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Maternal hemoglobin and hematocrit levels during pregnancy and childhood lung function and asthma. The Generation R Study

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Abstract

Objective: To examine the associations of maternal hemoglobin and hematocrit levels during pregnancy with childhood lung function and asthma, and whether adverse pregnancy outcomes and atopic predisposition modify the associations.

Methods: In a population-based prospective cohort study among 3672 subjects, we measured maternal hemoglobin and hematocrit levels in early pregnancy, and lung function by spirometry and current asthma by questionnaire at age 10 years.

Results: Higher maternal hematocrit levels, both continuously and categorized into clinical cut-offs, were associated with lower forced expiratory flow at 75% of forced vital capacity (FEF_{75}) in children (Z-score (95%CI): −0.04 (−0.07, −0.01), per increase of 1 SDS in hematocrit level; Z-score (95%CI) difference: −0.11 (−0.20, −0.03) compared with normal hematocrit levels, respectively), taking lifestyle and socio-economic factors into account. Adverse pregnancy outcomes and atopic predisposition did not modify the results. No associations of maternal hemoglobin and hematocrit with current asthma were observed.

Conclusion: Higher maternal hematocrit levels during pregnancy are associated with lower childhood lung function but not with risk of asthma. Adverse pregnancy outcomes and atopic predisposition do not modify these associations. Underlying mechanisms need to be further studied.

KEYWORDS
asthma, childhood, hematocrit, hemoglobin, lung function, pregnancy

1 | INTRODUCTION

Preterm birth and low birth weight are associated with lower lung function and increased risk of asthma in childhood.[1–5] These associations might be explained by intrauterine mechanisms such as an inadequate oxygen and nutrients supply from mother to the fetus[5–7] represented by lower or higher maternal hemoglobin and hematocrit status during pregnancy.[8–10] Lower or higher maternal hemoglobin and hematocrit levels could lead to insufficient oxygen supply to the fetus,[11] respectively, due to an increased hemodilution, or to an increased blood

viscosity resulting in an impaired blood flow through the placenta.[12] Subsequently, insufficient oxygen supply could affect fetal growth and lung development,[5,6,13–15] and increase the risk of chronic obstructive respiratory diseases in later life.[16,17]

Human cohort studies that examined associations of maternal hemoglobin and hematocrit status with asthma-related outcomes in childhood are scarce, and report inconsistent results.[16,17] A prospective cohort study among 597 children and their mothers showed that maternal anemia based on hemoglobin levels at any time during pregnancy was associated with an increased risk of infant wheezing and childhood asthma.[16] In contrast, we previously observed no association of maternal hemoglobin levels during pregnancy with wheezing from birth to age 6 years, ever physician-diagnosed asthma, or change in respiratory resistance (Rint) and fractional exhaled nitric oxide (FeNO) at 6 years.[17] Differences in results of these studies might be explained by differences in type of measurement of hemoglobin or hematocrit levels, definitions of anemia such as hemoglobin level lower than 11 or 10.5 g/dL, type of lung function measures such as Rint, FeNO, or peak expiratory flow, definitions of asthma such as wheezing, ever or current asthma, ages at time of assessment, or adjustment for confounders. Moreover, the role of socio-economic and lifestyle factors, adverse pregnancy outcomes and atopic predisposition on the association of maternal hemoglobin and hematocrit status during pregnancy with asthma-related outcomes in children is not fully clear.[4,18]

Therefore, we examined the associations of maternal hemoglobin and hematocrit levels during pregnancy with lung function measures and asthma among 3672 children participating in a population-based prospective cohort, and whether these associations were explained by lifestyle and socio-economic factors or modified by adverse pregnancy outcomes and atopic predisposition.

2 | METHODS

2.1 | Design

This study was embedded in the Generation R Study, a prospective population-based cohort study from early fetal life onwards in Rotterdam, the Netherlands.[19–21] The study was approved by the Medical Ethical Committee of the Erasmus MC, University Medical Centre Rotterdam, the Netherlands. Written informed consent was obtained from all participants. A total of 3672 mothers and their children were included for the current analyses (Fig. 1).

2.2 | Maternal hemoglobin and hematocrit levels during pregnancy

As previously described, we assessed maternal serum hemoglobin and hematocrit levels from fresh EDTA plasma samples obtained by maternal venous blood samples at enrolment.[15] Of all blood samples, >80% were obtained before 18 weeks of gestation (median 14.6 weeks [5-95% range: 11.0-22.7 weeks]). Samples were analyzed at STAR Medisch Diagnostisch Centrum (Rotterdam, the Netherlands).[19,22] Using the World Health Organization (WHO) criteria,[23] anemia was defined as a serum hemoglobin level (Hb) <6.83 mmol/L or hematocrit level (Ht) <33%, which in our study population reflected the lowest 12.3%. Similarly, elevated serum hemoglobin and hematocrit levels were defined as the upper 12.3% of the study population resulting in a hemoglobin higher than 8.19 mmol/L or hematocrit higher than 39%. Normal levels were defined as a serum hemoglobin level between 6.83 mmol/L and 8.19 mmol/L or hematocrit level between 33% and 39%. Standard deviation scores (SDS) were constructed for hemoglobin and hematocrit levels and used as continuous variables in our regression models to obtain a more reliable comparison of the hemoglobin and hematocrit levels within the data from the study population.

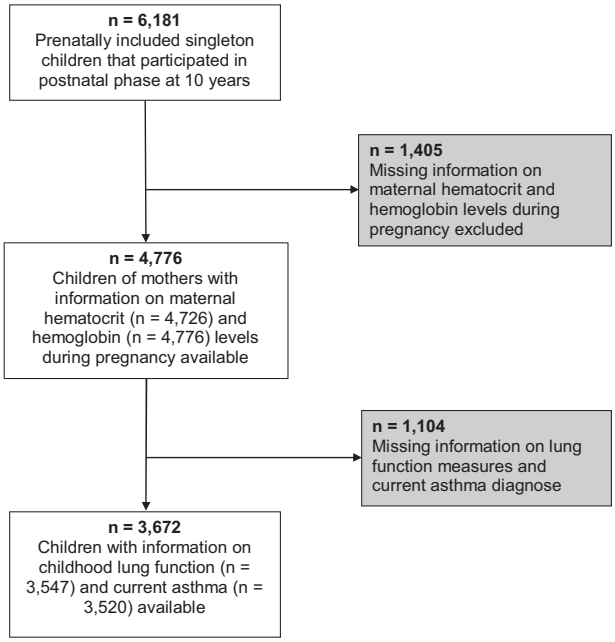

FIGURE 1 Flow chart of participants included for analysis

2.3 | Lung function and asthma

Spirometry was performed according to the American Thoracic Society and European Respiratory Society recommendations[24] in 9.8-year- (range: 8.6-12.0 years) old children. Lung function measures included forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), FEV_1/FVC, forced expiratory flow at 25-75% of FVC (FEF_{25-75}) and forced expiratory flow at 75% of FVC (FEF_{75}), and were converted into sex-, height-, age-, and ethnicity-adjusted Z-scores according to the Global Lung Initiative reference data.[25] Ever physician-diagnosed asthma, wheezing, and use of inhalant medication in the past 12 months were reported by a parental questionnaire at age 9.8 years, adapted from the International Study on Asthma and Allergy in Childhood (ISAAC).[26] Current asthma was defined as ever physician diagnosed asthma, with either wheezing or the use of inhalant medication in the past 12 months.

2.4 | Covariates

Information on maternal characteristics included age, parity, ethnicity, educational level, pre-pregnancy body mass index, smoking during pregnancy, history of asthma and atopy, gestational hypertensive disorders, folic acid supplementation during pregnancy, and alcohol consumption during pregnancy. All data were obtained from questionnaires during pregnancy or from midwife and hospital records at delivery.[19] Mode of delivery and child's sex, gestational age at birth, and birth weight were obtained from midwife and hospital records at birth.

2.5 | Statistical analysis

Independent *T*-test and chi-square test were used to compare all characteristics of our population under study with those with missing information on maternal hemoglobin and hematocrit levels during pregnancy or those with missing information of lung function measures and current asthma. For association analyses, we examined the associations of maternal hemoglobin and hematocrit levels during pregnancy with childhood lung function measures and asthma using linear and logistic regression analysis. First, this model was adjusted for gestational age at which maternal blood sampling occurred (crude model).[15] Second, we additionally adjusted for socio-economic factors such as maternal age, parity, educational level, and pre-pregnancy BMI (model 1), and lifestyle factors such as folic acid supplementation and alcohol consumption during pregnancy (model 2). Third, we adjusted for all socio-economic and lifestyle factors (full model). Confounders were selected based on literature, if they were associated with hemoglobin and hematocrit levels and with lung function or asthma, or if the effect estimate of the crude analyses changed ≥10% when we additionally adjusted for the confounder. Missing data in covariates were imputed by the multiple imputation method using chained equations to select the most likely value for a missing response.[27] When data are missing not at random, bias in analysis based on multiple imputation may be as large or larger than bias in analysis of complete cases Missing data of covariates were imputed to reduce bias and improve efficiency. Multiple imputation analysis aim to avoid bias only if enough variables predictive of missing values are included in the imputation model.[28] Therefore, we included variables used in the multivariate models, and additionally important variables that could correctly predict the missing values including socio-economic factors (family income, paternal education level, maternal ethnicity, and child day care attendance in the first year of life) and biological factors (maternal smoking, atopy and asthma family history, mode of delivery, gestational age at birth, breastfeeding, and maternal weight and height at enrolment). Non-linear associations were tested by adding the quadratic term for hemoglobin and hematocrit levels in the models. We used similar regression analyses for the associations of low and elevated hemoglobin and hematocrit levels with lung function measures and current asthma, using normal hemoglobin and hematocrit levels as reference categories. Last, effect modification due to adverse pregnancy outcomes (caesarian section, lower gestational age, or weight at birth), and atopic predisposition factors (maternal history of asthma or atopy and child's allergic sensitization) were examined in the fully adjusted models by adding the product term between the determinant and the covariate in the statistical models. We used the F-statistic to test the model fit of models with and without interaction terms. *P* values for F-statistics <0.05 were considered significant, indicating a different model fit when comparing the models with and without interaction terms. All measures of association are presented with their 95% Confidence Intervals (95%CI). Statistical analyses were performed using SPSS version 21.0 for Windows software (SPSS Inc, Chicago, IL).

3 | RESULTS

Maternal and child characteristics are presented in Table 1. Mean maternal hemoglobin and hematocrit levels were 7.56 mmol/L (SD ± 0.61) and 36% (SD ± 3.00), respectively. Based on hemoglobin and hematocrit levels, respectively, 12.2% and 17.5% of the mothers were anemic, and 15% and 16.8% had elevated Hb or Ht levels. Mean absolute values for FEV_1, FVC, FEV_1/FVC, FEF_{25-75}, and FEF_{75} of the children were 2.02 L (SD ± 0.30), 2.33 L (SD ± 0.36), 0.87 (SD ± 0.06), 2.71 L/s (SD ± 0.64), and 1.14 L/s (SD ± 0.34), respectively. Current asthma was observed in 7.2% of the participants. Compared with those included in our study, we observed that mothers not included in the study were younger, more often multiparous, had a higher pre-pregnancy BMI, more often had gestational hypertensive disorders, less often used folic acid supplementation and alcohol during pregnancy, more often had caesarean-section as mode of delivery of their child, and that children were born at an earlier gestational age (*P* values <0.01) (Table S1).

3.1 | Hemoglobin and hematocrit levels and respiratory outcomes

The crude model showed that higher maternal hemoglobin levels were associated with lower FEF_{25-75} and FEF_{75} in children (Z-score [95%CI]: −0.05 [−0.09, −0.01] and −0.05 [−0.08, −0.02], respectively, per increase of 1 SDS in hemoglobin level), and that higher maternal hematocrit levels were associated with lower FEV_1/FVC and FEF_{75} in children (−0.04 [−0.07, −0.01] and −0.05 [−0.08, −0.02], respectively, per increase of 1 SDS in hematocrit level) (Table 2). Maternal hemoglobin and hematocrit levels were not associated with FEV_1, FVC or current asthma. The effect estimates of associations of maternal hemoglobin with FEF_{25-75} and FEF_{75}, and of associations of maternal hematocrit level with FEV_1/FVC attenuated into significant when socio-economic (model 1) and lifestyle factors (model 2), respectively, were taken into account. After adjusting for both socio-economic and lifestyle factors (full model), only the association of a higher maternal hematocrit level during pregnancy with a lower childhood FEF_{75} remained (−0.04 [−0.07, −0.01], per increase of 1 SDS in hematocrit level). Based on hemoglobin clinical cut-offs, maternal anemia during pregnancy was associated with a high FEF_{25-75} (difference in Z-score [95%CI]: 0.16 [0.05, 0.27]) when compared to normal hemoglobin levels (Fig. 2D, Table S2). Based on hematocrit clinical cut-offs, an elevated

TABLE 1 Characteristics of mothers and their children (n = 3672)

	Original data	Imputed data
Maternal characteristics		
Age (years)	30.9 (4.84)	30.9 (4.84)
Parity (%)		
Nullipara	58.4 (2131)	58.4 (2145)
Multipara	41.6 (1518)	41.6 (1527)
Missing	0.6 (23)	
Ethnicity (%)		
European	65.2 (2346)	64.6 (2373)
Non-European	34.8 (1254)	35.4 (1299)
Missing	2.0 (72)	
Education level (%)		
Less than secondary level	48.8 (1702)	49.9 (1832)
Higher	51.2 (1786)	50.1 (1840)
Missing	5.0 (184)	
Prepregnancy body mass index (kg/m^2)	24.54 (4.23)	24.54 (4.23)
Smoking during pregnancy (%)		
No	76.2 (2513)	76.2 (2797)
Yes	23.8 (786)	23.8 (875)
Missing	5.0 (184)	
History of asthma or atopy (%)		
No	62.3 (2014)	–
Yes	37.7 (1220)	–
Missing	11.9 (438)	–
Gestational hypertensive disorders (%)		
No	96.3 (3366)	96.0 (3525)
Yes	3.7 (130)	4.0 (147)
Missing	4.8 (176)	
Folic acid supplementation (%)		
No	21.7 (621)	23.3 (856)
Start 1st 10 weeks of gestation	30.8 (879)	31.2 (1145)
Start periconceptional	47.5 (1357)	45.5 (1671)
Missing	22.2 (815)	
Alcohol consumption during pregnancy (%)		
None	42.6 (1390)	43.0 (1580)
Any	57.4 (1872)	57.0 (2091)
Missing	11.2 (410)	
Mode of delivery (%)		
Vaginal	88.1 (2941)	88.2 (3239)
Caeserean-section	11.9 (396)	11.8 (433)
Missing	9.1 (335)	
Hemoglobin (mmol/L)	7.56 (0.61)	–
Hemoglobin clinical categories (%)		
Anemia (<6.83 mmol/L)	12.0 (442)	–
Normal (6.83-8.20 mmol/L)	72.8 (2672)	–
Elevated hemoglobin (>8.20 mmol/L)	15.2 (558)	–
Hematocrit	0.36 (0.03)	–
Hematocrit clinical categories (%)		
Anemia (<33%)	17.6 (640)	–
Normal (33-39%)	65.4 (2376)	–
Elevated hematocrit (>39%)	16.9 (615)	–
Child's characteristics		
Female sex (%)	51.7 (1900)	51.7 (1900)
Gestational age at birth (weeks)	39.94 (1.71)	39.94 (1.71)
Birth weight (grams)	3442.39 (541.91)	3441.57 (543.2)
Breastfeeding (%)		
No	6.9 (209)	7.3 (267)
Yes	93.1 (2816)	92.7 (3405)
Missing	17.6 (647)	
Age	9.79 (0.34)	9.79 (0.34)
FEV1	2.02 (0.30)	–
FVC	2.33 (0.36)	–
FEV1/FVC	0.87 (0.06)	–
FEF25-75	2.71 (0.64)	–
FEF75	1.14 (0.34)	–
Current asthma		
No	92.7 (2781)	–
Yes	7.3 (219)	–
Missing	18.3 (672)	–

Values are means (SD) or valid percentages (absolute numbers).

maternal hematocrit level during pregnancy was associated with a low FEF_{75} (difference in Z-score [95%CI]: −0.11 [−0.20, −0.03]) when compared to normal hematocrit levels (Fig. 2E, Table S2). Maternal anemia or elevated hemoglobin or hematocrit levels were not associated with FEV_1, FVC, FEV_1/FVC, or current asthma in children (Fig. 2A-C, Table S2).

We did not observe any non-linear associations of maternal hemoglobin and hematocrit levels with childhood lung function or asthma (data not shown, P values for quadratic term >0.05). We did not observe modifying effects of maternal mode of delivery, child's gestational age and weight at birth, maternal history of asthma or atopy and child's allergic sensitization for the associations of maternal hemoglobin or hematocrit level with childhood lung function measures or current asthma (P values for interaction >0.05, P values for F-statistics >0.05).

4 | DISCUSSION

In this population-based prospective birth cohort study, we observed that higher maternal hematocrit levels during pregnancy, both continuously and categorized according to clinical cut-offs, were associated with

TABLE 2 Associations of maternal hemoglobin and hematocrit levels with childhood lung function measures and asthma

	FEV_1 Z-score (95%CI)	FVC Z-score (95%CI)	FEV_1/FVC Z-score (95%CI)	FEF_{25-75} Z-score (95%CI)	FEF_{75} Z-score (95%CI)	Current asthma OR (95%CI)
Hemoglobin (SDS)						
Crude model[a]	−0.02 (−0.05, 0.02)	−0.00 (−0.03, 0.03)	−0.03 (−0.07, 0.00)	−0.05 (−0.09, −0.01)*	−0.05 (−0.08, −0.02)**	1.00 (0.86, 1.17)
Model 1[b]	−0.02 (−0.06, 0.02)	−0.01 (−0.05, 0.02)	−0.02 (−0.06, 0.01)	−0.05 (−0.09, −0.01)*	-0.04 (-0.07, −0.01)*	1.01 (0.86, 1.18)
Model 2[c]	−0.01 (−0.04, 0.03)	0.00 (−0.03, 0.04)	−0.02 (−0.06, 0.01)	−0.04 (−0.08, 0.00)	−0.03 (−0.06, 0.00)	1.02 (0.87, 1.19)
Full model[d]	−0.02 (−0.05, 0.02)	−0.01 (−0.04, 0.03)	−0.02 (−0.05, 0.02)	−0.04 (−0.08, 0.00)	−0.03 (−0.07, 0.00)	1.01 (0.87, 1.18)
Hematocrit (SDS)						
Crude[a]	−0.02 (−0.05, 0.02)	0.00 (−0.03, 0.03)	−0.04 (−0.07, −0.01)*	−0.04 (−0.07, 0.00)	−0.05 (−0.08, −0.02)**	1.01 (0.87, 1.17)
Model 1[b]	−0.02 (−0.06, 0.01)	−0.01 (−0.04, 0.02)	−0.03 (−0.07, 0.00)	−0.03 (−0.07, 0.01)	−0.05 (−0.08, −0.02)**	1.01 (0.87, 1.17)
Model 2[c]	−0.01 (−0.04, 0.03)	0.01 (−0.03, 0.04)	−0.03 (−0.07, 0.00)	−0.02 (−0.06, 0.01)	−0.04 (−0.07, −0.01)*	1.02 (0.83, 1.19)
Full model[d]	−0.02 (0.02, 0.00)	−0.01 (−0.04, 0.03)	−0.03 (−0.06, 0.01)	−0.03 (−0.07, 0.01)	−0.04 (−0.07, −0.01)*	1.01 (0.87, 1.18)

Values reflect changes in Z-scores or odds ratios (OR) with their 95% Confidence Interval per increase of one SDS hemoglobin or hematocrit *P-value <0.05, **P-value <0.01.

[a]Adjusted for gestational age at maternal blood sampling.

[b]Crude model additionally adjusted for maternal age, parity, educational level, pre-pregnancy BMI.

[c]Crude model additionally adjusted for maternal folic acid supplementation and alcohol consumption during pregnancy.

[d]Adjusted for all confounders.

lower FEF_{75} in children taking lifestyle and socio-economic factors into account. Maternal anemia during pregnancy based on hemoglobin levels was associated with a high FEF_{25-75}, compared to normal hemoglobin levels. Results were not modified by adverse pregnancy outcomes and atopic predisposition. We did not observe any associations of maternal hemoglobin and hematocrit levels with current asthma, although residual confounders might affect our findings.

4.1 | Comparison with previous studies

Prospective cohort studies that assessed the association of maternal hemoglobin or hematocrit levels with childhood lung function and asthma are scarce,[16,17] and show inconsistent results. We aimed to address all factors that explained differences in results of the previous studies into account, and now used both hemoglobin and hematocrit levels, WHO clinical cut-off definitions, lung function measured by spirometry, which is an objective and reliable[29,30] method, took gestational age at blood sampling and multiple confounders into account, and explored intermediating and effect modifying factors. We observed associations of maternal hemoglobin and hematocrit levels measured in early pregnancy with lower childhood lung function, but not with asthma at age 10 years. A population based cohort study among 597 children and their mothers observed associations of maternal anemia in late pregnancy with wheezing and asthma until age 6 years, however we did not. Main differences between the studies that could explain the different findings are the period during pregnancy at which hemoglobin and hematocrit blood levels were measured, in the first pregnancy versus at delivery, the adjustment for gestational age at blood sampling or not, and the determinants definitions. Previous studies have shown that hemoglobin or hematocrit levels might change according to the pregnancy trimester evaluated[23] and that mothers are more likely to develop anemia in the third trimester.[31] Period in pregnancy at which maternal anemia is measured seems therefore important. Also, the age at which asthma was measured, and differences in study populations, a high-risk asthma population versus a population-based cohort, might have influenced differences in results.

Maternal iron status during pregnancy may be used as proxy of the overall maternal nutritional status and also as a proxy of maternal anemia, since iron deficiency is a very common cause of anemia.[8] A previous study that examined the association of iron levels during pregnancy with asthma-related outcomes in children[32] suggested that lower maternal iron levels during pregnancy are associated with increased risks of wheezing and atopic sensitization in children whereas higher maternal iron levels during pregnancy are related to better lung function measures. Therefore, future studies need to focus on maternal hemoglobin and hematocrit in combination with iron levels status during multiple trimesters of pregnancy and the association with childhood lung function and asthma.

4.2 | Interpretation of results

The associations of maternal hemoglobin and hematocrit levels during pregnancy with childhood lung function might be explained by the importance of adequate oxygen and nutrients supply from the mother to the fetus in order to guarantee an adequate general and lung growth and development.[5–7] Low maternal hemoglobin levels may represent an insufficient oxygen supply to the fetus since the hemoglobin molecule is the oxygen carrier in our body.[33] High hemoglobin or hematocrit levels during pregnancy may lead to an important increase in the blood viscosity inducing an impaired blood flow through the placenta and, therefore, an insufficient oxygen supply to the fetus. Animal models have shown that undernutrition and hypoxia lead to an impaired lung development.[34,35] In sheep, it has been shown that intrauterine exposure to hypoxia may inhibit lung growth and DNA synthesis.[34] A study in lambs fetuses showed that hypoxemia decreases pulmonary blood flow, which might lead to reduced oxygen and nutrients supply that could have an adverse impact on lung development.[35]

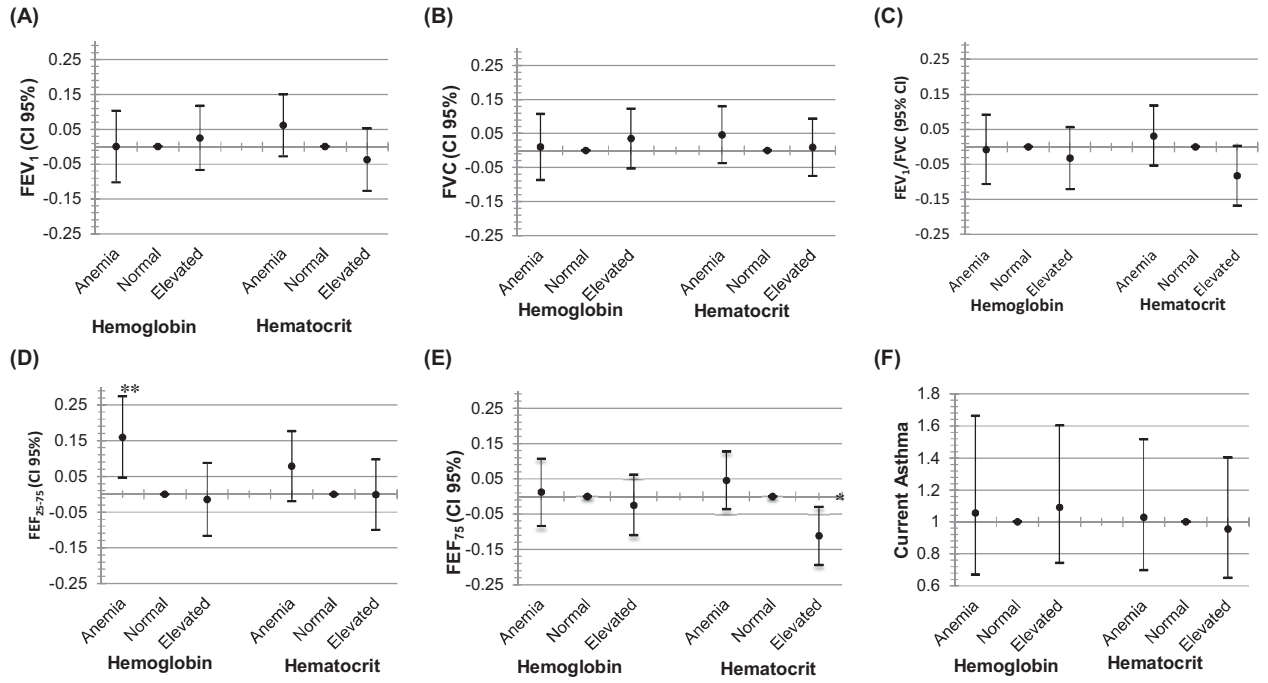

FIGURE 2 Associations of clinical cut-offs of maternal hemoglobin and hematocrit levels with childhood FEV_1 (A), FVC (B), FEV_1/FVC (C), FEF_{25-75} (D), FEF_{75} (E), and current asthma (F). Values reflect changes in Z-scores or odds ratios (OR) with their 95% Confidence Interval. Reference group are those mothers with a normal hemoglobin or hematocrit level. Models are adjusted for gestational age at maternal blood sampling, maternal age, parity, educational level, pre-pregnancy BMI, folic acid supplementation, and alcohol consumption during pregnancy. **P*-value < 0.05, ***P*-value < 0.01

It has been suggested that in some circumstances the physiological plasma-volume expansion that usually occur in pregnancy may fail which could lead to relatively elevated hemoglobin or hematocrit levels during pregnancy.[36,37] This would imply more viscous blood and could potentially lead to impaired placental perfusion and inappropriate blood and nutrients supply to the fetus. Our study showed that maternal elevated hematocrit levels were associated with lower lung function measures and might support the proposed hypothesis that maternal blood hyper viscosity during pregnancy influences fetal growth and lung development and later life lower lung function.

We observed that maternal anemia was associated with a high FEF_{25-75} in children. We analyzed hemoglobin and hematocrit levels mostly from blood samples obtained in the first trimester of pregnancy but hemoglobin or hematocrit levels might change according to the pregnancy trimester evaluated.[23] During early pregnancy, the mother is exposed to normal hemodynamic changes with an increase in the blood plasma disproportionally greater than increase of blood red cells numbers, which results in a physiologic anemia. Cohort studies have suggested that maternal physiologic anemia and iron deficiency anemia, which more often occurs in the third trimester,[31] might have different consequences to child health.[9,10,32] Iron deficiency seems associated with adverse child health outcomes while physiologic anemia seems to be associated with better outcomes for mother and child. Our results are in line with this hypothesis, although we were unable to assess the role of maternal iron status during pregnancy on lung function and asthma in children. It has also been suggested that the placenta might adapt to lower levels of hemoglobin or hematocrit leading to an increase in its vascular density.[11] This adaptive response could enhance fetal development, and our findings of maternal anemia leading to a high FEF_{25-75} could be attributed to this mechanism.

4.3 | Strengths and limitations

We used a population-based prospective cohort design from early pregnancy onwards, with detailed information on maternal and child characteristics and on maternal red blood status. Spirometry is the preferred method to assess lung function,[29,30,38] and to date this is the first study that uses this method as asthma-related outcome with maternal hemoglobin and hematocrit levels during pregnancy and long-term pulmonary outcomes under study. However, some limitations do apply. First, subjects lost to follow-up had less favorable social-economic status and lifestyle characteristics than those included in our study. This could have led to selection bias if the associations of maternal hemoglobin and hematocrit levels during pregnancy with childhood lung function and asthma would have been different between those subjects included and those lost to follow-up. This seems unlikely, but needs to be considered. Additionally, we did not observe any differences between complete case analysis and multiple imputation, which suggests that any non-random missing data did not affect our results.[28] Second, we used self-reported data to define current asthma in our study population. Although we used

questions previously validated in other cohorts studies and commonly used in epidemiological studies, we cannot exclude the possibility of under- or over-reporting, which could have led to misclassification of the asthma outcome and, thus, to under- or over-estimations of the true associations. Third, although we have included many potential confounders in our analysis, it is possible that residual confounding such as nutritional factors remains an issue in our study. Last, we observed most consistent associations of maternal hematocrit levels with FEF_{75} but not with other lung function measures or current asthma. It has been shown that FEF_{75} correlates more closely to mild or moderate asthma whereas FEV_1 with more severe asthma cases.[38] Therefore, in our population-based study, we might have had a higher prevalence of mild and moderate asthma, reflected by FEF_{75}, than severe asthma cases, which has consequences for the generalizability of our results. Furthermore, our current asthma definition did not include spirometry values, which might have been normal due to treatment. Also, current asthma was measured subjectively by parental reports while lung function objectively by spirometry, which might partly explain the absence of associations of maternal hemoglobin and hematocrit levels with asthma diagnose itself.

In conclusion, our study suggests that higher maternal hematocrit levels are associated with lower lung function, and maternal anemia in early pregnancy with higher lung function in childhood. Results are partly explained by lifestyle and socio-economic factors, and not modified by adverse pregnancy outcomes or atopic predisposition. Future studies are needed to explore whether maternal hemoglobin or hematocrit levels during pregnancy are associated with lung function and asthma in later life, and to explore potential underlying nutritional factors.

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CONFLICT OF INTEREST

None.

AUTHORS' CONTRIBUTION

SPJ, HD, LD contributed to the conception and design, acquisition of data, analyses and interpretation of the data, drafted the article, revised it critically for important intellectual content, and gave final approval of the version to be published. JJ, IR, ES, VJ contributed to the conception and design, acquisition of data, revised the drafted manuscript critically for important intellectual content, and gave final approval of the version to be published.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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