



**HAL**  
open science

## **Prenatal Hemoglobin Concentration and Long-Term Child Neurocognitive Development**

Michael Mireku, Michael Boivin, Romeo Zoumenou, Amanda Garrison, Michel Cot, Jules Alao, Nadine Fievet, Achille Massougbodji, Florence Bodeau-Livinec

### ► **To cite this version:**

Michael Mireku, Michael Boivin, Romeo Zoumenou, Amanda Garrison, Michel Cot, et al.. Prenatal Hemoglobin Concentration and Long-Term Child Neurocognitive Development. *American Journal of Tropical Medicine and Hygiene*, 2025, 112 (3), pp.692-698. <10.4269/ajtmh.24-0643>. <hal-04870650>

**HAL Id: hal-04870650**

**<https://hal.science/hal-04870650v1>**

Submitted on 15 Jan 2025

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC 4.0 - Attribution - Non-commercial use - International License

# Prenatal Hemoglobin Concentration and Long-Term Child Neurocognitive Development

Michael O. Mireku,<sup>1</sup> Michael J. Boivin,<sup>2</sup> Roméo Zoumenou,<sup>3,4</sup> Amanda Garrison,<sup>5</sup> Michel Cot,<sup>3</sup> Jules Alao,<sup>6</sup> Nadine Fievet,<sup>3,7</sup> Achille Massougboji,<sup>7</sup> and Florence Bodeau-Livinec<sup>3,5\*</sup>

<sup>1</sup>College of Health and Science, University of Lincoln, Lincoln, United Kingdom; <sup>2</sup>Department of Psychiatry, Michigan State University, East Lansing, Michigan; <sup>3</sup>UMR 261-MERIT, Institut de Recherche pour le Développement (IRD), Université Paris Cité, Paris, France; <sup>4</sup>Institute of Psychology, Laboratoire de Psychopathologie et Processus de Santé, Boulogne, France; <sup>5</sup>Université Rennes, EHESP, Inserm, IRSET (Institut de Recherche en Santé, Environnement et Travail)–UMR\_S 1085, Rennes, France; <sup>6</sup>Service de Pédiatrie, CHU de la Mère et de l'Enfant-Lagune de Cotonou, Cotonou, Benin; <sup>7</sup>Institut de Recherche Clinique du Bénin (IRCB), Abomey-Calavi, Benin

**Abstract.** Anemia in pregnancy, defined by a hemoglobin level (Hb) of less than 110 g/L, contributes to infant mortality and morbidity in sub-Saharan Africa. Maternal Hb changes physiologically and pathologically during pregnancy. However, the impact of these changes on long-term child neurocognitive function is unknown. This study therefore investigates the association between Hb at specific antenatal care visits and prenatal Hb trajectories during pregnancy and long-term child neurocognitive function. We analyzed data from a prospective cohort study that included 6-year-old singleton children born to women enrolled before 29 weeks of gestation into an antimalarial drug clinical trial. Hemoglobin level was analyzed from venous blood collected at least twice during pregnancy and at delivery. We used group-based trajectory modeling to identify distinct prenatal Hb trajectories. In total, 478 children (75.1% of eligible children) had assessment of cognitive and motor functions at 6 years of age. Three distinct Hb trajectories were identified: persistently anemic (Hb <110 g/L throughout the second and third trimesters), anemic to nonanemic (Hb <110 g/L at second trimester with increasing Hb toward the third trimester to Hb >110 g/L), and persistently nonanemic (Hb >110 g/L throughout the second and third trimesters). Children of women in the persistently anemic and anemic-to-nonanemic groups had significantly lower neurocognitive scores than children of women in the persistently nonanemic group ( $\beta = -6.8$ , 95% CI:  $-11.7$  to  $-1.8$ ; and  $\beta = -6.3$ , 95% CI:  $-10.4$  to  $-2.2$ , respectively). The study shows that maintaining an elevation of Hb above 110 g/L from the second to third trimester may be associated with optimal long-term child neurocognitive function.

## INTRODUCTION

About 14% of children experience poor cognitive function globally.<sup>1</sup> However, sub-Saharan Africa (SSA) accounts for the highest prevalence of poor child cognitive function, with 44% of children failing to meet the threshold for good cognitive function.<sup>1</sup> Although a multitude of factors relating to the post-natal environment, such as malnutrition, infections, and poverty, may partly explain the disproportionately elevated levels of poor neurocognitive function, exposure to some of these insults may occur even earlier, from the onset of conception throughout the prenatal period, for many SSA children.<sup>2,3</sup>

The prenatal period is a phase of rapid neurological development that sets the foundation for the core cognitive and social skills needed to achieve an individual's potential. The precarity of extensive serial neurological processes during this period, including synaptogenesis and hippocampal formation, makes the brain vulnerable to insults that could alter its development and function.<sup>4</sup> Existing literature shows that exposure to prenatal insults, such as nutrient deficiencies, infections, and anemia, may impair neurocognitive functions in the first 2 years postpartum.<sup>5-7</sup> Few studies have investigated potential neurocognitive consequences of prenatal insults beyond those found 2 years postpartum.<sup>8-10</sup> However, the influence of anemia or changes in prenatal hemoglobin concentration (Hb) on long-term neurocognitive outcomes remains unclear.

Anemia in pregnancy (AiP), defined as Hb below 110 g/L, remains prevalent in SSA, affecting around 17 million women.<sup>11</sup> Although a decline in Hb may be indicative of a pathological condition or nutrient deficiency, physiological changes in plasma volume during pregnancy also result in a decline in Hb

during the second trimester.<sup>12</sup> These physiological changes are necessary to permit adequate supply of oxygen, iron, and essential nutrients to the fetus, especially during the second and third trimesters of pregnancy.<sup>13</sup> Indeed, in a birth cohort in Benin, we found that children of mothers with elevated and extremely low Hb levels during the second antenatal care visit (ANC) had poor motor function.<sup>14</sup> Thus, for the present study, we sought to investigate whether observed associations between prenatal Hb and early child development at 1 year persist later in childhood, at 6 years, in the Beninese cohort. Furthermore, we aimed to identify distinct Hb trajectories over the second and third trimesters of pregnancy in this cohort using a trajectory-based approach and to assess the relationship between Hb trajectory groups and long-term cognitive and behavioral child cognitive development.

## MATERIALS AND METHODS

### Population.

Participants are singleton 6-year-old children born to pregnant women enrolled in a malaria clinical trial called Malaria in Pregnancy Preventive Alternative Drugs (MiPPAD; clinical trial no. NCT00811421) in Benin, West Africa. The MiPPAD trial was set up to investigate the efficacy of sulfadoxine-pyrimethamine (SP) and mefloquine (MQ) as intermittent preventive treatment of malaria in pregnancy (IPTp).<sup>15</sup> Briefly, HIV-negative pregnant women attending their first ANC were recruited into MiPPAD. Surviving singleton children born to MiPPAD mothers were then followed until 1 year of age<sup>16</sup> and subsequently at 6 years, when they were assessed for neurocognitive and behavioral development.<sup>17</sup>

### Prenatal hematological and other biological assessments.

Four-milliliter venous blood samples taken from pregnant women at inclusion into the MiPPAD trial, hereinafter referred

\* Address correspondence to Florence Bodeau-Livinec, UMR 261-MERIT, Institut de Recherche pour le Développement (IRD), Université Paris Cité, 75004 Paris, France. E-mail: florence.bodeau-livinec@ehesp.fr

to as the first ANC, underwent biological analysis. After sample collection at the first ANC, each pregnant woman received either IPTp-MQ or IPTp-SP, anthelmintic therapy, oral ferrous sulfate (200 mg daily), and folic acid (5 mg daily) as part of the ANC package in Benin. Pregnant women received micronutrient supplements throughout pregnancy without charge. We analyzed a 10- $\mu$ L aliquot of blood samples for Hb by using a Hemo-Control photometer. Blood sample collection and Hb analysis were repeated during the second scheduled ANC and at delivery.

Blood samples collected at the three visits were also assessed for ferritin concentration, inflammation, and malaria infection by using standard protocols<sup>18,19</sup>; details of these assessments are explained elsewhere.<sup>14,16</sup> The presence of *Plasmodium falciparum* was determined from thick and thin blood film stains as well as placental biopsy specimens according to standard, quality-controlled procedures at each ANC and at delivery.<sup>15</sup> A selection of paired peripheral and placental blood stains was analyzed for *P. falciparum* density using quantitative polymerase chain reaction tests. *Plasmodium falciparum* parasitemia was quantified by extrapolation of cycle thresholds from a standard curve.<sup>20</sup> Samples with no cycle thresholds were considered to have negative malaria status, whereas those with a density of at least 2 parasites/ $\mu$ L were positive for malaria status.

#### Neurocognitive and motor function assessments at 6 years.

The principal outcomes in the present study are neurocognitive function and motor proficiency assessed when children were 6 years old. Detailed neurocognitive and motor function assessments were carried out by trained research nurses and psychologists using the Kaufman Assessment Battery for Children, second edition (KABC-II),<sup>21</sup> and the Bruininks-Oseretsky Test of Motor Proficiency Edition 2 (BOT-2),<sup>22</sup> respectively.

The KABC-II is an individualized test that measures cognitive and processing ability of 3- to 18-year-olds.<sup>21</sup> The French version of the KABC-II test (Pearson, France) was used in this study. The construct validity of the translated version was assessed in a sample of Beninese children before being approved for use in this study.<sup>17</sup> In this study, we used the age-standardized mental processing index (MPI) score, a composite measure of global cognitive ability. The normative mean of the standardized MPI score is 100 (SD = 15), with higher scores indicating higher cognitive ability.

The BOT-2 is a standardized, individually administered test of 53 items for measuring motor functions of 4- to 21-year-olds.<sup>22</sup> We computed the total motor composite (TMC), which combines age-standardized scores of all items to generate an overall motor proficiency for statistical analysis. Higher TMC scores indicate greater motor proficiency.

#### Sociodemographic characteristics.

Maternal sociodemographic characteristics, including occupation, level of education, age, gestational age, gravidity, weight, and height, were collected during the first ANC. Family socioeconomic status was determined by scores generated from familial possessions and amenities using computation explained elsewhere.<sup>23</sup> Gestational age was determined using fundal height, and child sex was recorded at delivery. Childhood environment was assessed using the Home Observatory Measurement of the Environment (HOME) inventory,<sup>24</sup> and postnatal depression (using the Edinburgh Postnatal Depression Scale [EPDS])<sup>25</sup> was measured at 1 year postpartum.

## STATISTICAL ANALYSES

We used linear regression analysis to investigate the relationship between prenatal anemia (and Hb) at each of the ANCs and at delivery and the MPI and TMC scores, separately. To investigate whether earlier findings of inverted U-shaped relationships between prenatal Hb concentration and motor functions at 1 year<sup>14</sup> were replicable at 6 years, we applied quadratic terms to the Hg concentration variable at each ANC in linear regression models. We adjusted for maternal age, educational level, gravidity, prepregnancy body mass index (BMI), HOME scores, and EPDS scores in multiple linear regression models.

To identify prenatal Hb trajectory groups, we selected women with complete Hb assessments at the second and third trimesters. We then used a group-based modeling approach for longitudinal data using the Stata *traj* function to identify distinct Hb trajectories during pregnancy.<sup>26</sup> We have outlined details of the statistical approach in Supplemental Appendix 1. Briefly, each trajectory was statistically identified on the basis of the similarities of Hb changes over the second and third trimesters considering the gestational age at assessment. We used the Bayesian information criterion to determine the optimal number of trajectory groups and shape. After identifying distinct trajectories, we estimated the posterior predicted probability for each woman to belong to each trajectory, after which they were assigned to the group to which they had the highest posterior probability.

We then used multiple linear regression models to establish the associations between Hb trajectory groups and MPI and TMC scores with adjustment for earlier-mentioned confounders. To ensure robustness of results, we performed sensitivity analysis by further adjusting for common risk factors for prenatal anemia, i.e., malaria infection, helminth infection and identification, and child's cognitive/motor assessment.

## RESULTS

Of 636 children eligible for cognitive assessment at 6 years, 158 (24.8%) were lost to follow-up. Of the remainder, we excluded 5 from analyses due to severe neurological disorder or fever (temperatures  $\geq 37.5^\circ\text{C}$ ) during assessment (Figure 1). As shown in Supplemental Table 1, maternal and child characteristics were similar between those lost to follow-up between the ages of 1 and 6 years and those assessed for neurocognitive functions at 6 years, except for gravidity, where the proportion of children lost to follow-up to primigravida mothers (28.5%) was significantly higher than that of children retained in the study 6 years after childbirth (15.1%). Thus, most women of eligible children entered the MiPPAD study as multi-gravida (85.2%) and during the second trimester (93.4%) of pregnancy (Table 1). Table 1 describes the sociodemographic characteristics of mother-child dyads. One-third of mothers were administered SP during the MiPPAD trial. Mean (SD) gestational age at first ANC was 21.8 (4.0) weeks. The prevalence of AiP was highest at the first ANC (65.3%) and declined at delivery to 37.9%. Likewise, the mean Hb was lowest at the first ANC (104.5 g/L) and highest at delivery (112.2 g/L), as shown in Supplemental Table 2.

Maternal anemia status at both ANCs and at delivery was not associated with MPI score or TMC score (Table 2). Whereas Hb concentrations at the first and second ANCs

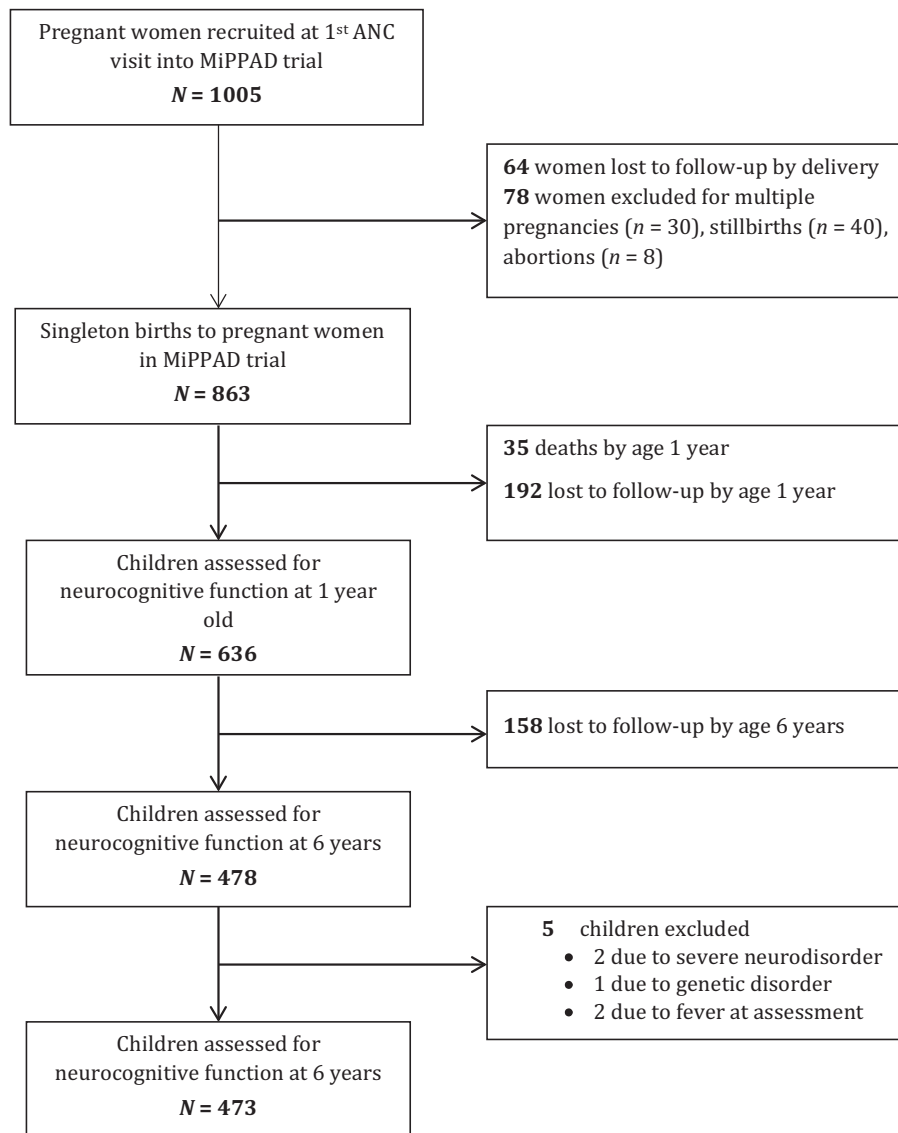


FIGURE 1. Flow chart of study participants' follow-up. ANC = antenatal care; MiPPAD = Malaria in Pregnancy Preventive Alternative Drugs (clinical trial).

were crudely associated with MPI score ( $\beta = 0.1$ , 95% CI: 0.0 to 0.2 for both), the association was no longer significant after adjustment for confounders. Likewise, there were no statistically significant quadratic relationships between prenatal Hb at any of the ANCs and offspring MPI scores or TMC scores at 6 years (Table 2).

Through group-based trajectory modeling, we found three distinct Hb trajectories from the second to third trimester, as shown in Figure 2. After visual inspection, we labeled the three trajectory groups as “persistently anemic” (anemic at second trimester with minimal to no change by the third trimester, representing 14.9% of women), “anemic to non-anemic” (anemic at second trimester with increasing Hb toward the third trimester to nonanemic levels, 76.3%), and “persistently nonanemic” (never anemic from second to third trimester, 8.8%). Participants’ individual trajectories for the three groups are shown in Supplemental Figure 1.

Supplemental Table 3 shows the estimated mean Hb levels in grams per liter for each of the groups at the second and third trimesters. Supplemental Table 4 shows the relationships between Hb trajectory groups and sociodemographic characteristics of mother-child dyads. Overall, the persistently nonanemic group had the highest family possession score and HOME score. The anemic-to-nonanemic group had the lowest proportion of employment and the lowest family possession score.

Although the TMC scores of children of women in the anemic-to-nonanemic group were significantly lower ( $\beta = -2.8$ , 95% CI:  $-6.0$  to  $-0.5$ ) than those of children of women in the persistently nonanemic group in the crude analysis, the association attenuated and was no longer significant after adjustment for confounders (Table 3). However, there was a statistically significant relationship between Hb trajectory groups and MPI scores, which remained significant after

TABLE 1  
Sociodemographic characteristics of mother-child dyads,  $N = 473$

Characteristics	Number (%)
Age at 1st ANC visit (years)*	26.4 (5.6)
Family possession score* <sup>†</sup>	5.6 (2.7)
Prepregnancy BMI (kg/m <sup>2</sup> )*	21.2 (3.2)
Gravidity	
Primigravida	70 (14.8)
Multigravida	403 (85.2)
Education	
Never schooled	322 (68.1)
Primary or more	151 (31.9)
Occupation	
Housewives	246 (52.0)
Used	227 (48.0)
Gestational age at 1st ANC (weeks)*	21.9 (4.0)
1st trimester (0–13)	5 (1.1)
2nd trimester (14–27)	442 (93.4)
3rd trimester (28–43)	26 (5.5)
Hemoglobin genotype	
AA	345 (72.9)
AS	97 (20.5)
AC	26 (5.5)
CC/SC/SS	5 (1.1)
Sex	
Boy	233 (49.3)
Girl	240 (50.7)
HOME score,* <sup>†</sup> $N = 471$	26.9 (2.3)
EPDS score,* <sup>†</sup> $N = 471$	8.1 (4.0)
MiPPAD trial treatment	
Mefloquine	322 (68.1)
Sulfadoxine-pyrimethamine	151 (31.9)

ANC = antenatal care; BMI = body mass index; EPDS = Edinburgh Postnatal Depression Scale; HOME = Home Observatory Measurement of the Environment; MiPPAD = Malaria in Pregnancy Preventive Alternative Drugs (clinical trial).

\* Displayed as mean (SD).

<sup>†</sup>  $N = 471$ .

adjustment for confounders. Specifically, in the adjusted model, children of women in the persistently anemic and anemic-to-nonanemic groups had significantly lower MPI scores than children of women in the persistently nonanemic group ( $\beta = -6.8$ , 95% CI:  $-11.7$  to  $-1.8$ , and  $\beta = -6.3$ , 95% CI:  $-10.4$  to  $-2.2$ , respectively). The associations remained statistically significant albeit attenuated after adjustment for prenatal iron deficiency anemia status, malaria status and helminth infection status at baseline, and neurocognitive function scores of children at 1 year in a sensitivity analysis (Table 3).

## DISCUSSION

This study set out to investigate the relationship between Hb trajectories of pregnant women and neurocognitive and motor functions of children at 6 years of age. Using a trajectory-based approach, we identified three distinct trajectory groups when considering Hb changes from the second to third trimester: persistently anemic, anemic to nonanemic, and persistently nonanemic. We found that children of mothers in both the anemic-to-nonanemic and persistently anemic trajectory groups had worse cognitive function than those of mothers in the persistently nonanemic group, although there were no differences in motor proficiency between the Hb trajectory groups. We also found no relationships, linear or quadratic, between anemia or Hb at any of the ANCs and long-term neurocognitive functions of the children. These findings show that the observed inverted U-shaped relationship between prenatal Hb at specific ANCs and infant gross motor proficiency at 12 months<sup>14</sup> was no longer present when

TABLE 2  
Relationship between prenatal anemia/Hb concentration and child neurocognitive function and motor proficiency scores at 6 years

Anemia or Hb Concentration	KABC-II MPI Score		BOT-2 TMC Score	
	Crude $\beta$ (95% CI)	Adjusted $\beta$ (95% CI)*	Crude $\beta$ (95% CI)	Adjusted $\beta$ (95% CI)*
<b>Anemia</b>				
1st ANC visit				
No anemia	Reference	Reference	Reference	Reference
Anemia	-1.0 (-3.3 to 1.3)	-0.6 (-2.8 to 1.7)	-0.6 (-2.4 to 1.1)	0.0 (-1.6 to 1.7)
2nd ANC visit				
No anemia	Reference	Reference	Reference	Reference
Anemia	-1.1 (-3.4 to 1.2)	-0.7 (-3.0 to 1.5)	0.1 (-1.6 to 1.9)	0.7 (-0.9 to 2.4)
Delivery				
No anemia	Reference	Reference	Reference	Reference
Anemia	-1.4 (-3.7 to 0.9)	-1.0 (-3.4 to 1.3)	-0.8 (-2.6 to 0.9)	0.2 (-1.5 to 1.8)
<b>Hb concentration<sup>†</sup></b>				
1st ANC visit	0.1 (0.0 to 0.2)	0.1 (-0.0 to 0.2)	0.1 (-0.0 to 0.1)	0.0 (-0.0 to 0.1)
2nd ANC visit	0.1 (0.0 to 0.2)	0.1 (-0.0 to 0.2)	0.0 (-0.1 to 0.1)	-0.0 (-0.1 to 0.1)
Delivery	0.1 (-0.0 to 0.1)	0.1 (-0.0 to 0.1)	0.0 (-0.0 to 0.1)	-0.0 (-0.1 to 0.1)
<b>Hb concentration<sup>‡</sup></b>				
1st ANC visit	$3 \times 10^{-4}$ ( $-5 \times 10^{-3}$ to $5 \times 10^{-3}$ )	$6 \times 10^{-4}$ ( $-6 \times 10^{-3}$ to $4 \times 10^{-3}$ )	$2 \times 10^{-4}$ ( $-4 \times 10^{-3}$ to $4 \times 10^{-3}$ )	$-3 \times 10^{-4}$ ( $-4 \times 10^{-3}$ to $3 \times 10^{-3}$ )
2nd ANC visit	$-1 \times 10^{-3}$ ( $-8 \times 10^{-3}$ to $5 \times 10^{-3}$ )	$-2 \times 10^{-3}$ ( $-9 \times 10^{-3}$ to $4 \times 10^{-3}$ )	$-3 \times 10^{-4}$ ( $-5 \times 10^{-3}$ to $5 \times 10^{-3}$ )	$-4 \times 10^{-4}$ ( $-5 \times 10^{-3}$ to $4 \times 10^{-3}$ )
Delivery	$2 \times 10^{-3}$ ( $-1 \times 10^{-3}$ to $4 \times 10^{-3}$ )	$1 \times 10^{-3}$ ( $-2 \times 10^{-3}$ to $4 \times 10^{-3}$ )	$-6 \times 10^{-4}$ ( $-3 \times 10^{-3}$ to $2 \times 10^{-3}$ )	$-8 \times 10^{-4}$ ( $-3 \times 10^{-3}$ to $1 \times 10^{-3}$ )

ANC = antenatal care; BOT-2 = Bruininks-Oseretsky Test of Motor Proficiency Edition 2; Hb = hemoglobin; HOME = Home Observatory Measurement of the Environment; KABC-II = Kaufman Assessment Battery for Children, second edition; MPI = mental processing index; TMC = total motor composite.

\* Adjusted for maternal education, maternal occupation, gravidity, family possession score, postnatal depression, child sex, HOME score, and gestational age at Hb assessment.

<sup>†</sup>  $\beta$  refers to change in neurocognitive scores for 1-g/L increase in maternal Hb concentration.

<sup>‡</sup> Values are for  $\beta$  (95% CI) of the squared/quadratic term for Hb.

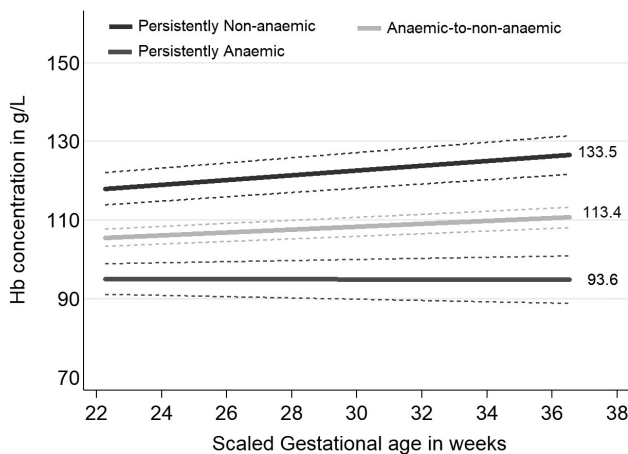


FIGURE 2. Hemoglobin (Hb) concentration trajectories of pregnant women (95% CI) from the second to third trimester. The y axis shows gestational age in weeks estimated by trajectory models. Values represent the mean Hb concentrations at delivery for each trajectory group. This figure appears in color at [www.ajtmh.org](http://www.ajtmh.org).

children were 6 years old. Further, there were no associations between prenatal Hb or anemia at any ANC and neurodevelopment at 6 years.

Several studies, including a meta-analysis, appear to support associations between prenatal Hb concentration and birth outcomes and early child development by 24 months.<sup>7,27-31</sup> However, the association between prenatal Hb and long-term neurocognitive development beyond 2 years postpartum is understudied. To our knowledge, the limited literature on long-term neurodevelopmental or cognitive correlates of prenatal Hb has emerged from high-income countries.<sup>10,32</sup> One of these found that a prenatal anemia diagnosis was associated with increased odds of autism spectrum disorder between 6 and 29 years in a Swedish population.<sup>10</sup> Another found Hb in the ninth gestational month to be consistently associated with poor academic performance and education achievement of offspring from 14 to 31 years.<sup>32</sup> In contrast, the only study from SSA found no association between prenatal Hb or anemia (at least once) and cognitive or motor functions at 5 years in Uganda.<sup>33</sup> This finding is consistent with the lack of association we observed for Hb or anemia during specific ANCs and cognitive or motor function in our sample.

The timing of Hb assessment during pregnancy may account for inconsistent findings of associations between prenatal Hb and maternal and child health outcomes.<sup>31,34</sup>

Thus, identifying and comparing outcomes by distinct Hb trajectory groups may provide a better assessment of the relationship between Hb during pregnancy and offspring health. Of the three Hb trajectory groups, the persistently anemic and anemic-to-nonanemic groups were both associated with poor offspring neurocognitive function at 6 years of age in comparison with those in the persistently nonanemic group even after accounting for known risk factors of anemia and child neurocognitive scores at 1 year. Although the biochemical mechanism of prenatal Hb trajectory and neurodevelopment is understudied, a structural neuroimaging study of 2- to 3-year-old children in South Africa showed significant linear relationships between prenatal Hb and caudate, right hemisphere, putamen left hemisphere, and corpus callosum brain volumes.<sup>35</sup>

The present study takes advantage of an ongoing longitudinal study with multiple biological assessments (including Hb) during pregnancy and child neurocognitive assessments, which is rare in SSA, a region that accounts for the highest prevalence of prenatal anemia<sup>11</sup> and the highest burden of poor child cognitive function.<sup>1</sup> Although about a quarter of children were lost to follow-up between ages 1 and 6 years, the characteristics of the children lost to follow-up did not notably differ from those assessed at age 6 years. In addition, we accounted for relevant confounding variables in the analysis, including known risk factors of anemia and cognitive function scores in sensitivity analysis. In this cohort, preventable factors such as nutrient deficiencies (folic acid, iron, vitamin B12) and parasitic infections (malaria and helminths) were associated with anemia at the first ANC when they were newly recruited in the clinical trial.<sup>36</sup> However, during the trial, only iron and folic acid deficiencies were associated with an increased risk of anemia at the second ANC after intermittent preventive malaria and anthelmintic treatments.<sup>36,37</sup>

The present study has limitations. Although we identified distinct Hb trajectories in this sample, they were limited to the second and third trimesters of pregnancy. The Hb in the first trimester was missing for most women because the first ANC for most women in this region was later, given that the inclusion criteria of the parent study, MiPPAD, permitted women of gestational age of 29 weeks or less to participate. Also, the three group-based trajectories of Hb from the second to the third trimester identified in this study may be limited to the size of the study sample. Thus, minor possible trajectories that may have not been detected in this Beninese cohort may be visible in a study with a larger sample size. Since MiPPAD was a clinical trial, pregnant women were carefully

TABLE 3  
Relationship between Hb trajectories from second to third trimester and child neurocognitive development scores at 6 years\*

Hb Trajectory Classes	KABC-II MPI Score			BOT-2 TMC Score		
	Model I	Model II	Model III	Model I	Model II	Model III
Persistently anemic	-7.9 (-12.9 to -2.9)	-6.8 (-11.7 to -1.8)	-6.0 (-11.0 to -1.0)	-2.5 (-6.4 to 1.3)	-1.9 (-5.4 to 1.6)	-2.1 (-5.6 to 1.3)
Anemic to nonanemic	-8.0 (-12.2 to -3.8)	-6.3 (-10.4 to -2.2)	-5.8 (-10.0 to -1.6)	-2.8 (-6.0 to -0.5)	-1.9 (-4.8 to 1.0)	-2.0 (-4.9 to 0.9)
Persistently nonanemic	Reference	Reference	Reference	Reference	Reference	Reference

BOT-2 = Bruininks-Oseretsky Test of Motor Proficiency Edition 2; Hb = hemoglobin; HOME = Home Observatory Measurement of the Environment; KABC-II = Kaufman Assessment Battery for Children, second edition; MiPPAD = Malaria in Pregnancy Preventive Alternative Drugs (clinical trial); MPI = mental processing index; TMC = total motor composite.

\* All values are presented as regression coefficient (95% CI). Model I = crude/unadjusted model. Model II = adjusted model, i.e., model I adjusted for maternal education, maternal occupation, gravidity, family possession score, postnatal depression, child sex, HOME score, and gestational age at Hb assessment. Model III = sensitivity model, i.e., model II further adjusted for iron deficiency status, malaria status and helminth infection status at baseline, MiPPAD clinical trial treatment, and neurocognitive function scores at 1 year (for KABC-II mental processing index score) or child motor score at 1 year (for BOT-2 total motor composite score).

monitored, including at least three ANC visits, and provided iron supplementation, preventative treatment for malaria, and treatment for helminths. These interventions may have improved maternal health and subsequently child health beyond what is available for most women and may explain the small proportion of women in the persistently anemic group. Further, the absence of Hb assessment at the first trimester limits the appreciation of the starting Hb of pregnant women in each of the three trajectories.

This study does not confirm the hypothesis that the inverted U-shaped relationship between prenatal Hb at specific ANC visits and offspring neurocognitive development at 1 year persists in late childhood. However, the study shows that maintaining an Hb above 110 g/L between the second and third trimesters may be associated with long-term neurocognitive function in offspring.

Received September 25, 2024. Accepted for publication October 25, 2024.

Published online Month 00, 2024

Note: Supplemental material appears at [www.ajtmh.org](http://www.ajtmh.org).

**Acknowledgments:** We thank all mothers and children who participated in this study. We also thank the staff of the three health centers (Allada, Atogon, and Sékou). Finally, we thank the entire TOVI, Malaria in Pregnancy Preventive Alternative Drugs clinical trial (MiPPAD), and EXPLORE field personnel.

**Financial support:** This work was supported by the Fondation de France (grant number 2015 00060746), the Eunice Kennedy Shriver National Institute of Child Health and Human Development (grant number NIH/NICHD- R21-HD060524), and the European and Developing Countries Clinical Trials Partnership (grant number EDCTP-IP.07.31080.002). The funders had no role in study design, conduct, or reporting of the results. The views expressed are those of the authors and not necessarily those of the funders.

**Disclosures:** All relevant data related to this study are within the manuscript and supporting supplemental documents. MiPPAD data cannot be shared publicly because of ethical restrictions. Access to MiPPAD study data may be available upon request through The MiPPAD Executive Committee (Raquel Gonzalez at +34-932-275-400 or [raquel.gonzalez@cresib.cat](mailto:raquel.gonzalez@cresib.cat)).

We sought ethical approvals from the institutional ethical review boards of the Faculté des Sciences de la Santé and the Applied Biomedical Sciences Institute (CER-ISBA) in Benin, New York University in New York, New York, and the Research Institute for Development's (IRD) Consultative Ethics Committee in France. We obtained informed consent from all mothers in accordance with the World Medical Association Declaration of Helsinki.

**Authors' contributions:** M. O. Mireku, M. J. Boivin, M. Cot, and F. Bodeau-Livinec conceptualized the study. M. O. Mireku completed the literature review, performed the statistical analysis, and wrote the first draft of the manuscript. M. O. Mireku, R. Zoumenou, and A. Garrison performed data cleaning. All authors had full access to all the study findings, contributed to interpretation of the results and revision of the manuscript, and were responsible for the decision to submit the report for publication.

Current contact information: XXX.

## REFERENCES

- McCoy DC, Peet ED, Ezzati M, Danaei G, Black MM, Sudfeld CR, Fawzi W, Fink G, 2016. Early childhood developmental status in low- and middle-income countries: National, regional, and global prevalence estimates using predictive modeling. *PLoS Med* 13: e1002034.
- Walker SP, Chang SM, Powell CA, Grantham-McGregor SM, 2005. Effects of early childhood psychosocial stimulation and nutritional supplementation on cognition and education in growth-stunted Jamaican children: Prospective cohort study. *Lancet* 366: 1804–1807.
- Mburu W, Conroy AL, Cusick SE, Bangirana P, Bond C, Zhao Y, Opoka RO, John CC, 2021. The impact of undernutrition on cognition in children with severe malaria and community children: A prospective 2-year cohort study. *J Trop Pediatr* 67: fmb091.
- Tau GZ, Peterson BS, 2010. Normal development of brain circuits. *Neuropsychopharmacology* 35: 147–168.
- Shonkoff JP, Garner AS, Siegel BS, Dobbins MI, Earls MF, Garner AS, McGuinn L, Pascoe J, Wood DL; The Committee on Psychosocial Aspects of Child and Family Health, Committee on Early Childhood, Adoption, and Dependent Care, Section on Developmental and Behavioral Pediatrics, 2012. The life-long effects of early childhood adversity and toxic stress. *Pediatrics* 129: e232–e246.
- Mireku MO, Boivin MJ, Davidson LL, Ouédraogo S, Koura GK, Alao MJ, Massougbdji A, Cot M, Bodeau-Livinec F, 2015. Impact of helminth infection during pregnancy on cognitive and motor functions of one-year-old children. *PLoS Negl Trop Dis* 9: e0003463.
- Chang S, Zeng L, Brouwer ID, Kok FJ, Yan H, 2013. Effect of iron deficiency anemia in pregnancy on child mental development in rural China. *Pediatrics* 131: e755–e763.
- Garrison A, Boivin MJ, Fiévet N, Zoumenou R, Alao JM, Massougbdji A, Cot M, Bodeau-Livinec F, 2021. The effects of malaria in pregnancy on neurocognitive development in children at one and six years of age in Benin: A prospective mother-child cohort. *Clin Infect Dis* 74: 766–775.
- Garrison A, Boivin M, Khoshnood B, Courtin D, Alao J, Mireku M, Ibikounle M, Massougbdji A, Cot M, Bodeau-Livinec F, 2021. Soil-transmitted helminth infection in pregnancy and long-term child neurocognitive and behavioral development: A prospective mother-child cohort in Benin. *PLOS Negl Trop Dis* 15: e0009260.
- Wieggersma AM, Dalman C, Lee BK, Karlsson H, Gardner RM, 2019. Association of prenatal maternal anemia with neurodevelopmental disorders. *JAMA Psychiatry* 76: 1294–1304.
- McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B, 2009. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993–2005. *Public Health Nutr* 12: 444–454.
- Churchill D, Nair M, Stanworth SJ, Knight M, 2019. The change in haemoglobin concentration between the first and third trimesters of pregnancy: A population study. *BMC Pregnancy Childbirth* 19: 359.
- Hutter D, Kingdom J, Jaeggi E, 2010. Causes and mechanisms of intrauterine hypoxia and its impact on the fetal cardiovascular system: A review. *Int J Pediatr* 2010: e401323.
- Mireku MO, Davidson LL, Koura GK, Ouédraogo S, Boivin MJ, Xiong X, Accrombessi MMK, Massougbdji A, Cot M, Bodeau-Livinec F, 2015. Prenatal hemoglobin levels and early cognitive and motor functions of one-year-old children. *Pediatrics* 136: e76–e83.
- González R, et al., 2014. Intermittent preventive treatment of malaria in pregnancy with mefloquine in HIV-negative women: A multicentre randomized controlled trial. *PLoS Med* 11: e1001733.
- Mireku MO, Davidson LL, Boivin MJ, Zoumenou R, Massougbdji A, Cot M, Bodeau-Livinec F, 2016. Prenatal iron deficiency, neonatal ferritin, and infant cognitive function. *Pediatrics* 138: e20161319.
- Bodeau-Livinec F, Davidson LL, Zoumenou R, Massougbdji A, Cot M, Boivin MJ, 2019. Neurocognitive testing in West African children 3–6 years of age: Challenges and implications for data analyses. *Brain Res Bull* 145: 129–135.
- World Health Organization, 2019. *Bench Aids for the Diagnosis of Intestinal Parasites*, Second Edition. Paris, France: WHO.
- Planche T, Krishna S, Kombila M, Engel K, Faucher JF, Ngoumilama E, Kreamsner PG, 2001. Comparison of methods for the rapid laboratory assessment of children with malaria. *Am J Trop Med Hyg* 65: 599–602.
- Ndam NT, et al., 2017. Resisting and tolerating *P. falciparum* in pregnancy under different malaria transmission intensities. *BMC Med* 15: 130.

21. Kaufman AS, Kaufman NL, 2014. *Kaufman Assessment Battery for Children*, Second Edition. Circle Pines, MN: American Guidance Service.
22. Bruininks BD, Bruininks RH, 2005. *Bruininks-Oseretsky Test of Motor Proficiency (BOT-2)*, Second Edition. Minneapolis, MN: Pearson Products, Inc.
23. Mireku MO, Cot M, Massougbdji A, Bodeau-Livinec F, 2020. Relationship between stunting, wasting, underweight and geophagy and cognitive function of children. *J Trop Pediatr* 66: 517–527.
24. Caldwell BM, Bradley RH, 2001. *Home Inventory Administration Manual*, Third Edition. Little Rock, AR: University of Arkansas for Medical Sciences.
25. Cox JL, Holden JM, Sagovsky R, 1987. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. *Br J Psychiatry* 150: 782–786.
26. Jones BL, Nagin DS, 2013. A note on a Stata plugin for estimating group-based trajectory models. *Social Methods Res* 42: 608–613.
27. Sekhavat L, Davar R, Hosseini-dezoki S, 2011. Relationship between maternal hemoglobin concentration and neonatal birth weight. *Hematology* 16: 373–376.
28. Stephansson O, Dickman PW, Johansson A, Cnattingius S, 2000. Maternal hemoglobin concentration during pregnancy and risk of stillbirth. *JAMA* 284: 2611–2617.
29. Liu D, et al., 2022. Maternal hemoglobin concentrations and birth weight, low birth weight (LBW), and small for gestational age (SGA): Findings from a prospective study in Northwest China. *Nutrients* 14: 858.
30. Randall DA, Patterson JA, Gallimore F, Morris JM, McGee TM, Ford JB; Obstetric Transfusion Steering Group, 2019. The association between haemoglobin levels in the first 20 weeks of pregnancy and pregnancy outcomes. *PLoS One* 14: e0225123.
31. Young MF, Oaks BM, Rogers HP, Tandon S, Martorell R, Dewey KG, Wendt AS, 2023. Maternal low and high hemoglobin concentrations and associations with adverse maternal and infant health outcomes: An updated global systematic review and meta-analysis. *BMC Pregnancy Childbirth* 23: 264.
32. Fararouei M, Robertson C, Whittaker J, Sovio U, Ruokonen A, Pouta A, Hartikainen A-L, Jarvelin M-R, Hyppönen E, 2010. Maternal Hb during pregnancy and offspring's educational achievement: A prospective cohort study over 30 years. *Br J Nutr* 104: 1363–1368.
33. Nampijja M, Mutua AM, Elliott AM, Muriuki JM, Abubakar A, Webb EL, Atkinson SH, 2022. Low hemoglobin levels are associated with reduced psychomotor and language abilities in young Ugandan children. *Nutrients* 14: 1452.
34. Janbek J, Sarki M, Specht IO, Heitmann BL, 2019. A systematic literature review of the relation between iron status/anemia in pregnancy and offspring neurodevelopment. *Eur J Clin Nutr* 73: 1561–1578.
35. Wedderburn CJ, et al., 2022. Association of maternal and child anemia with brain structure in early life in South Africa. *JAMA Netw Open* 5: e2244772.
36. Ouédraogo S, Koura GK, Accrombessi MMK, Bodeau-Livinec F, Massougbdji A, Cot M, 2012. Maternal anemia at first antenatal visit: Prevalence and risk factors in a malaria-endemic area in Benin. *Am J Trop Med Hyg* 87: 418–424.
37. Ouédraogo S, Koura GK, Bodeau-Livinec F, Accrombessi MMK, Massougbdji A, Cot M, 2013. Maternal anemia in pregnancy: Assessing the effect of routine preventive measures in a malaria-endemic area. *Am J Trop Med Hyg* 88: 292–300.