	11.1 (9.8, 12.0)	0.096
Blood pressure at enrollment (mmHg)			
Systolic	110 (104, 118)	110 (104, 119)	0.887
Diastolic	66 (60, 72)	65.5 (60, 72)	0.832
Blood pressure at delivery (mmHg)			
Systolic	111 (106, 118)	111 (106.25, 118)	0.921
Diastolic	70 (62, 76)	66 (61, 75)	**0.012**
Socioeconomic Status			0.768
Low	392 (33.4)	100 (35.0)	
Middle	395 (33.7)	91 (31.8)	
High	386 (32.9)	95 (33.2)	
Educational Status			0.174
Low	364 (31.0)	71 (24.8)	
Medium	626 (53.4)	166 (58.0)	
High	183 (15.6)	49 (17.1)	
Antenatal Malaria			
Any PCR+ throughout pregnancy	727 (61.0)	193 (67.9)	0.074
PCR+ before 24 weeks gestation	457 (42.6)	136 (51.3)	**0.013**
Primigravid	382 (32.5)	111 (38.8)	**0.050**
Neonatal Sex (female)	589 (50.1)	150 (52.6)	0.095
Birth Weight (g)	3000 (2785, 3200)	2600 (2400, 2900)	**<0.001**
Small-for-Gestational Age[c]	132 (11.6)	22 (8.2)	0.132
Preterm birth[d]			
Moderate to late preterm (32 to <37 weeks)	–	271 (94.8)	
Very preterm (28 to <32 weeks)	–	13 (4.5)	
Extremely preterm (<28 weeks)		2 (0.7)	

[a]Median [IQR], Mann-Whitney. Number (%), Pearson's Chi-Squared.
[b]BMI and hemoglobin data available for n = 1364 (missing 9%). Sex was undetermined for one neonate (0.07%). Gestational age at enrollment (n = 1416; missing 5.5%), PCR status (any, n = 1456 [missing 0.4%]; <24 weeks gestation, n = 1337 [missing 8.5%]), socioeconomic and education status (n = 1459; missing 0.2%), blood pressure at delivery (n = 1381; missing 5.5%), small-for-gestational age (n = 1407; missing 3.8%), and birth weight (n = 1407; missing 3.8%) are also missing data.
[c]Defined according to parent trial [19].
[d]Defined according to current WHO guidelines [https://www.who.int/news-room/fact-sheets/detail/preterm-birth]. The majority of preterm deliveries (n = 250; 87%) occurred after visit 2 (34 to <37 weeks).

[aRR = 3.10 (95% CI: 1.97, 5.08), p < 0.001] and sEng [aRR = 2.17 (95% CI: 1.45, 3.42), p < 0.001] (Figure 2). The study was underpowered to analyze the relative risk of very early (n = 15) versus moderate-late preterm birth by analyte.

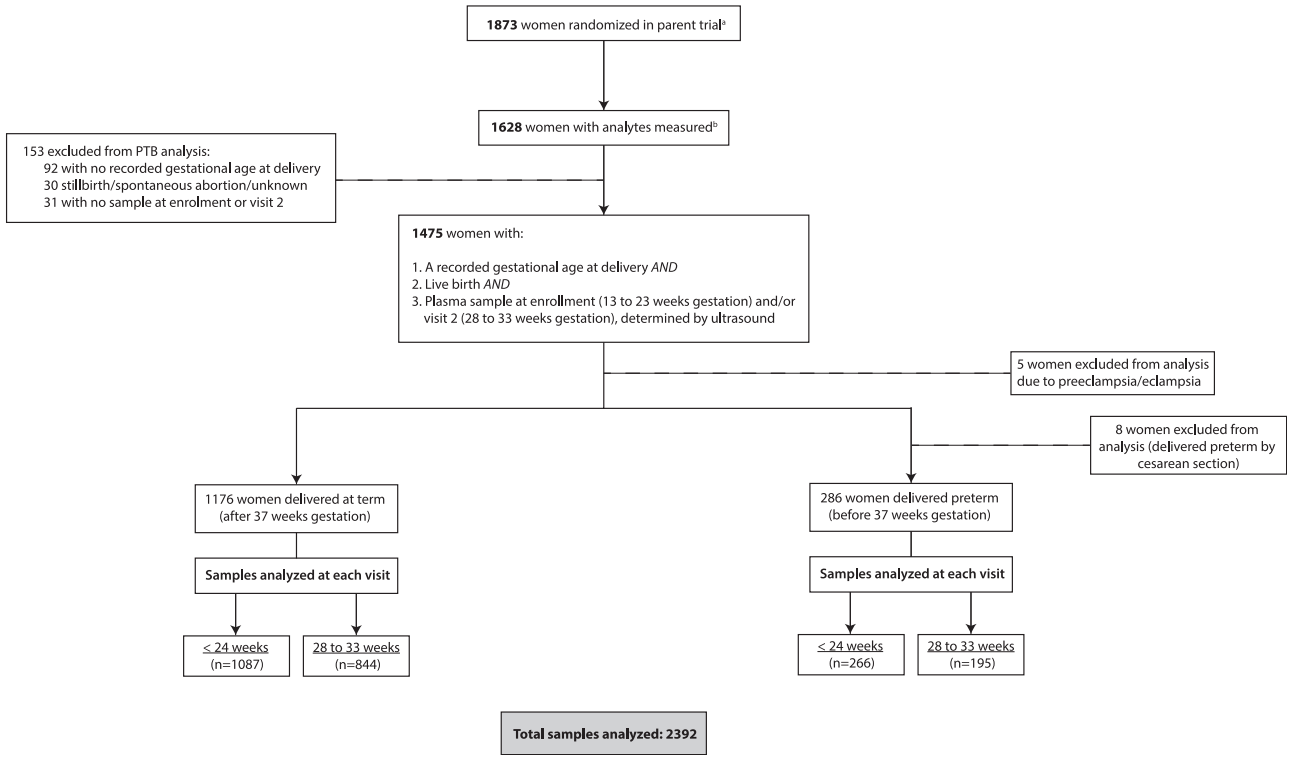


Figure 1. Flow chart of the patient population

Breakdown of patients and samples included in the current study. [a] Madanitsa and colleagues [20]. [b] Elphinstone and colleagues [7].

Women in the highest quartile of pro-inflammatory markers at enrollment (13–23 weeks' gestation) who were also in the highest quartile of anti-angiogenic markers at a subsequent visit (visit 2: 28–33 weeks' gestation) accounted for up to 16% of preterm pregnancies and under 7% of term pregnancies (Figure S1).

Angiogenic factors mediate the relationship between early inflammation and gestational age at delivery

Given the temporal link between early inflammation (enrollment), subsequent dysregulation of angiogenic factors important for placental vascular development (visit 2), and risk of PTB, we used mediation analysis to statistically assess a causal link (Figure 3, Tables S2, and S3). Path diagrams and regression estimates (with 95% CI) for each section of the path are presented in Figure 3. Regression analyses showed that one unit increases in log-transformed enrollment concentrations of the inflammatory analytes sTNFR2 (a_1: 0.17 [0.07, 0.27], $p < 0.001$; Figure 3A) and CHI3L1 (a_1: 0.14 [0.06, 0.22], $p < 0.001$; Figure 3B), but not IL18BP (Figure 3C), were significantly associated with an increased (anti-angiogenic) ratio of sFlt1/PlGF. sTNFR2 at enrollment was also associated with increased sEng at visit 2 (a_2: 0.06 [0.01, 0.11], $p < 0.05$; Figure 3A). In concordance with the relative risk analysis, increasing concentrations of both sFlt1/PlGF and sEndoglin were associated with earlier gestational age at delivery, after controlling for inflammation (b_1 and b_2, $p < 0.001$; Figures 3A–3C).

Mediation analysis showed that the effect of sTNFR2 at enrollment on gestational age at delivery was mediated through both sFlt1/PlGF (a_1b_1: mean gestation age at delivery = −0.051 [-0.10, −0.02], $p < 0.01$) and sEng (a_2b_2: −0.03 [-0.07, −0.01], $p < 0.05$). Together, the indirect effects of sTNFR2 through angiogenic mediators accounted for 43.1% of the total effect of sTNFR2 on gestational age at delivery (Figure 3A). sFlt1/PlGF at visit 2 also mediated the effect of CHI3L1 at enrollment on gestational age at delivery (a_1b_1: −0.04 [-0.09, −0.02], $p < 0.01$); this indirect effect through sFlt-1/PlGF accounted for 50.6% of the total effect of CHI3L1 on gestational age at delivery (Figure 3B). The negative mediation effects indicate that gestational age at delivery is reduced as a result of sTNFR2 and CHI3L1's effect on angiogenic mediators. IL18BP at enrollment had a significant direct effect on gestational age at delivery (c': −0.28 [-0.46, −0.12], $p < 0.01$; Figure 3C), but its effect

Table 2. Median biomarker concentrations (ng/mL) at each visit in term (≥37 weeks gestation) and preterm (<37 weeks gestation) pregnancies

	Term Birth			Preterm Birth	
Analyte[a]	n	Median [IQR]	N	Median [IQR]	*p* value[b]
Enrollment (weeks)[c]		**19.7 [18.0, 21.6]**		**21.3 [19.7, 22.4]**	**<0.001**
Angpt-2	1087	14.60 [10.36, 21.39]	266	14.65 [10.18, 20.40]	0.735
Angpt-1	1087	7.67 [3.65, 13.55]	266	6.61 [3.48, 13.26]	0.471
Angptl3	1090	16.04 [9.70, 25.26]	266	17.43 [10.62, 26.25]	0.088
sEng	1087	2.50 [1.85, 3.30]	266	2.53 [1.82, 3.70]	0.500
sFlt-1	1087	2.01 [1.31, 2.99]	266	2.25 [1.40, 3.50]	0.011
PlGF[c]	1087	45.98 [26.51, 77.95]	266	62.09 [32.65, 116.05]	**<0.001**
CRP[d]	1036	3.38 [1.56, 7.51]	249	4.03 [1.66, 11.15]	0.016
CHI3L1	1090	17.68 [9.67, 35.00]	266	19.61 [11.42, 39.68]	0.018
sTNFR2	1090	2.44 [1.57, 4.05]	266	2.83 [1.84, 4.74]	**0.001**
IL18BP	1089	14.68 [10.37, 21.60]	266	16.56 [12.43, 23.90]	**<0.001**
Visit 2 (weeks)[c]		**31.0 [30.3, 31.6]**		**31.6 [30.7, 32.0]**	**<0.001**
Angpt-2	844	10.77 [7.14, 15.40]	195	9.79 [6.64, 14.16]	0.080
Angpt-1	844	10.06 [5.27, 17.32]	195	10.77 [5.50, 18.72]	0.435
Angptl3	844	20.38 [12.02, 30.81]	195	20.36 [11.92, 31.39]	0.966
sEng	844	2.68 [2.06, 3.59]	195	3.34 [2.42, 4.80]	**<0.001**
sFlt-1	844	2.82 [1.92, 3.88]	195	3.32 [2.51, 4.63]	**<0.001**
PlGF[d]	844	159.50 [84.62, 270.77]	195	98.20 [46.22, 220.21]	**<0.001**
CRP[e]	812	2.50 [0.95, 5.20]	182	2.54 [1.23, 5.25]	0.594
CHI3L1	844	17.42 [10.84, 34.06]	195	17.58 [10.53, 29.77]	0.729
sTNFR2	844	2.46 [1.87, 3.36]	195	2.26 [1.68, 3.05]	0.018
IL18BP	839	15.72 [12.47, 20.61]	194	17.25 [11.91, 22.12]	0.597

[a]Angiopoietin-1 (Angpt-1), Angiopoietin-2 (Angpt-2), Angiopoietin-like 3 (Angptl3), soluble Endoglin (sEng), soluble Fms-like tyrosine kinase 1 (sFlt-1), Placental Growth Factor (PlGF), Chitinase-3-Like Protein-1 (CHI3L1), soluble Tumor Necrosis Factor Receptor 2 (sTNFR2), C-reactive protein (CRP), Interleukin-18 Binding Protein (IL18BP).
[b]Wilcoxon rank-sum test with continuity correction, bolded p values indicate $p < 0.003$ after Bonferroni correction ($p = 0.05/20$ comparisons).
[c]Gestational age in weeks at plasma collection (Median [IQR]).
[d]pg/mL.
[e]ug/mL.

was not mediated through sFlt1/PlGF or sEng. A sensitivity analysis of our mediation approach indicated that those excluded from our cohort (Figure 1) did not significantly affect our findings (Table S3).

DISCUSSION

A comprehensive understanding of the pathobiology of PTB may help inform the development of risk stratification tools and more effective interventions. Here, we longitudinally assess angiogenic and inflammatory markers that are essential for placental development and function in 2392 plasma samples collected from a cohort of 1462 Malawian women. Women with a pro-inflammatory profile at enrollment (13–24 weeks gestation), or an anti-angiogenic profile between 28 and 33 weeks gestation had an increased relative risk of preterm delivery. Mediation analysis supported a putative causal link between early inflammation and subsequent dysregulation of angiogenesis. These findings provide temporal and mechanistic insights into the relationship between inflammation and angiogenesis in pregnancy, and suggest that the pathobiology of PTB may begin early in pregnancy.

Our data confirm, in a large longitudinal cohort with a high burden of PTB, that the critical balance between pro- and anti-angiogenic factors and inflammatory biomarkers is disrupted in women who deliver preterm. Placental vascular development and function, essential to support oxygen and nutrient requirements of the

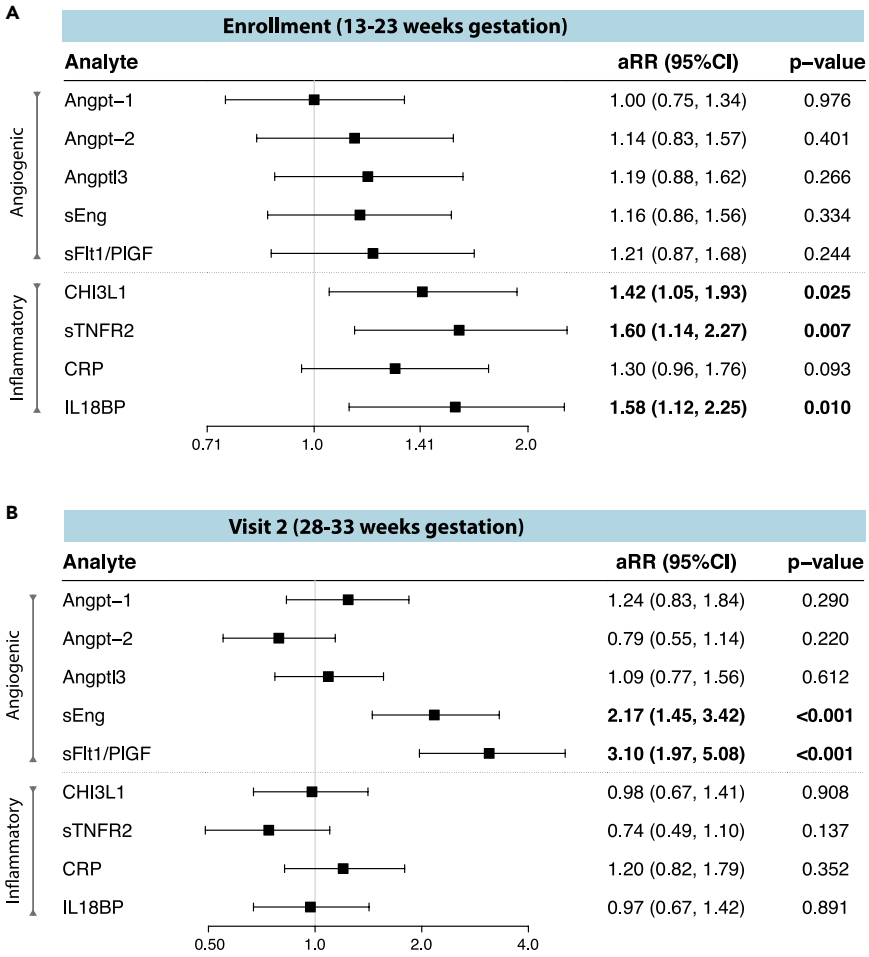


Figure 2. Forest plot of adjusted relative risk (and 95% confidence interval) for preterm birth at each visit, according to inflammatory and angiogenic mediator concentration

The adjusted relative risk (95% CI) for preterm birth for women in the highest quartile of each marker, relative to women in the lowest quartile, at each visit: (A) enrollment (13–23 weeks gestation) or (B) visit 2 (28–33 weeks gestation). Models were adjusted for blood pressure (systolic) at enrollment, gestational age at plasma collection for each respective visit, treatment group, maternal gravidity (primigravid: yes/no), socioeconomic status, and malaria during pregnancy (yes/no by PCR). Abbreviations: Adjusted relative risk (aRR), Angiopoietin-1 (Angpt-1), Angiopoietin-2 (Angpt-2), Angiopoietin-Like 3 (Angptl3), Chitinase-3-Like Protein-1 (CHI3L1), C-reactive protein (CRP), Interleukin-18 Binding Protein (IL18BP), Placental Growth Factor (PlGF), soluble Endoglin (sEng), soluble Fms-like tyrosine kinase 1 (sFlt-1), soluble Tumor Necrosis Factor Receptor 2 (sTNFR2).

growing fetus, relies on the co-ordinated and dynamic regulation of angiogenic and inflammatory pathways throughout pregnancy.[23] Abnormal placental function is a shared hallmark of pregnancy pathologies characterized by inflammation and/or dysregulated angiogenesis including preeclampsia, PTB, fetal growth restriction, and stillbirth.[19,23–31] However, few studies have investigated inflammation, angiogenesis, and the temporal and mechanistic relationship between these pathways in a single cohort.

Inflammation is closely tied to pregnancy and PTB.[11] A large body of evidence has confirmed the role of cytokines in the induction of term and preterm labor, including decidual and cervical maturation, myometrial contractions, and preterm premature rupture of membranes (reviewed in[11,32–34]). Its dual role in both term and preterm labor has led to a concentration of research investigating inflammation late in pregnancy. In an effort to achieve early identification of at-risk pregnancies, some research has linked inflammatory proteins early in pregnancy, including TNF and sTNFRs, IFN-γ, CHI3L1, IL18BP, IL-6, and CRP, with PTB.[14,35–38] However, these studies were cross-sectional and despite some evidence for early inflammation in pregnancies at risk for PTB, the mechanism by which it may drive PTB is not well established.

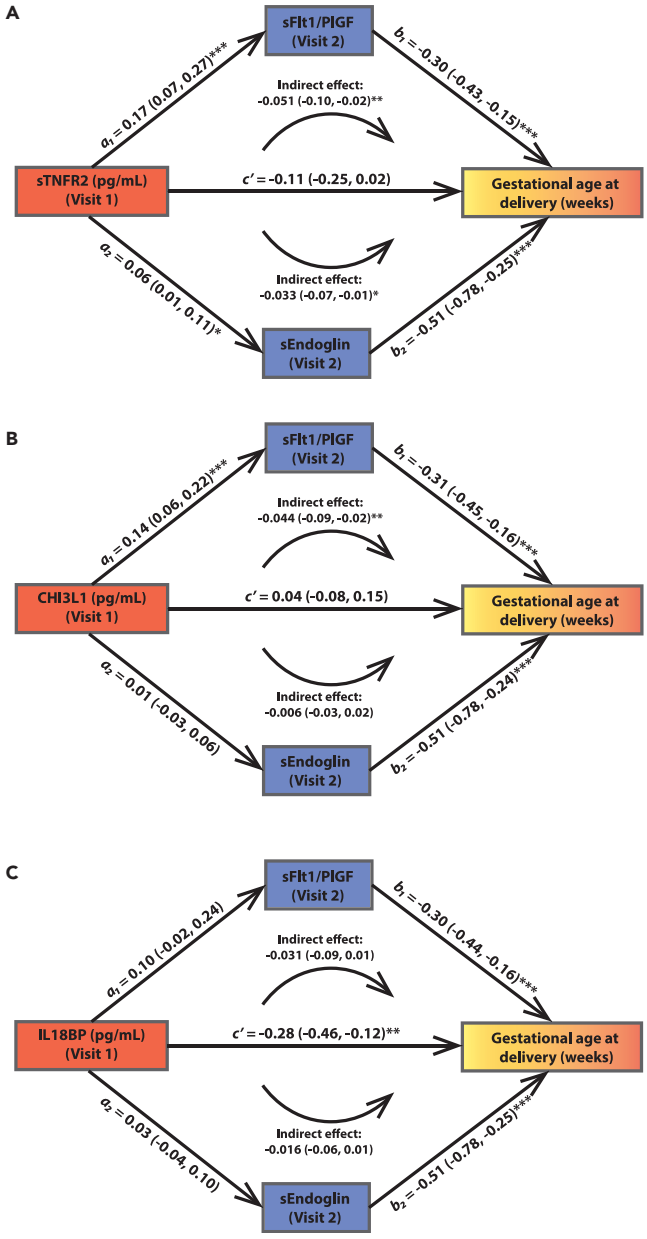


Figure 3. Path diagrams for multiple mediator analysis of inflammatory analytes through angiogenic mediators

Regression coefficients with 95% bootstrapped confidence intervals for each path in the multiple mediator models. Indirect (mediation) effects were calculated using the product of coefficients method (a_1b_1 or a_2b_2). c' represents the direct effect of the inflammatory analytes (A) sTNFR2, (B) CHI3L1, or (C) IL18BP on gestational age at delivery, after controlling for mediators. Gestational age and blood pressure (systolic) at enrollment, as well as malaria during pregnancy (by PCR), were included as covariates. Positive estimates indicate the increase in outcome with a one unit increase in predictor. Negative estimates indicate the decrease in outcome with a one unit increase in predictor. *p < 0.05, **p < 0.01, ***p < 0.001. Abbreviations: Chitinase-3-Like Protein-1 (CHI3L1), Interleukin-18 Binding Protein (IL18BP), Placental Growth Factor (PlGF), soluble Endoglin (sEng), soluble Fms-like tyrosine kinase 1 (sFlt-1), soluble Tumor Necrosis Factor Receptor 2 (sTNFR2).

One way by which early maternal systemic inflammation could lead to PTB is via disruption of placental vasculogenesis and angiogenesis at critical points in placental vascular development and re-modeling. Inflammatory and angiogenic pathways display considerable crosstalk and co-regulation.[21,22] The three

inflammatory markers we observed associated with PTB in this study, IL18BP, sTNFR2, and CHI3L1, have all previously been implicated in the regulation of angiogenesis. At enrollment, all three were associated with an increased relative risk of preterm delivery, and all modulate angiogenesis in part by regulating expression of angiogenic factors including the VEGF and the angiopoietin-Tie2 families of proteins and their receptors.[21,39–41] Disruption to angiogenic factors during pregnancy is strongly associated with adverse birth outcomes. Cross-sectional and longitudinal profiling of pathological pregnancy states including preeclampsia, fetal growth restriction, PTB, and fetal death have reported a common theme of imbalanced pro-angiogenic (i.e. PlGF) and anti-angiogenic (i.e. sFlt-1, sEndoglin) mediators.[10,14–16,20,42–47] In particular, longitudinal studies demonstrate a shift to an anti-angiogenic profile consisting of increased sEndoglin and sFlt-1, and decreased PlGF during the second and third trimester of pathologic pregnancies including spontaneous PTB.[10,20,46] Dysregulated angiogenesis has been linked to compromised placental function and inadequate placental vascular development required to support the rapidly growing fetus and leading to adverse birth outcomes.[19,48,49] Therefore, downstream disruption of angiogenesis is a biologically plausible mechanism by which early inflammation could contribute to placental insufficiency and PTB.

In our cohort, we observed a longitudinal progression from inflammation to dysregulated angiogenesis supporting the hypothesis that early maternal inflammation may impair placental vascular development in the pathobiology of PTB. Specifically, inflammatory markers (IL18BP, CHI3L1, and sTNFR2) were elevated early in pregnancy in women who delivered preterm (13–23 weeks gestation), shifting to an anti-angiogenic profile (sEndoglin, sFlt1/PlGF) later in pregnancy (28–33 weeks gestation). Mediation analysis supported a putative causal relationship between CHI3L1 and sTNFR2 at enrollment, increased sFlt1/PlGF and sEndoglin at visit 2, and decreased gestational age at delivery. While 43% of the effect of sTNFR2 on gestational age at delivery was mediated through sFlt1/PlGF and sEndoglin, the effect of IL18BP on gestational age was not mediated through angiogenic factors at all. This indicates the likelihood of multiple etiologies of PTB, and supports the existence of additional, unmeasured mediators that are contributing to PTB.

Several risk factors for PTB are known to trigger systemic inflammation,[11] supporting dysregulated inflammation and angiogenesis as a common pathway underlying PTB, regardless of precipitating event. Few studies have investigated pregnancy-related inflammation and angiogenesis in LMICs, where the burden of PTB is highest. In LMICs like Malawi, a major underlying driver of high PTB rates are maternal infections, of which malaria and HIV are two principal contributors.[9,50–55] Malaria and HIV are both thought to cause PTB by inducing systemic inflammation and dysregulated angiogenesis.[9,10,56] Our analysis was conducted in HIV-negative women, and malaria status was controlled for in the multivariate analyses. Therefore, our results indicate that even after controlling for two important contributors to adverse birth outcomes in LMICs, dysregulated inflammation and angiogenesis across pregnancy were still associated with PTB.

The strengths of this study include the large sample size, repeated sample collection across pregnancy, and the detailed tracking (e.g. ultrasound gestational dating) and follow-up of women enrolled in the parent trial. The size of the cohort and the high prevalence of preterm birth allowed the robust evaluation of both angiogenic and inflammatory pathways at multiple points in pregnancy in the pathobiology of PTB. Furthermore, our analysis of both inflammatory and angiogenic proteins in a single large, repeated measures cohort gives preliminary insight into temporal relationships between inflammation and mediators of placental vasculature in preterm pregnancies.

Here, we present findings from a large cohort of Malawian women with repeated samples collected across pregnancy, in a setting with the highest rates of PTB globally. Our findings support the hypothesis that early dysregulation of inflammatory and angiogenic mediators of placental vascular development is associated with PTB. In particular, our data suggest that inflammation early in pregnancy and a consequent shift to an anti-angiogenic profile later in pregnancy may lead to preterm delivery. Advancing mechanistic knowledge of PTB is necessary to inform strategies that prevent the devastating long-term and intergenerational effects associated with being born preterm. These findings provide mechanistic insight into the relationship of inflammation and angiogenesis in the pathobiology of PTB, and provide pathways that could be studied as putative targets early in pregnancy to help mitigate the global burden of PTB.

Limitations of the study

Our study population was weighted toward moderate to late preterm birth (32 to <37 weeks gestation; n = 271/286) and therefore our findings are limited in their applicability to the etiology of early preterm birth (<32 weeks gestation; n = 15). Although we considered covariates related to PTB such as maternal age, BMI, blood pressure at enrollment, education and socioeconomic status, gravidity, and malaria infection, our conclusions are limited by the observational nature of the study and the potential for residual confounding by unmeasured variables (e.g. intrauterine infection, etc.). Furthermore, the generalizability of our findings to populations with lower rates of maternal infections including malaria (for example, in high-income settings) may be limited and further studies in diverse populations are required to assess the commonality of this mechanism to the global burden of PTB. Future prospective studies are also needed to link the longitudinal dynamics of inflammatory and angiogenic factors across pregnancy with placental vascular structure and functional placental vascular read-outs such as vascular resistance.[19,57] While our data support one mechanism of moderate to late PTB, additional studies are required to explore the implications of early inflammation (e.g. IL18BP) whose effects do not appear to be mediated through angiogenic dysregulation, an expanded panel of inflammatory mediators that could represent alternate therapeutically targetable pathways (e.g., IL-1α, IFN- γ, C5a, etc.),[19,21] and the generalizability of our findings to early PTB.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - Human studies
- METHOD DETAILS
  - Inflammatory and angiogenic analyte measurement
- QUANTIFICATION AND STATISTICAL ANALYSIS
- ADDITIONAL RESOURCES

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.106912.

ACKNOWLEDGMENTS

We would like to acknowledge all the mothers who participated in this study and the hardworking staff at the trial sites in Malawi. This study was supported by the Canadian Institutes of Health Research (CIHR FDN-148439 to K.C.K.), Canada Research Chair (K.C.K.), CIHR Doctoral Award GSD-157907 (A.M.W.) and Open Philanthropy. The funders had no role in study design; collection, analysis, and interpretation of data; writing of the report; or decision to submit the article for publication.

AUTHOR CONTRIBUTIONS

Conceptualization, A.M.W., R.E.E., C.R.M., A.L.C., K.C.K.; Formal Analysis, A.M.W., J.M.S.; Investigation, R.E.E., A.M.W., C.R.M., V.T., K.Z., M.M., L.K.P., A.L.C.; Data Curation, R.E.E., A.M.W., C.K.; Writing – Original Draft, A.M.W., K.C.K.; Writing – Review & Editing, all authors; Visualization, A.M.W., K.C.K., F.O.K.; Project Administration, R.E., M.M.; Resources, M.M., L.K.P., V.M., F.O.K., K.C.K.; Supervision, C.R.M., K.C.K.; Funding Acquisition, V.M., F.O.K., K.C.K.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

Received: June 8, 2022
Revised: November 11, 2022
Accepted: May 12, 2023
Published: May 19, 2023

REFERENCES

1. Liu, L., Oza, S., Hogan, D., Chu, Y., Perin, J., Zhu, J., Lawn, J.E., Cousens, S., Mathers, C., and Black, R.E. (2016). Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet *388*, 3027–3035. https://doi.org/10.1016/S0140-6736(16)31593-8.

2. Blencowe, H., Cousens, S., Oestergaard, M.Z., Chou, D., Moller, A.B., Narwal, R., Adler, A., Vera Garcia, C., Rohde, S., Say, L., and Lawn, J.E. (2012). National , regional , and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries : a systematic analysis and implications. Lancet *379*, 2162–2172. https://doi.org/10.1016/S0140-6736(12)60820-4.

3. Saigal, S., and Doyle, L.W. (2008). An overview of mortality and sequelae of preterm birth from infancy to adulthood. Lancet *371*, 261–269. https://doi.org/10.1016/S0140-6736(08)60136-1.

4. Luyckx, V.A. (2017). Preterm birth and its impact on renal health. Semin. Nephrol. *37*, 311–319. https://doi.org/10.1016/j.semnephrol.2017.05.002.

5. Karnati, S., Kollikonda, S., and Abu-Shaweesh, J. (2020). Late preterm infants – changing trends and continuing challenges. Int. J. Pediatr. Adolesc. Med. *7*, 36–44. https://doi.org/10.1016/J.IJPAM.2020.02.006.

6. Ijaz, S., Shamsher, M., Ismail, I., and Ayub, A. (2022). A descriptive study of frequency of short term complications in late preterm neonates at a tertiary care hospital Rawalpindi. J. Pakistan Med. Assoc. *72*, 1775–1778. https://doi.org/10.47391/JPMA.4569.

7. Chawanpaiboon, S., Vogel, J.P., Moller, A.B., Lumbiganon, P., Petzold, M., Hogan, D., Landoulsi, S., Jampathong, N., Kongwattanakul, K., Laopaiboon, M., et al. (2019). Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. Lancet Global Health *7*, e37–e46. https://doi.org/10.1016/S2214-109X(18)30451-0.

8. Lawn, J.E., Kinney, M. v, Belizan, J.M., Mason, E.M., McDougall, L., Larson, J., Lackritz, E., Friberg, I.K., and Howson, C.P.; Born Too Soon Preterm Birth Action Group (2013). Born Too Soon: accelerating actions for prevention and care of 15 million newborns born too soon. Reprod. Health *10*, S6. https://doi.org/10.1186/1742-4755-10-S1-S6.

9. Elphinstone, R.E., Weckman, A.M., McDonald, C.R., Tran, V., Zhong, K., Madanitsa, M., Kalilani-Phiri, L., Khairallah, C., Taylor, S.M., Meshnick, S.R., et al. (2019). Early malaria infection, dysregulation of angiogenesis, metabolism and inflammation across pregnancy, and risk of preterm birth in Malawi: a cohort study. PLoS Med. *16*, e1002914. https://doi.org/10.1371/journal.pmed.1002914.

10. Conroy, A.L., McDonald, C.R., Gamble, J.L., Olwoch, P., Natureeba, P., Cohan, D., Kamya, M.R., Havlir, D. v, Dorsey, G., and Kain, K.C. (2017). Altered angiogenesis as a common mechanism underlying preterm birth, small for gestational age, and stillbirth in women living with HIV. Am. J. Obstet. Gynecol. *217*, 684.e1–684.e17. https://doi.org/10.1016/j.ajog.2017.10.003.

11. Goldenberg, R.L., Culhane, J.F., Iams, J.D., and Romero, R. (2008). Epidemiology and causes of preterm birth. Lancet *371*, 75–84. https://doi.org/10.1016/S0140-6736(08)60074-4.

12. Gomez-Lopez, N., StLouis, D., Lehr, M.A., Sanchez-Rodriguez, E.N., and Arenas-Hernandez, M. (2014). Immune cells in term and preterm labor. Cell. Mol. Immunol. *11*, 571–581. https://doi.org/10.1038/cmi.2014.46.

13. Ferguson, K.K., McElrath, T.F., Chen, Y.H., Mukherjee, B., and Meeker, J.D. (2014). Longitudinal profiling of inflammatory cytokines and C-reactive protein during uncomplicated and preterm pregnancy. Am. J. Reprod. Immunol. *72*, 326–336. https://doi.org/10.1111/aji.12265.

14. McDonald, C.R., Darling, A.M., Conroy, A.L., Tran, V., Cabrera, A., Liles, W.C., Wang, M., Aboud, S., Urassa, W., Fawzi, W.W., and Kain, K.C. (2015). Inflammatory and angiogenic factors at mid-pregnancy are associated with spontaneous preterm birth in a cohort of Tanzanian women. PLoS One *10*, e0134619. https://doi.org/10.1371/journal.pone.0134619.

15. Romero, R., Chaiworapongsa, T., Erez, O., Tarca, A.L., Gervasi, M.T., Kusanovic, J.P., Mittal, P., Ogge, G., Vaisbuch, E., Mazaki-Tovi, S., et al. (2010). An imbalance between angiogenic and anti-angiogenic factors precedes fetal death in a subset of patients: results of a longitudinal study. J. Matern. Fetal Neonatal Med. *23*, 1384–1399. https://doi.org/10.3109/14767051003681121.

16. Romero, R., Nien, J.K., Espinoza, J., Todem, D., Fu, W., Chung, H., Kusanovic, J.P., Gotsch, F., Erez, O., Mazaki-Tovi, S., et al. (2008). A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. J. Matern. Fetal Neonatal Med. *21*, 9–23. https://doi.org/10.1080/14767050701830480.

17. Kim, Y.M., Bujold, E., Chaiworapongsa, T., Gomez, R., Yoon, B.H., Thaler, H.T., Rotmensch, S., and Romero, R. (2003). Failure of physiologic transformation of the spiral arteries in patients with preterm labor and intact membranes. Am. J. Obstet. Gynecol. *189*, 1063–1069. https://doi.org/10.1067/S0002-9378(03)00838-X.

18. Parker, S.E., and Werler, M.M. (2014). Epidemiology of ischemic placental disease: a focus on preterm gestations. Semin. Perinatol. *38*, 133–138. https://doi.org/10.1053/j.semperi.2014.03.004.

19. Conroy, A.L., Silver, K.L., Zhong, K., Rennie, M., Ward, P., Sarma, J.V., Molyneux, M.E., Sled, J., Fletcher, J.F., Rogerson, S., and Kain, K.C. (2013). Complement activation and the resulting placental vascular insufficiency drives fetal growth restriction associated with placental malaria. Cell Host Microbe *13*, 215–226. https://doi.org/10.1016/j.chom.2013.01.010.

20. Chaiworapongsa, T., Romero, R., Tarca, A., Kusanovic, J.P., Mittal, P., Kim, S.K., Gotsch, F., Erez, O., Vaisbuch, E., Mazaki-Tovi, S., et al. (2009). A subset of patients destined to develop spontaneous preterm labor has an abnormal angiogenic/anti-angiogenic profile in maternal plasma: evidence in support of pathophysiologic heterogeneity of preterm labor derived from a longitudinal study. J. Matern. Fetal Neonatal Med. *22*, 1122–1139. https://doi.org/10.3109/14767050902994838.

21. Naldini, A., and Carraro, F. (2005). Role of inflammatory mediators in angiogenesis. Curr. Drug Targets - Inflamm. Allergy *4*, 3–8.

22. Girardi, G., Yarilin, D., Thurman, J.M., Holers, V.M., and Salmon, J.E. (2006). Complement activation induces dysregulation of angiogenic factors and causes fetal rejection and growth restriction. J. Exp. Med. *203*, 2165–2175. https://doi.org/10.1084/jem.20061022.

23. Chen, D.B., and Zheng, J. (2014). Regulation of placental angiogenesis. Microcirculation *21*, 15–25. https://doi.org/10.1111/micc.12093.

24. Arias, F., Victoria, A., Cho, K., and Kraus, F. (1997). Placental histology and clinical characteristics of patients with preterm premature rupture of membranes. Obstet. Gynecol. *89*, 265–271. https://doi.org/10.1016/S0029-7844(96)00451-6.

25. Noori, M., Donald, A.E., Angelakopoulou, A., Hingorani, A.D., and Williams, D.J. (2010). Prospective study of placental angiogenic factors and maternal vascular function before and after preeclampsia and gestational hypertension. Circulation *122*, 478–487.

https://doi.org/10.1161/CIRCULATIONAHA.109.895458.

26. Soto, E., Romero, R., Kusanovic, J.P., Ogge, G., Hussein, Y., Yeo, L., Hassan, S.S., Kim, C.J., and Chaiworapongsa, T. (2012). Late-onset preeclampsia is associated with an imbalance of angiogenic and anti-angiogenic factors in patients with and without placental lesions consistent with maternal underperfusion. J. Matern. Fetal Neonatal Med. *25*, 498–507. https://doi.org/10.3109/14767058.2011.591461.

27. Korzeniewski, S.J., Romero, R., Chaiworapongsa, T., Chaemsaithong, P., Kim, C.J., Kim, Y.M., Kim, J.S., Yoon, B.H., Hassan, S.S., and Yeo, L. (2016). Maternal plasma angiogenic index-1 (placental growth factor/soluble vascular endothelial growth factor receptor-1) is a biomarker for the burden of placental lesions consistent with uteroplacental underperfusion: a longitudinal case-cohort study. Am. J. Obstet. Gynecol. *214*, 629.e1–629.e17. https://doi.org/10.1016/j.ajog.2015.11.015.

28. Cotechini, T., Komisarenko, M., Sperou, A., Macdonald-Goodfellow, S., Adams, M.A., and Graham, C.H. (2014). Inflammation in rat pregnancy inhibits spiral artery remodeling leading to fetal growth restriction and features of preeclampsia. J. Exp. Med. *211*, 165–179. https://doi.org/10.1084/jem.20130295.

29. Kwak-Kim, J., Bao, S., Lee, S.K., Kim, J.W., and Gilman-Sachs, A. (2014). Immunological modes of pregnancy loss: inflammation, immune effectors, and stress. Am. J. Reprod. Immunol. *72*, 129–140. https://doi.org/10.1111/aji.12234.

30. Misra, V.K., Hobel, C.J., and Sing, C.F. (2009). Placental blood flow and the risk of preterm delivery. Placenta *30*, 619–624. https://doi.org/10.1016/j.placenta.2009.04.007.

31. Cotechini, T., and Graham, C.H. (2015). Aberrant maternal inflammation as a cause of pregnancy complications: a potential therapeutic target? Placenta *36*, 960–966. https://doi.org/10.1016/j.placenta.2015.05.016.

32. Romero, R., Gotsch, F., Pineles, B., and Kusanovic, J.P. (2007). Inflammation in pregnancy: its roles in reproductive physiology, obstetrical complications, and fetal injury. Nutr. Rev. *65*, S194–S202. https://doi.org/10.1111/j.1753-4887.2007.tb00362.x.

33. Romero, R., Dey, S.K., and Fisher, S.J. (2014). Preterm labor: one syndrome, many causes. Science *345*, 760–765. https://doi.org/10.1126/science.1251816.

34. Keelan, J.A. (2018). Intrauterine inflammatory activation, functional progesterone withdrawal, and the timing of term and preterm birth. J. Reprod. Immunol. *125*, 89–99. https://doi.org/10.1016/j.jri.2017.12.004.

35. Lynch, A.M., Wagner, B.D., Deterding, R.R., Giclas, P.C., Gibbs, R.S., Janoff, E.N., Holers, V.M., and Santoro, N.F. (2016). The relationship of circulating proteins in early pregnancy with preterm birth. Am. J. Obstet. Gynecol. *214*, 517.e1–517.e8. https://doi.org/10.1016/j.ajog.2015.11.001.

36. Wallenstein, M.B., Jelliffe-Pawlowski, L.L., Yang, W., Carmichael, S.L., Stevenson, D.K., Ryckman, K.K., and Shaw, G.M. (2016). Inflammatory biomarkers and spontaneous preterm birth among obese women. J. Matern. Fetal Neonatal Med. *29*, 3317–3322. https://doi.org/10.3109/14767058.2015.1124083.

37. Pitiphat, W., Gillman, M.W., Joshipura, K.J., Williams, P.L., Douglass, C.W., and Rich-Edwards, J.W. (2005). Plasma C-reactive protein in early pregnancy and preterm delivery. Am. J. Epidemiol. *162*, 1108–1113. https://doi.org/10.1093/aje/kwi323.

38. Curry, A.E., Thorsen, P., Drews, C., Schendel, D., Skogstrand, K., Flanders, W.D., Hougaard, D., Olsen, J., and Vogel, I. (2009). First-trimester maternal plasma cytokine levels, pre-pregnancy body mass index, and spontaneous preterm delivery. Acta Obstet. Gynecol. Scand. *88*, 332–342. https://doi.org/10.1080/00016340802702219.

39. Libreros, S., Garcia-Areas, R., and Iragavarapu-Charyulu, V. (2013). CHI3L1 plays a role in cancer through enhanced production of pro-inflammatory/pro-tumorigenic and angiogenic factors. Immunol. Res. *57*, 99–105. https://doi.org/10.1007/s12026-013-8459-y.

40. Badimon, L., Peña, E., Arderiu, G., Padró, T., Slevin, M., Vilahur, G., and Chiva-Blanch, G. (2018). C-reactive protein in atherothrombosis and angiogenesis. Front. Immunol. *9*, 430. https://doi.org/10.3389/FIMMU.2018.00430.

41. Volin, M.v., and Koch, A.E. (2011). Interleukin-18: a mediator of inflammation and angiogenesis in rheumatoid arthritis. J. Interferon Cytokine Res. *31*, 745–751. https://doi.org/10.1089/jir.2011.0050.

42. Darling, A.M., McDonald, C.R., Conroy, A.L., Hayford, K.T., Liles, W.C., Wang, M., Aboud, S., Urassa, W.S., Kain, K.C., and Fawzi, W.W. (2014). Angiogenic and inflammatory biomarkers in midpregnancy and small-for-gestational-age outcomes in Tanzania. Am. J. Obstet. Gynecol. *211*, 509.e1–509.e8. https://doi.org/10.1016/j.ajog.2014.05.032.

43. Levine, R.J., Lam, C., Qian, C., Yu, K.F., Maynard, S.E., Sachs, B.P., Sibai, B.M., Epstein, F.H., Romero, R., Thadhani, R., et al. (2006). Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N. Engl. J. Med. *355*, 992–1005. https://doi.org/10.1056/NEJMoa055352.

44. Levine, R.J., Maynard, S.E., Qian, C., Lim, K.-H., England, L.J., Yu, K.F., Schisterman, E.F., Thadhani, R., Sachs, B.P., Epstein, F.H., et al. (2004). Circulating angiogenic factors and the risk of preeclampsia. N. Engl. J. Med. *350*, 672–683. https://doi.org/10.1056/NEJMoa031884.

45. Mijal, R.S., Holzman, C.B., Rana, S., Karumanchi, S.A., Wang, J., and Sikorskii, A. (2012). Mid-pregnancy levels of angiogenic markers as indicators of pathways to preterm delivery. J. Matern. Fetal Neonatal Med. *25*, 1135–1141. https://doi.org/10.3109/14767058.2011.625458.

46. Straughen, J.K., Misra, D.P., Helmkamp, L., and Misra, V.K. (2017). Preterm delivery as a unique pathophysiologic state characterized by maternal soluble fms-like tyrosine kinase 1 and uterine artery resistance during pregnancy: a longitudinal cohort study. Reprod. Sci. *24*, 1583–1589. https://doi.org/10.1177/1933719117698574.

47. Chaiworapongsa, T., Romero, R., Erez, O., Tarca, A.L., Conde-Agudelo, A., Chaemsaithong, P., Kim, C.J., Kim, Y.M., Kim, J.S., Yoon, B.H., et al. (2017). The prediction of fetal death with a simple maternal blood test at 24-28 weeks: a role for angiogenic index-1 (PlGF/sVEGFR-1 ratio). Am. J. Obstet. Gynecol. *217*, 682.e1–682.e13. https://doi.org/10.1016/j.ajog.2017.10.001.

48. Mcdonald, C.R., Cahill, L.S., Gamble, J.L., Elphinstone, R., Gazdzinski, L.M., Zhong, K.J.Y., Philson, A.C., Madanitsa, M., Kalilani-Phiri, L., Mwapasa, V., et al. (2018). Malaria in pregnancy alters l-arginine bioavailability and placental vascular development. Sci. Transl. Med. *10*, eaan6007. https://doi.org/10.1126/scitranslmed.aan6007.

49. Tran, V., Weckman, A.M., Crowley, V.M., Cahill, L.S., Zhong, K., Cabrera, A., Elphinstone, R.E., Pearce, V., Madanitsa, M., Kalilani-Phiri, L., et al. (2021). The Angiopoietin-Tie2 axis contributes to placental vascular disruption and adverse birth outcomes in malaria in pregnancy. EBioMedicine *73*, 103683. https://doi.org/10.1016/J.EBIOM.2021.103683.

50. Ticconi, C., Mapfumo, M., Dorrucci, M., Naha, N., Tarira, E., Pietropolli, A., and Rezza, G. (2003). Effect of maternal HIV and malaria infection on pregnancy and perinatal outcome in Zimbabwe. J. Acquir. Immune Defic. Syndr. *34*, 289–294. https://doi.org/10.1097/00126334-200311010-00005.

51. Ayisi, J.G., van Eijk, A.M., ter Kuile, F.O., Kolczak, M.S., Otieno, J.A., Misore, A.O., Kager, P.A., Steketee, R.W., and Nahlen, B.L. (2003). The effect of dual infection with HIV and malaria on pregnancy outcome in western Kenya. AIDS *17*, 585–594. https://doi.org/10.1097/00002030-200303070-00014.

52. Kapisi, J., Kakuru, A., Jagannathan, P., Muhindo, M.K., Natureeba, P., Awori, P., Nakalembe, M., Ssekitoleko, R., Olwoch, P., Ategeka, J., et al. (2017). Relationships between infection with Plasmodium falciparum during pregnancy, measures of placental malaria, and adverse birth outcomes NCT02163447 NCT. Malar. J. *16*, 400. https://doi.org/10.1186/s12936-017-2040-4.

53. Zash, R., Jacobson, D.L., Diseko, M., Mayondi, G., Mmalane, M., Essex, M., Petlo, C., Lockman, S., Makhema, J., and Shapiro, R.L. (2017). Comparative safety of antiretroviral treatment regimens in pregnancy. JAMA Pediatr. *171*, e172222. https://doi.org/10.1001/JAMAPEDIATRICS.2017.2222.

54. Wedi, C.O.O., Kirtley, S., Hopewell, S., Corrigan, R., Kennedy, S.H., and Hemelaar, J.

(2016). Perinatal outcomes associated with maternal HIV infection: a systematic review and meta-analysis. Lancet. HIV *3*, e33–e48. https://doi.org/10.1016/S2352-3018(15)00207-6.

55. Lufele, E., Umbers, A., Ordi, J., Ome-Kaius, M., Wangnapi, R., Unger, H., Tarongka, N., Siba, P., Mueller, I., Robinson, L., and Rogerson, S. (2017). Risk factors and pregnancy outcomes associated with placental malaria in a prospective cohort of Papua New Guinean women. Malar. J. *16*, 427. https://doi.org/10.1186/s12936-017-2077-4.

56. Fried, M., Kurtis, J.D., Swihart, B., Pond-Tor, S., Barry, A., Sidibe, Y., Gaoussou, S., Traore, M., Keita, S., Mahamar, A., et al. (2017). Systemic inflammatory response to malaria during pregnancy is associated with pregnancy loss and preterm delivery. Clin. Infect. Dis. *65*, 1729–1735. https://doi.org/10.1093/cid/cix623.

57. Moeller, S.L., Nyengaard, J.R., Larsen, L.G., Nielsen, K., Bygbjerg, I.C., Msemo, O.A., Lusingu, J.P.A., Minja, D.T.R., Theander, T.G., and Schmiegelow, C. (2019). Malaria in early pregnancy and the development of the placental vasculature. J. Infect. Dis. *220*, 1425–1434. https://doi.org/10.1093/infdis/jiy735.

58. Madanitsa, M., Kalilani, L., Mwapasa, V., van Eijk, A.M., Khairallah, C., Ali, D., Pace, C., Smedley, J., Thwai, K.-L., Levitt, B., et al. (2016). Scheduled intermittent screening with rapid diagnostic tests and treatment with dihydroartemisinin-piperaquine versus intermittent preventive therapy with sulfadoxine-pyrimethamine for malaria in pregnancy in Malawi: an open-label randomized controlled tr. PLoS Med. *13*, e1002124. https://doi.org/10.1371/journal.pmed.1002124.

59. Rosseel, Y. (2012). Lavaan: an R package for structural equation modeling. J. Stat. Software *48*, 1–36. https://doi.org/10.18637/jss.v048.i02.

60. van Buuren, S., and Groothuis-Oudshoorn, K. (2011). Mice: multivariate imputation by chained equations in R. J. Stat. Software *45*, 1–67. https://doi.org/10.18637/JSS.V045.I03.

61. Zeisler, H., Llurba, E., Chantraine, F., Vatish, M., Staff, A.C., Sennström, M., Olovsson, M., Brennecke, S.P., Stepan, H., Allegranza, D., et al. (2016). Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia. N. Engl. J. Med. *374*, 13–22. https://doi.org/10.1056/NEJMoa1414838.

62. Fairchild, A.J., and McDaniel, H.L. (2017). Best (but oft-forgotten) practices: mediation analysis. Am. J. Clin. Nutr. *105*, 1259–1271. https://doi.org/10.3945/AJCN.117.152546.

63. Roh, M.E., Kuile, F.O.T., Rerolle, F., Glymour, M.M., Shiboski, S., Gosling, R., Gutman, J., Kakuru, A., Desai, M., Kajubi, R., et al. (2020). Overall, anti-malarial, and non-malarial effect of intermittent preventive treatment during pregnancy with sulfadoxine-pyrimethamine on birthweight: a mediation analysis. Lancet Global Health *8*, e942–e953. https://doi.org/10.1016/S2214-109X(20)30119-4.

64. Alwin, D.F., and Hauser, R.M. (1975). The decomposition of effects in path analysis. Am. Socio. Rev. *40*, 37. https://doi.org/10.2307/2094445.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Peripheral plasma samples; collected as part of parent clinical trial	Madanitsa et al., 2016[58]	https://doi.org/10.1186/ISRCTN69800930
Critical commercial assays		
Multianalyte Luminex Human Discovery Assay (Angiopoietin-1, Angiopoietin-2, soluble Endoglin, sFlt-1, PlGF)	R&D Systems	Custom Plate: #LXSAHM-01
Multianalyte Luminex Human Discovery Assay (Angiopoietin-like protein-3, soluble Tumor Necrosis Factor Receptor 2, and Chitinase-3-like protein-1)	R&D Systems	Catalog Number: #LXSAHM-5
DuoSet ELISA (CRP)	R&D Systems	Catalog Number: #DY1707
DuoSet ELISA (IL18BP)	R&D Systems	Catalog Number: #DY119
Deposited data		
Raw analyte and demographic data	This paper; Elphinstone et al., 2019[9]	https://doi.org/10.1371/journal.pmed.1002914.
Software and algorithms		
RStudio v4.0.3	R Foundation for Statistical Computing	https://www.R-project.org/
lavaan: An R Package for Structural Equation Modeling	Rosseel, 2012[59]	https://www.jstatsoft.org/v48/i02/
mice: Multivariate Imputation by Chained Equations in R	van Buuren & Groothuis-Oudshoorn, 2011[60]	https://www.jstatsoft.org/v45/i03/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Kevin C. Kain (kevin.kain@uhn.ca).

Materials availability

This study did not generate any new unique reagents.

Data and code availability

- All analyte and clinical data (anonymized) from the parent analyte trial (a subset of which was used for the analyses presented in this paper) are publicly available and have been published at https://doi.org/10.1371/journal.pmed.1002914.
- This paper does not report original code. Publicly available R software and packages used for statistical analysis in this paper have been listed and sourced in the key resources table.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Human studies

This was a secondary analysis of previously collected samples. The samples tested in this study were prospectively collected as part of a larger randomized-controlled trial (RCT) of malaria prevention strategies in pregnant women.[58] Briefly, HIV-negative women attending their first antenatal visit were randomized to receive either 3–4 doses of intermittent preventative therapy in pregnancy with sulfadoxine-pyrimethamine (IPTp-SP), or intermittent screening with malaria rapid diagnostic tests (RDT) and treatment with dihydroartemisinin-piperaquine (ISTp-DP). Women in the ISTp-DP group with a positive RDT (First Response Malaria

pLDH/HRP2 Combo Test, Premier Medical Corporation) received a standard 3-day course of DP.[58] Follow-up included 3–4 scheduled visits every 4–6 weeks until delivery. Women who felt ill or had concerns about their pregnancy were encouraged to make unscheduled visits. Malaria was assessed at each visit by RDT (ISTp-DP group), microscopy, and real-time polymerase chain reaction (PCR). Women in the ISTp-SP group with uncomplicated clinical malaria (fever or history of fever and positive RDT) received artemether lumefantrine (AL); in the ISTp-DP group, women with uncomplicated malaria received DP, or AL if they had received DP within the preceding 4 weeks[58] Venous blood samples for analyte measurement were collected at each visit (same day), processed, and stored at −80C until shipping. Plasma samples were shipped on dry ice and stored at −80C until analysis. Gestational age was determined by ultrasound at enrollment in the trial.[58] Ultrasound images were obtained using a portable Sonosite™ S180 machine (SonoSite, Inc, Bothell, Washington, USA). Trained research staff obtained ultrasound images of three biometric parameters (biparietal diameter, femur length, and abdominal circumference), and the Hadlock formula was used to calculate gestational age.

The sample size was determined based on availability of plasma samples from the parent trial from women meeting our inclusion criteria. Inclusion criteria from the parent RCT for the present secondary observational cohort study included: enrollment in the parent trial before 24 weeks gestation; availability of a plasma sample from their enrollment visit (13 to 23 weeks) and/or a second visit (28 to 33 weeks)[9]; live birth; and a recorded gestational age at delivery (Figure 1). We focused on these two visits to study the early pathobiology of preterm birth and avoid sampling bias due to delivery before a third visit (34 to 37 weeks gestation). In this cohort, maternal and/or fetal indicated preterm deliveries were carried out via cesarean section; women who delivered preterm by cesarean section (n=8) were excluded from our analysis (Figure 1). All preterm deliveries included in this cohort were spontaneous and therefore PTB refers to spontaneous preterm labour and delivery throughout the manuscript. There were no documented cases of preterm premature rupture of membranes (PPROM); however, preeclampsia or eclampsia were reported as serious adverse events in five cases (n=5/1475; 0.3%). These cases were also excluded from our analysis (Figure 1). Clinical characteristics for those included versus excluded from the larger analyte cohort[9] for the reasons outlined above are outlined in Table S1.

All study participants provided written informed consent. This study protocol was reviewed and approved by ethical review boards at the Liverpool School of Tropical Medicine, the Malawian Health Science Research Committee, and the University of Toronto-UHN.

METHOD DETAILS

Inflammatory and angiogenic analyte measurement

Analyte data were derived as previously described (also presented below) and reanalyzed for this study.[9] While the relationship of these biomarker data with malaria in pregnancy has been reported previously,[9] their temporal relationship to each other, upstream of preterm birth, has not been reported. Briefly, EDTA plasma samples were analyzed for angiogenic and inflammatory factors previously implicated in the pathogenesis of adverse pregnancy outcomes.[14,15,42] Samples were analyzed by multi-analyte Luminex assays (R&D Systems, Minneapolis, MN) for: Angiopoietin-1 (Angpt-1), Angiopoietin-2 (Angpt-2), sEng, sFlt-1, and PlGF (all at 1:2 dilution); Angiopoietin-like protein-3 (Angptl3), soluble Tumor Necrosis Factor Receptor 2 (sTNFR2), and Chitinase-3-like protein-1 (CHI3L1) (all at 1:25 dilution). Samples were also analyzed for C-reactive protein (CRP; 1:50,000 dilution) and Interleukin-18 binding protein (IL18BP; 1:20 dilution) using commercially available enzyme-linked immunosorbent assay (ELISA) kits (DuoSet, R&D Systems, Minneapolis, MN). Assay characteristics (i.e., % coefficient of variance, range of detection) have been previously reported.[9] All laboratory personnel were blinded to the outcome.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis was performed using R version 4.0.3 (R Core Team 2020, The R Foundation for Statistical Computing, Vienna, Austria). Population baseline characteristics were compared between women who delivered preterm and at term by Pearson Chi-square or Wilcoxon rank-sum test. Relative risk (with 95% confidence intervals [CI]) of PTB for women in the highest quartile of each analyte (with the lowest quartile as a reference category) was calculated using log-binomial regression. In cases where the model did not converge, a log-Poisson regression was used. The regression models were adjusted for baseline variables associated with PTB, including blood pressure (systolic) at enrollment, gestational age at plasma collection

for each respective visit, primigravidity (yes/no), socioeconomic status (as a continuous variable), and malaria during pregnancy (by PCR). Treatment group was included as a covariate in all models to account for parent trial study design. Due to their agonist/antagonist relationship and evidence that their ratio may be a stronger predictor of adverse pregnancy outcomes than either marker alone,[61] sFlt1 and PlGF (sFlt1/PlGF) were presented as a ratio for multivariate and mediation analysis.

We conducted multiple mediator analyses to test the potential for causal relationships between inflammation, subsequent dysregulation of angiogenesis, and preterm birth. The predictors were inflammatory analytes at enrolment (13–23 weeks' gestation), the mediators were angiogenic analytes at a subsequent visit (visit 2; 28-33 weeks' gestation), and the outcome was gestational age at delivery. We focused on inflammatory and angiogenic analytes that were associated with increased risk for preterm birth by log-binomial regression. We used the lavaan package in R to run the mediation analyses.[59] To handle missing data, we used full-information maximum likelihood (FIML) estimation. Multiple imputation for missing data (with 10 iterations), using the mice package in R,[60] delivered similar results. To estimate mediation (indirect) effects, we fitted two models per inflammatory marker: (1) regressing both mediators (a_1 and a_2) on each inflammatory marker, and (2) regressing gestational age at delivery on the mediators (b_1 and b_2) and each inflammatory marker (c', which represents the direct effect of inflammation on gestational age at delivery). We investigated interactions between exposure (inflammatory analyte) and mediator (angiogenic analyte) by testing exposure-mediator interaction terms in Equation 2.[62] An interaction term was considered nonzero and added to the final model if its p-value was < 0.05, as previously described.[63] Mediation (indirect) effects of inflammation on gestational age at delivery via angiogenic mediators were estimated using the product of coefficients approach (a_1b_1 and a_2b_2) with 95% bootstrapped confidence intervals. The proportion of total effect accounted for by the indirect effects of inflammatory mediators through angiogenic mediators was calculated as ab/(ab+c'). In the case of inconsistent mediation (ab and c' with opposite signs), absolute values were used to calculate the proportion of total effect, as previously suggested.[64] We included gestational age and blood pressure (systolic) at enrolment, as well as malaria infection during pregnancy (by PCR) as covariates. Indices of model fit are presented in the supplementary (Table S2). Analyte data were log-transformed. We performed a sensitivity analysis of our mediation, using the same analysis on the larger parent analyte cohort (n=1628) to assess whether those excluded from our final cohort (n=1462) significantly influenced the results (Table S3). Statistical significance was defined as p-value < 0.05. Exact p-values have been presented everywhere except in Figure 3 (due to space constraints), which uses asterisks as follows: *p<0.05, **p<0.01, ***p<0.001.

Statistical details for each experiment can also be found in the Table and Figure legends.

ADDITIONAL RESOURCES

The parent malaria prevention trial was registered in the Pan African Clinical Trials Registry PACTR201103000280319 and ISRCTN: ISRCTN69800930.