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Haematological changes from conception to childbirth: An indicator of major pregnancy complications

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Abstract

Background: About 800 women die every day worldwide from pregnancy-related complications, including excessive blood loss, infections and high-blood pressure (World Health Organization, 2019). To improve screening for high-risk pregnancies, we set out to identify patterns of maternal hematological changes associated with future pregnancy complications.

Methods: Using mixed effects models, we established changes in 14 complete blood count (CBC) parameters for 1710 healthy pregnancies and compared them to measurements from 98 pregnancy-induced hypertension, 106 gestational diabetes and 339 postpartum hemorrhage cases.

Results: Results show interindividual variations, but good individual repeatability in CBC values during physiological pregnancies, allowing the identification of specific alterations in women with obstetric complications. For example, in women with uncomplicated pregnancies, haemoglobin count decreases of 0.12 g/L (95% CI −0.16, −0.09) significantly per gestation week (p value <.001). Interestingly, this decrease is three times more pronounced in women who will develop pregnancy-induced hypertension, with an additional decrease of 0.39 g/L (95% CI −0.51, −0.26). We also confirm that obstetric complications and white CBC predict the likelihood of giving birth earlier during pregnancy.

Conclusion: We provide a comprehensive description of the associations between haematological changes through pregnancy and three major obstetric complications to support strategies for prevention, early-diagnosis and maternal care.

KEYWORDS
clinical haematology, complete blood count, longitudinal data, maternal health, mixed effects model

David Baud and Matthew R. Robinson denotes equal contribution.

1 | INTRODUCTION

During pregnancy, women experience physiological changes to facilitate the growing fetus and to prepare for labor.[1] Understanding these changes as well as improving prevention, early-diagnosis and care for women during pregnancy, labor, and postpartum, requires increased efforts to generate data and large reference samples. While reference values for maternal health are established to avoid unnecessary interventions,[2,3] very few studies focus on blood cell count changes from conception to childbirth. Physiological changes, including haematological changes, that may be perceived as pathological outside of pregnancy are poorly understood as the participation of pregnant women is extremely limited in clinical trials[4–6] and large-scale cohort data are lacking. It is therefore important to fully characterize what makes a healthy pregnancy as the pregnancy progresses, to facilitate identification of unusual patterns of obstetric complications and improve the stratification of high-risk pregnancies.

In this study, we used data on 14 complete blood count (CBC) parameters, collected from 2003 to 2009 at the Lausanne University Hospital (CHUV), to establish haematological changes during pregnancy. CBC is routinely performed to assess any abnormal fluctuations in blood values that help to screen for clinical risk factors associated with pregnancy.[7] For example, gestational thrombocytopenia in pregnant women, a common haematologic complication of pregnancy, is identified by a platelet count below 150 000/μl no earlier than 100 days before delivery.[8] Intra- and inter-individual CBC may thus fluctuate during pregnancy and be influenced by maternal life-style factors and pregnancy-related complications (Supplementary Figure S1). To characterize haematological changes, we first set up a reference for the 14 CBC parameters from healthy pregnancies. We then assessed differences in the variation of CBC during pregnancies with three major complications: (i) hypertensive disorders of pregnancy (HDP) including pregnancy-induced hypertension, preeclampsia, hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome and unspecified maternal hypertension, (ii) gestational diabetes mellitus (GDM) and (iii) postpartum hemorrhage (PPH). Finally, we estimate the association of CBC parameters and pregnancy-related complications, namely HDP and GDM, on birth timing using a Cox proportional hazards model.

2 | METHODS

2.1 | Study design and participants

The CHUV maternity cohort aims to study maternal health, and maternal and fetal outcomes. We obtained local canton ethical approval from CER-VD[9] under the project ID 2019-00280 for reuse of data initially collected for a serological surveillance study to investigate the prevalence of maternal and fetal toxoplasmosis infections between 2009 and 2014. The data consists of maternal medical record information for 4347 pregnancies including haematological measures of which CBC taken at prenatal visits and described in Table 1. Our central laboratory uses an automated blood counter (Sysmex XN®), which rely on complementary techniques to determine CBC; (i) photometry after total red blood cell lysis for HB; (ii) impedance for ERY and PLATE counts (cell type being discriminated by size cutoffs), MCV, and MPV; and (iii) flow-cytometry for leucocyte (LEUC) counts and differentiation. Other CBC parameters (HT, MCH, MCHC, RDW) are derived from these measures. The data also includes (i) intrapartum measures (reason for admission, age, maternal weight, maternal height, blood pressure, gestational age, fetal position, fundal height, method of delivery, contraction number, interventions to assist with birth, delivery date and delivery time), (ii) newborn measures (sex, birth weight, birth height, pH of umbilical cord, Apgar score), (iii) ICD-10 classification for diagnosis and medical procedures, and (iv) blood samples from the mother and from the umbilical cord collected at delivery.

TABLE 1 Cell blood counts (unit) included in the study

Type	Name	Description
Red blood cells	ERY (T/L)	Red blood cell count
	HB (g/L)	Total amount of the oxygen-carrying protein in the blood
	HT (%)	Volume percentage of red blood cells in blood
	MCV (fl)	Mean cell volume
	MCH (pg)	Mean corpuscular haemoglobin
	MCHC (g/L)	Mean corpuscular haemoglobin concentration
	RDW (%)	Red blood cell distribution width
White blood cells	LEUC (G/L)	White blood cell count
	ANEUT (G/L)	Absolute number of neutrophils
	ALYMPH (G/L)	Absolute number of lymphocytes
	AMONO (G/L)	Absolute number of monocytes
	AEOSI (G/L)	Absolute number of eosinophils
Platelets	PLATE (G/L)	Platelet count
	MPV (fl)	Mean platelet volume

In this study, we focus on the CBC measures taken throughout pregnancy (Table 1). We considered a longitudinal cohort study of 2253 pregnancies with a single live birth (ICD10 Z370). From these, 42 pregnant women have data for two consecutive pregnancies. The study includes 1710 control pregnancies with single spontaneous full-term uncomplicated delivery live births (ICD10 O80), 98 cases of HDP including pregnancy-induced hypertension, preeclampsia, HELLP syndrome and unspecified maternal hypertension (ICD10 codes O13, O14 and O16), 106 cases of GDM (ICD10 O24) and 339 cases of PPH (ICD10 O72). Still births (ICD10 Z371), liveborn twins (ICD10 Z372), multiple pregnancies (ICD10 O30) and complications specific to multiple gestation (ICD10 O31) were excluded from the analysis. Women with ICD-10 classifications unrelated to pregnancy, childbirth and the puerperium were filtered out excluding pre-pregnancy diseases of which pre-existing hypertension (ICD10 O100). To better compare cases of HDP, GDM and PPH in our analysis, pregnancies

with two or all three complications studied and women with additional ICD-10 classifications for edema, proteinuria, and HDP, delivery and puerperium (O10-O16), other maternal disorders primarily related to pregnancy (O20-O29), polyhydramnios (O40), other amniotic fluid and membrane disorders (O41), placental disorders (O43-O44), and maternal care for fetal abnormality (O35-O36), were excluded. In addition to the ICD10 codes, we also used reports at delivery. Women with an indication of hypertension were added to HDP cases and those with blood loss greater than 500 ml following delivery to PPH cases. The distributions for maternal age at birth, maternal weight at birth, gestational age, parity and nationality of the selected participants are shown in Supplementary Figures S2,S3. Distributions for each CBC parameter included in our study are shown in Supplementary Figure S4 and reported in Supplementary Table S1 and Table S2.

2.2 | Statistical analysis

2.2.1 | Haematological changes throughout low- and high-risk pregnancies

We applied a cubic polynomial regression model with a random intercept for each woman to (i) define a reference for the evolution of CBC measures taken throughout control pregnancies and (ii) to assess CBC changes in women with major obstetric complications. For each CBC measure, we have:

$$\begin{aligned} CBC_{ij} = {} & \gamma_0 + \gamma_1 \cdot I(group_i = GDM) + \gamma_2 \cdot I(group_i = HDP) + \gamma_3 \cdot I(group_i = PPH) + \left(\beta_1 \cdot week_{ij} + \beta_2 \cdot week_{ij}^2 + \beta_3 \cdot week_{ij}^3\right) \\ & + \left(\beta_4 \cdot week_{ij} \cdot I(group_i = GDM) + \beta_5 \cdot week_{ij}^2 \cdot I(group_i = GDM) + \beta_6 \cdot week_{ij}^3 \cdot I(group_i = GDM)\right) \\ & + \left(\beta_7 \cdot week_{ij} \cdot I(group_i = HDP) + \beta_8 \cdot week_{ij}^2 \cdot I(group_i = HDP) + \beta_9 \cdot week_{ij}^3 \cdot I(group_i = HDP)\right) \\ & + \left(\beta_{10} \cdot week_{ij} \cdot I(group_i = PPH) + \beta_{11} \cdot week_{ij}^2 \cdot I(group_i = PPH) + \beta_{12} \cdot week_{ij}^3 \cdot I(group_i = PPH)\right) + Z_i\xi + u_i + \varepsilon_{ij}, \end{aligned}$$

where CBC is the outcome variable, $i = 1, \ldots N$ and $j = 1, \ldots, n_i$, with N the number of women and n_i the number of measurements done for women i. Variable $u_i \sim N(0, \sigma_u^2)$ is the random intercept for individual i and $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$ is the random error for individual's i j th measurement. Z are the covariate values and ξ is a vector with each of the covariate parameters including maternal age at birth, maternal weight at birth, parity, gestational age at birth and nationality. Of these, 0.09% of women had missing nationality, 0.04% had missing values for gestational age and 12.2% of pregnancies had no maternal weight reported. Nationality is categorized into European coded as 0 and non-European coded as 1. Variable *week* is the timing of the CBC measure in gestation week (GW). γ_0 is the intercept parameter, describing the woman's initial blood count value of CBC measure at the start of a control pregnancy. γ_1, γ_2 and γ_3 are the fixed-effect regression coefficients describing the difference from the intercept in GDM, HDP and PPH pregnancy groups. $\beta_1 - \beta_{12}$ are the fixed-effects regression coefficients of the polynomial terms in each pregnancy group.

When dividing CBC measurements by trimester, 581 measurements were taken up to GW 14, 480 between GW 15 and 28 and 3196 after GW 28 in control pregnancies. We included cases of HDP with 35, 44, and 677 CBC measurements collected in trimesters 1, 2, and 3, respectively; cases of GDM with 47, 31, and 289 CBC measurements collected in trimesters 1, 2, and 3, respectively; and cases of PPH with 112, 102, and 1229 CBC measurements collected in trimesters 1, 2, and 3, respectively (Supplementary Figure S4b). To assess CBC changes in women with the latter obstetric complications, we specified an interaction term between the timing of CBC measurements and a categorical variable specifying the pregnancy group. CBC measures, parity, gestational age, maternal age and weight at birth were centered and scaled with respect to their standard deviation prior analysis. Missing values were imputed using multiple imputation with predictive mean matching in the R package *mice*[10] and including case–control groups, maternal age at birth, the newborn's weight and height and a binary variable for premature birth (gestational age <37 weeks) in the imputation model. We analyzed 5 sets of complete data and pooled the results. Differences in the evolution of CBC between control and high-risk pregnancies are shown in Figure 1. Complete results from each polynomial regression model are reported in Supplementary Table S3 and S4. We primarily chose the cubic polynomial as it had the lowest Akaike information criterion (AIC) and Bayesian information criterion (BIC) for most CBC, reflecting a flexible but parsimonious number of parameters (Supplementary Table S5). We additionally applied a linear mixed effect model with random intercepts for each pregnancy describing woman's initial blood count value, similarly to the cubic polynomial model, as follows:

$$\begin{aligned} CBC_{ij} = {} & \gamma_0 + \gamma_1 \cdot I(group_i = GDM) + \gamma_2 \cdot I(group_i = HDP) \\ & + \gamma_3 \cdot I(group_i = PPH) + \beta_1 \cdot week_{ij} \\ & + \beta_2 \cdot week_{ij} \cdot I(group_i = GDM) \\ & + \beta_3 \cdot week_{ij} \cdot I(group_i = HDP) \\ & + \beta_4 \cdot week_{ij} \cdot I(group_i = PPH) + Z_i\xi + u_i + \varepsilon_{ij}, \end{aligned}$$

where the coefficients $\beta_1 - \beta_4$ give us an overview of the direction of change during pregnancy and the additional effect of having one of the three obstetric complications (Figure 2 and Supplementary

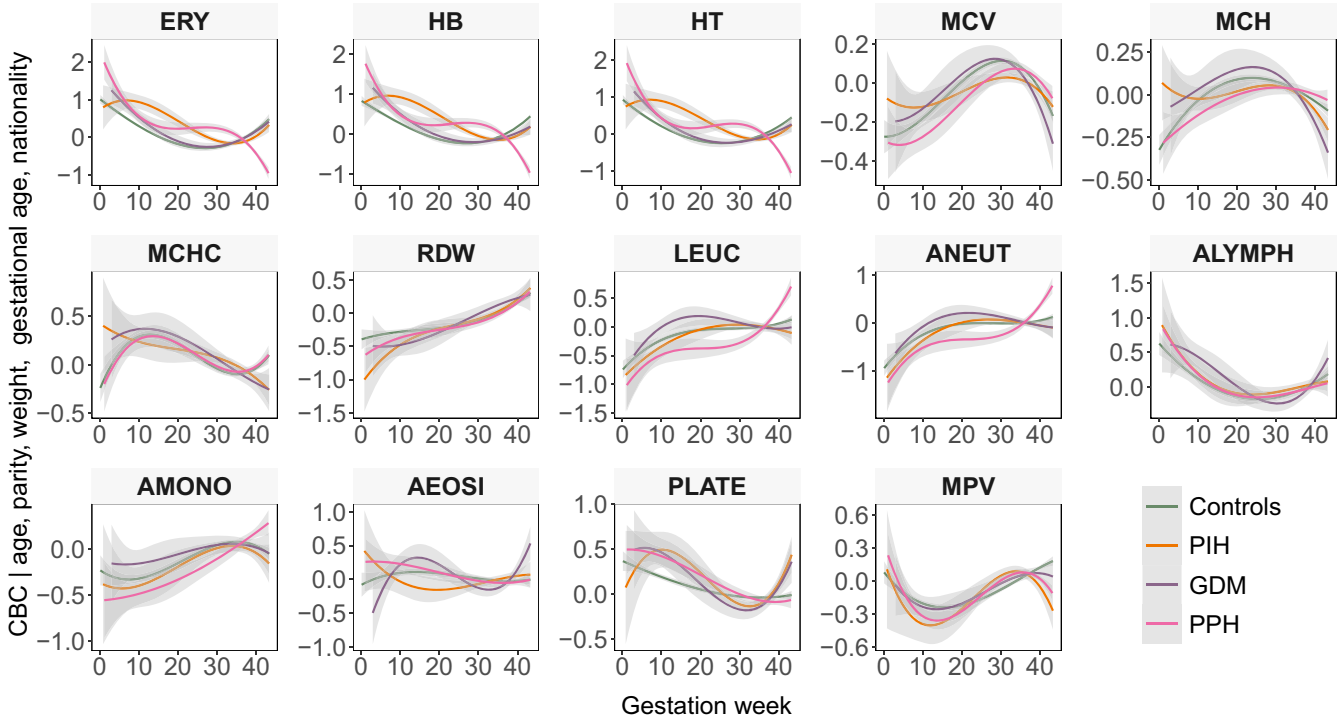

FIGURE 1 Haematological changes in major pregnancy complications. Cubic polynomial slopes showing the course of 14 cell blood counts (CBC) in control pregnancies, pregnancies with gestational diabetes mellitus (GDM), pregnancies with induced hypertension (HDP) and pregnancies resulting in postpartum hemorrhage (PPH). Slopes are adjusted for maternal age and weight at delivery, parity, gestational age and nationality. 95% CI are displayed in grey around the slopes. CBC parameters and covariates are centered and scaled with respect to their standard deviation. In control pregnancies, red CBC (ERY) and platelets (PLATE) decrease compared to white CBC (LEUC), which gradually increases overtime. Results show different patterns for HDP, GDM and PPH with a greater difference between the course of CBC in control and in HDP and PPH pregnancies. For instance, we observe non-overlapping 95% CI in ERY, HB and HT mean counts between control pregnancies and pregnancies complicated by HDP or by PPH.

Figure S5). Regression coefficients from each linear mixed effect model are reported in Supplementary Table S6. Linear and polynomial models are fit using the R package *lme4*.[11]

2.2.2 | Effect of CBC, HDP, and GDM on birth timing

We fit a time-dependent covariate Cox proportional-hazards model[12] to describe how CBC parameters and obstetric complications jointly influence the hazard rate of birth at a particular point in time. The model is expressed as follows:

$$h_i(t) = h_0(t) \cdot exp\left(\beta_1 \cdot X_1^i + \beta_2 \cdot X_2^i + \ldots + \beta_p \cdot X_p^i\right),$$

where t represents the pregnancy time, $h_i(t)$ is the hazard function for individual i, which can vary overtime and can be interpreted as the risk of labor at time t. The regression coefficients $\beta_1, \beta_2, \ldots, \beta_p$ measure the effect size of p covariates. h_0 is the baseline hazard describing how the risk of birth changes over time at baseline levels of covariates. To select the most adequate predictors to include in the model, we applied a backward stepwise selection model using the BIC. Backward stepwise selection performs model comparison by removing predictors included in the model and evaluating the BIC of models of decreasing complexity until the most optimal model is reached. Maternal age at birth, maternal weight at birth, parity, nationality, the 14 CBC parameters in Table 1 and a categorical variable for controls, GDM and HDP pregnancies, were considered in the stepwise analysis. Maternal weight and parity were selected and included in the multivariate analysis as time-constant covariates. From the CBC parameters, HB, RDW, LEUC, absolute lymphocyte (ALYMPH), absolute neutrophil (ANEUT), and PLATE levels were retained. The pregnancy groups were also added to the multivariate analysis setting the control group as the reference. Complete results from the multivariate Cox proportional hazards model are reported in Supplementary Table S7 and the hazard ratios (HR) are shown in Figure 3. The HR measures the likelihood of women whose pregnancy is complicated by GDM or HDP, to give birth at time t compared to controls. For the continuous variables, the HR reflects the hazard of birth at time t if the variable in question increases by one unit. Cox proportional-hazards models are fit using the R package *survival*.[13]

Finally, we further investigated significant associations found between time to birth, pregnancy complications and CBC measurements by exploring the number of C-sections and oxytocin

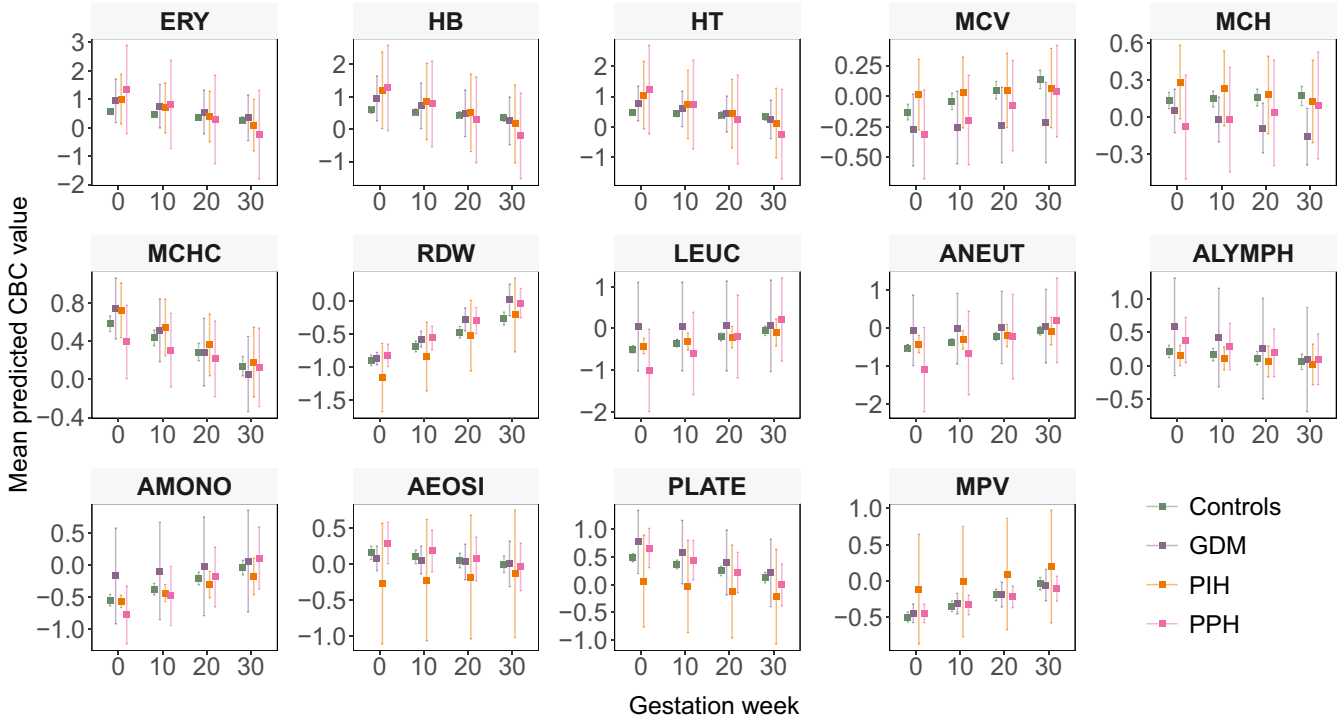

FIGURE 2 CBC prediction of each group across pregnancy. Mean predicted CBC values of control, gestational diabetes mellitus (GDM), hypertensive disorders in pregnancy (HDP) and postpartum hemorrhage (PPH) pregnancy groups at week 0, 10, 20, and 30. The error bars show the variance of the prediction error, except at week 0, where we show (i) the estimated mean intercept and 95% CI for the control group and (ii) the predicted mean values and the prediction error variance for the case groups by adding the estimated effect of pregnancies complicated by either GDM, HDP or PPH to the estimated control intercept. At week 10, 20, and 30, the predicted CBC value in control groups is computed by adding the estimated intercept and the estimated effect of time in weeks times gestation week = (10 or 20 or 30). To predict values in a pregnancy affected by one of the three complications, we also sum the effect of time in weeks for the group of interest times gestation week = (10 or 20 or 30). We observe clear differences in the groups, that is, in the count of AMONO in case pregnancies compared to the reference mean predicted values in green, as pregnancy progresses.

administration in pregnancy complications. We also explored the proportion of obstetric infections and premature rupture of membranes (PROM) using ICD-10 codes reported in each pregnancy group (Supplementary Figure S6).

3 | RESULTS

3.1 | Haematological changes throughout low- and high-risk pregnancies

Firstly, we investigated haematological changes during pregnancies with single spontaneous full-term uncomplicated delivery live birth (ICD10 code O80) and compared them to values in pregnancies complicated by either GDM, HDP or PPH. For this purpose, a cubic polynomial regression was applied with (i) an interaction between time and a categorical variable indicating the pregnancy group, (ii) a number of covariates and (iii) random intercepts for each pregnancy to model individual-level differences in repeated blood count values. We identify the following changes at a p value <.0036 adjusting the significance level with the Bonferroni correction for the 14 phenotypes tested (Supplementary Table S1, S2, S3).

Figure 1 depicts how CBC parameters change from the first to the third trimester, while Figure 2 shows CBC predictions at GW 0, 10, 20, and 30 using estimates from the linear mixed effect model. In control pregnancies, we observe a decrease in the second trimester followed by an increase in the third trimester for erythrocyte parameters (HB, HT, and ERY), a decrease in platelets (PLATE), and an increase in LEUC. The same patterns are delineated when the linear mixed effect model is applied to estimate an overall direction of haematological changes during pregnancy (Supplementary Figure S5). First, we found that HB levels significantly decrease by 0.008 (95% CI −0.011, −0.006), HT by 0.005 (95% CI −0.007, −0.003) and ERY by 0.01 (95% CI −0.012, −0.008) per GW. We also observe that the mean corpuscular hemoglobin (MCH) is not significantly altered, while the mean red cell volume (MCV) shows a marginal increase, explaining a marginal decrease in the MCH concentration (MCHC) across pregnancy. Lastly, red blood cell distribution width (RDW), which measures the change in red blood cell size, increases continuously as pregnancy progresses. Second, PLATE count also decreases by 0.012 (95% CI −0.014, −0.010), whereas the mean platelet volume (MPV) decreases and then increases in the second trimester. And third, LEUC counts increase by 0.015 (95% CI 0.012, 0.017). Among leucocytes, the polynomial slope for ANEUT count increases early in pregnancy

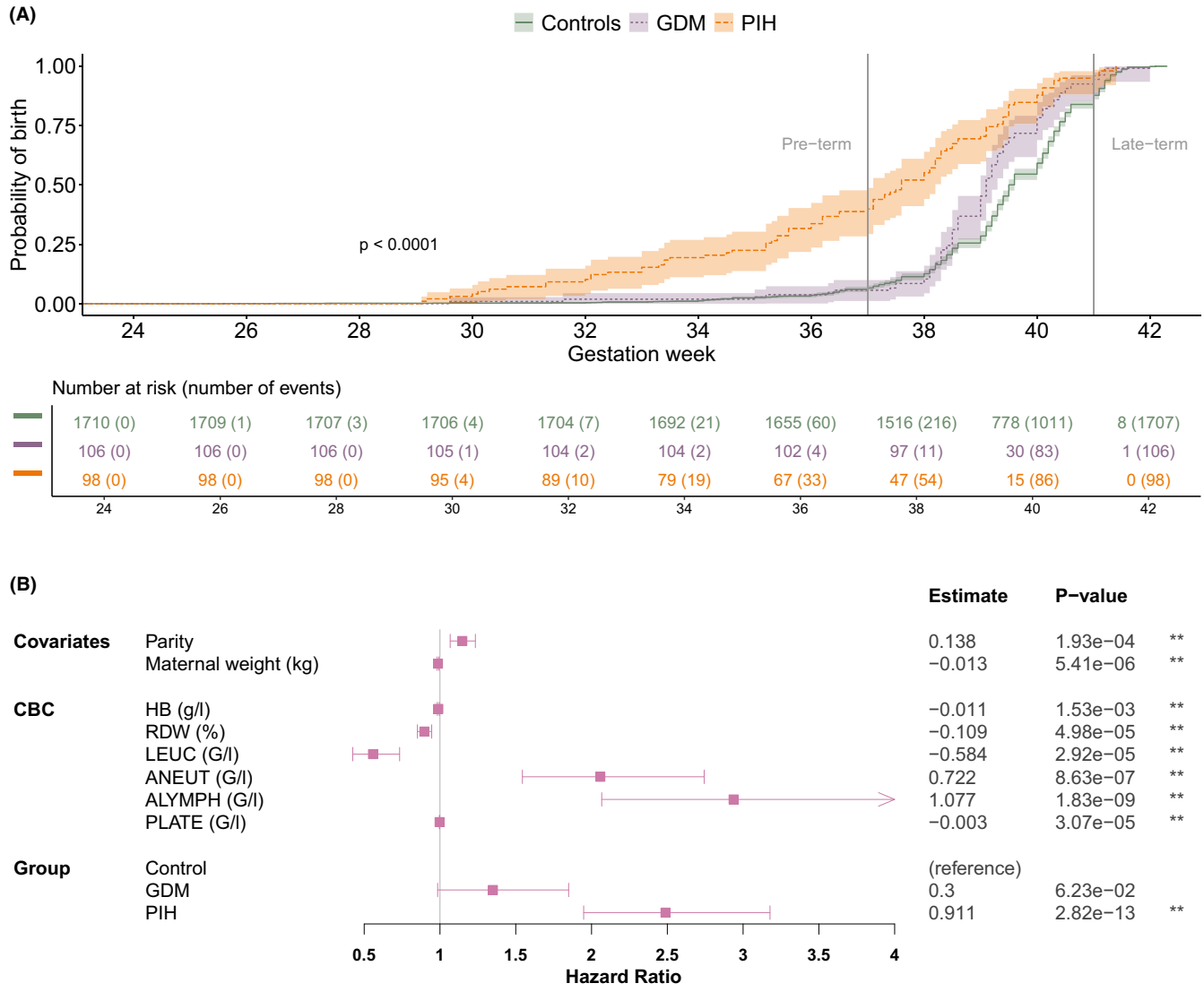

FIGURE 3 Time-to-birth analysis. (A) Cumulative incidence plot showing the probability of giving birth throughout controls and high-risk pregnancies including gestational diabetes mellitus (GDM) and pregnancy-induced hypertension (HDP). The cut-off point for a preterm and late-term delivery is indicated by the grey lines. The plot also includes a risk table with the number of women susceptible to give birth and the cumulative number of events at a given gestation week. The log-rank test *p* value <.0001 indicates that birth timing is significantly different between our groups. Pregnancies with HDP are found to have a higher probability of preterm birth. (B) Forest plot showing the hazard ratio with 95% confidence intervals, estimates and p-values associated with variables included in the cox proportional hazards model. HDP pregnancies give birth 2.5× the rate per unit time compared to control pregnancies (p value $= 6.33e-13$). We also observe that lymphocyte (ALYMPH) and neutrophil (ANEUT) counts increase the probability of giving birth, with a hazard by a factor of 2.9× ($p-$value $= 2.97e-09$) and 2× (p value $= 1.09e-06$) respectively. This might reflect the presence of an infection, which is one of the main causes of spontaneous preterm delivery, or a reactive neutrophilia and lymphocytosis accompanying the causal event for birth.

while absolute monocyte (AMONO) count increases from the second trimester onward. ALYMPH count decreases marginally early in pregnancy. The linear estimation confirms these trends with a significant increase by 0.015 (95% CI 0.013, 0.018) in ANEUT, 0.017 (95% CI 0.015, 0.019) in AMONO, and a decrease by 0.005 (95% CI −0.008, −0.003) in both ALYMPH and absolute eosinophil count (AEOSI) per GW.

In pregnancies complicated by either GDM, HDP or PPH, we find statistically significant differences at *p* value <.0036, in the polynomial terms for all CBC except ALYMPH and RDW (Supplementary Table S1). We observe group specific differences in the polynomial slopes, especially early in pregnancy, between GW 10 and 20 (Figure 1 and Supplementary Figure S7). As with the polynomial curves, CBC values, predicted from the linear mixed-effect model, vary overtime and are subject to changes specific to women with at-risk pregnancies (Figure 2). For pregnant women who will develop HDP, we find that erythrocyte parameters are approximately 2-fold higher compared to controls at GW 0, and that they decrease significantly faster during pregnancy with the following interaction effect sizes: −0.026 (95% CI −0.035, −0.018) for HB, −0.026 (95% CI -0.035, −0.17) for HT

and − 0.021 (95% CI −0.029, −0.012). PLATE count is significantly lower by −0.42 (95% CI −0.70, −0.14) at the start of pregnancy but although not significant, the decrease is slower in women who will develop HDP compared to controls (Figure 2, Supplementary Figure S5). We find similar trends in pregnancies leading to PPH with approximately 3-fold higher values in erythrocyte parameters at GW 0, which also decrease significantly faster: −0.041 (95% CI −0.046, −0.036) for HB, −0.044 (95% CI -0.049, −0.039) for HT, −0.043 (95% CI −0.048, −0.038) for ERY. PPH pregnancies also have 2-fold lower values LEUC and ANEUT counts at GW 0. As pregnancy progresses, these counts and AMONO increase significantly faster with interaction effect sizes: 0.026 (95% CI 0.020, 0.031) for LEUC, 0.028 (95% CI 0.023, 0.033) for ANEUT and 0.013 (95% CI 0.007, 0.018) for AMONO (Figure 2, Supplementary Figure S5). Finally, women diagnosed with GDM are distinguished by approximately 2-fold higher LEUC and ANEUT counts at GW 0. Interestingly, LEUC count remains constant throughout the pregnancy as it significantly decreases by 0.014 (95% CI: −0.022, −0.005) counteracting the increase of 0.015 found in the control pregnancies. We also note that their number of HB and HT decreases by −0.015 (95% CI −0.023, −0.007) and − 0.013 (95% CI −0.021, −0.004) with each passing week of pregnancy (Figure 2, Supplementary Figure S5).

Random intercepts for individuals fitted within each polynomial model revealed that 30.9%–84.6% of the variance is attributed to inter-individual differences indicating that the pregnant women differ in their initial blood cell count (Supplementary Table S5). The proportion of total variance attributable to this term also informs us about individual repeatability, that is, how similar observations of an individual are as compared to the rest of the population, as the pregnancy progresses.[14] For instance, 84.6% of the variance in MCV measurements would be explained by the random intercept and thus the rate of change for those measurements are likely to be the same for all women. We also find variation in blood measure with maternal age and weight at delivery, parity, nationality and gestational age at birth (Supplementary Figure S8). Estimates of the effect of nationality on the 14 CBC have a wider confidence interval than the other covariates, which could imply a broader genetic background in individuals than that described by their nationality. Compared to other CBC, RDW is more affected by higher parity with an increase of 0.16 (95% CI 0.13, 0.20). We also observe a negative effect with a 1-SD increase in gestational age on LEUC count which appears to be driven by ANEUT and ALYMPH with a decrease of −0.21 (95% CI −0.25, −0.17) and −0.18 (95% CI −0.22, −0.14) respectively. Finally, maternal age and weight show significant opposite effects on ERY, MCV, MCH, MCHC, RDW, and ALYMPH counts.

3.2 | Effect of CBC, HDP, and GDM on birth timing

Using a time-dependent covariate Cox-proportional hazards model, we assess how CBC measurements taken throughout pregnancy, GDM and HDP obstetric complications jointly influence birth timing measured as gestational age at birth. To select the most adequate multivariate model, we used backward step-wise selection and the final model used for the analysis included maternal weight, parity, HB, RDW, LEUC, ALYMPH, ANEUT, and PLATE CBC parameters, and the case–control categorical variable for HDP, GDM, and control pregnancies.

Kaplan Meier curves in Figure 3A describe the probability of birth from GW 24 to 42 in control pregnancies and in cases of GDM and HDP. The median gestational age at delivery is 39.55 (95% CI 39.50, 39.60) in controls and 39.10 (95% CI 39.00, 39.30) in GDM-complicated pregnancies, both being within the limits of what is considered to be full-term delivery; as opposed to 37.60 GW (95% CI 37.10, 38.20) in HDP-complicated pregnancies, which lies very close to the cutoff for preterm delivery. Indeed, 39.8% of HDP-complicated pregnancies resulted in birth ≤37 GW and, although ICD10 code O80 indicates single spontaneous full-term uncomplicated delivery in controls, only 6.6% of healthy and 5.7% of GDM-complicated pregnancies delivered prematurely. Furthermore, at GW 41, 16.1% of controls, 7.5% of GDM and 5.1% of HDP pregnancies are still ongoing and can be classified as late deliveries. In agreement with the Kaplan Meier curves, we find that the hazard of birth is 2.49 (95% CI 1.95, 3.18; p value = 2.82e-13) times higher in pregnancies complicated by HDP and, although not significant, the hazard of birth was found to be 1.35 (95% CI 0.98, 1.85) times higher in pregnancies complicated by GDM (Figure 3B). Moreover, the hazard of giving birth at a given time rises by 2.94 for 1 G/L increase in ALYMPH (95% CI 2.07, 4.17; p value = 1.83e-09) and by 2.06 for 1 G/L increase in ANEUT (95% CI 1.54, 2.74; p value = 8.63e-07). We also find that for one unit increase in HB, RDW and LEUC cell counts, the hazard of giving birth falls by 0.99 (95% CI 0.983, 0.996; p value = 1.53e-03), 0.90 (95% CI 0.85, 0.95; p value = 4.98e-05) and 0.56 (95% CI 0.42, 0.73; p value = 2.92e-05) respectively. Finally, the hazard of birth increases by 1.15 (95% CI 1.07, 1.23; p value = 1.93e-04) for one unit increase in parity and decreases by 0.99 (95% CI 0.98, 0.99; p value = 5.41e-06) for one unit increase in maternal weight.

We further investigate the association found between time to birth and HDP complication by exploring the number of C-sections and oxytocin administration reported. Of the 98 HDP cases in our study, 67.3% resulted in a C-section, 22.6% received oxytocin to induce or to accelerate labor and 10.2% gave birth without either intervention. Out of 39 preterm deliveries in the HDP group, only 3 infants were born by natural labor. We also investigated ANEUT and ALYMPH associations with time to birth, looking at the proportion of obstetric infections and PROM in each pregnancy group (Supplementary Figure S6). Using the reported ICD-10 codes in our sample, 0.7% of patients had an infection during pregnancy and we find ≤2% of infections per pregnancy group. On the other hand, we observe 15.3% of PROM, of which 7.9% are preterm PROM.

4 | DISCUSSION

In this study, we extensively describe the haematological changes that occur during healthy pregnancies and compare them with pregnancies involving three major obstetric complications, namely GDM, HDP and

PPH. Maternal data are also analysed in a novel manner, by exploring the effect of obstetric complications and CBC measurements throughout pregnancy, on gestational age at delivery.

Our results are in line with previous studies on haematological changes in healthy pregnancy. Blood volume is known to increase by about 1.5 L throughout pregnancy. Erythrocyte count increases due to a greater erythropoietin production[15]; however, the volume of plasma increases proportionally more, thus pregnant women present a net decrease in red blood cell parameters, notably haemoglobin and haematocrit in the second trimester, followed by a stabilization in the third.[1,15] Leucocyte counts have been shown to rise mainly due to neutrophilia and monocytosis caused by the physiologic stress imposed during pregnancy, alongside a decrease in lymphocytes.[15–17] Finally, previous studies have also shown a decrease in platelet count.[1,15,18] Here, we confirm and refine the description of these physiological changes. More importantly, we explore how repeated monitoring of CBC can help identify complications. Compared to healthy pregnancies, we found significant differences in the variation of CBC parameters throughout pregnancies complicated by GDM, HDP or PPH, each having a unique alteration pattern. We thus demonstrate that assessing haematological changes has the potential to timely identify women who will develop obstetric complications. Recent findings show that CBC can help predict the risk of PPH[14] and GDM.[19] Regarding HDP, few studies have used platelet count to predict preeclampsia alone.[20–22] An increase in hematocrit count between the first and second trimester has also been shown to be predictive of preeclampsia as well as other pregnancy outcomes such as fetal growth restriction.[23,24] In our analysis, we identify the 10–20 week of gestation as the most informative period for identifying these complications. This information brings novelty to the field as it indicates a specific time frame, early in pregnancy, that we can focus on to predict and identify maternal complications occurring weeks later and improve prenatal care. For instance, we demonstrate that routine red blood cell and platelet count are sensitive enough to identify pathophysiological mechanism occurring early in the course of the pregnancy that will later lead to the development of HDP. The observed pattern of significantly accelerated decrease in red blood cell and platelet counts, is consistent with low-grade thrombotic microangiopathy, which takes many forms during pregnancy including preeclampsia and the HELLP syndrome[25,26]; however further research is needed to establish this hypothesis. In current clinical practice, it is not usual to continuously measure CBC values during pregnancy. However, our results indicate a pattern of change in CBC values that differs in women with negative pregnancy outcomes, as opposed to healthy pregnancies. This suggests that more routine CBC testing throughout pregnancy may contribute to earlier diagnosis. Finally, as in[14], we observe that postlabor complications can also be related to what happens during pregnancy.

When exploring associations between time to birth, pregnancy complications and CBC measurements, we find that HDP significantly increases the hazard of birth and thus may shorten pregnancy duration compared to controls regardless of the GW. Among the HDP cases, we observe that few pregnant women, three of whom gave birth before GW 37, delivered without C-section or use of oxytocin. It is thus likely that our results reflect medically indicated births due to obstetric guidelines, recommending induction of labor in women who develop hypertension; and that onset of HDP before GW 37 increases the likelihood of preterm delivery. With regards to the CBC results, we show that the hazard of giving birth rises with higher values of ALYMPH and ANEUT. As both cell types are mobilized by the immune system in the presence of pathogens,[27,28] results may reflect an underlying subclinical infection. Using reported ICD-10 codes, we find less than 1% of obstetric infections. However, we observe 15.3% of PROM, a complication associated with infections in pregnancy.[29,30] Following PROM, if labor does not begin spontaneously within 24 h, obstetric guidelines recommend induction of labor. Of the reported cases, 52% are preterm PROM thus resulting in preterm birth. Additionally, physiological changes during labor that have been described as inflammatory reactions,[31,32] and treatments such as steroids in preparation of preterm birth, are additional factors that may lead to significant changes in white blood cell counts. We believe, further research is needed to investigate hematological changes specifically during labor and in complications that play a key role in the timing of birth. With regards to parity, a recent study showed that women in their first pregnancy are at greater risk of spontaneous preterm birth compared to women in their second pregnancy; and that the risk increases steadily in multiparous women.[33] We find that for every new pregnancy, the hazard of birth increases by 14.7% and so, it would be interesting to deconstruct our analysis and compare different parity status in a larger sample size.

Although the CHUV maternity cohort is phenotypically rich, the main limitation in our study is the heterogeneity in the data collection. First, although we have repeated measures throughout pregnancy, these have not been collected at similar time points for each pregnancy and 75.1%–89.6% of measures are taken in the third trimester (Supplementary Figure S4b). Our results are thus more reliable in the end of pregnancy where we can confidently establish specific ranges for each cell blood count (Supplementary Table S1), and we believe that a more complete dataset from the beginning of pregnancy would show a more pronounced difference between the groups. Second, the time of onset of the various pregnancy complications was not reported in the CHUV maternity cohort. We have also used a broad definition of HDP including unspecified hypertension in pregnancy to optimize the number of cases in our study. With a more complete data set and an increase in sample size, we would be able to further investigate whether haematologic changes in early pregnancy are also dependent on the timing of pregnancy complications. A larger sample size is also required to determine the prediction accuracy when predicting these complications from longitudinal CBC measures taken between the 10 and 20 week of gestation. Third, the data was collected 10 years ago and guidelines in maternal health have evolved since then. These three limitations emphasize the importance of data collection in a rapidly changing field. Finally, as obstetric complications have a multi-factorial etiology including a number of medical interventions, bigger sample sizes of diverse ancestries and genetic data are

required to (i) investigate maternal risk factors, (ii) better predict and understand obstetric complications, (iii) stratify women that are at risk and (iv) develop risk specific guidelines. Fortunately, maternal health is increasingly becoming part of the research agenda. Available data and collaborations to improve maternal care are increasing and we are currently filling the gaps in the field.

AUTHOR CONTRIBUTIONS

MP and MRR conceived and designed the study. MP conducted the analysis with contributions from MRR, DB, MS, SEO and ZK. MRR and DB provided study oversight, and DB contributed data. MP drafted the paper and all authors reviewed and approved the final manuscript prior to submission.

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Data sources are available through joint research agreements with the corresponding authors. All summary data from the manuscript are available and shared in the supplementary tables.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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