


ORIGINAL ARTICLE

Lower iron stores were associated with suboptimal gross motor scores in infants at 3–7 months

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Funding information

This work was funded by Vestfold Hospital Trust.

Abstract

Aim: To investigate associations between iron status and gross motor scores in infants aged 3–7 months.

Methods: In a prospective study, 252 infants aged 3–7 months were examined using the age-standardised Alberta Infant Motor Scale (AIMS) prior to analysing iron status in 250 infants. Combined AIMS and ferritin results were assessed in 226 infants, whereas AIMS and reticulocyte haemoglobin (ret-Hb) results were obtained for 61 infants. We used logistic regressions and receiver operator characteristics to analyse our data.

Results: With AIMS z-score <10th percentile as outcome measure, optimal cut-off value for ferritin was 51 µg/L (sensitivity 86%, specificity 81%) and 28 pg for ret-Hb (sensitivity 86%, specificity 85%). The area under the curve for ferritin and ret-Hb was 0.886 and 0.896, respectively ($n = 61$). Ferritin <51 µg/L predicted an AIMS z-score <10th percentile in a logistic regression (OR 3.3, 95% CI 1.4–7.5, $p = 0.006$, $n = 226$). Six of 14 (43%) infants with ret-Hb <28 pg scored <10th percentile on AIMS compared to 1/47 (2.1%) infants with ret-Hb ≥ 28 µg/L (Exact, $p < 0.001$).

Conclusion: Reticulocyte haemoglobin of <28 pg and ferritin <51 µg/L were associated with suboptimal gross motor scores in infants 3–7 months.

KEYWORDS

ferritin, infant nutrition, iron, motor development, reticulocyte haemoglobin

1 | INTRODUCTION

Iron is an essential micronutrient for the developing brain and is needed for myelination and maturation of the brain and for cognitive functions.¹ Iron deficiency in infancy is common in both high-income and low-income countries. The infants depend on sufficient availability of micronutrients to achieve rapid growth and development. Early identification and prevention of iron deficiency is especially

important since early iron supplementation has been shown to result in better motor skills at 9 and 12 months of age when compared to non-supplemented infants.^{2–4}

Ferritin levels are widely used as a biomarker of iron stores.⁵ Since ferritin exhibits large age-dependent variations during the first 6 months of life,⁶ as well as being an acute-phase protein increasing with infection and inflammation,⁷ reticulocyte haemoglobin (ret-Hb) is increasingly used in this age group to estimate iron stores.

Abbreviations: AIMS, Alberta Infant Motor Scale; CI, confidence interval; OR, odds ratio; ret-Hb, reticulocyte haemoglobin.

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Ret-Hb depicts the availability of iron for haemoglobin production in the bone marrow and predicts the state of bone marrow iron stores better than ferritin and mean corpuscular volume.⁸ Ret-Hb is used as a functional marker of iron sufficiency when ferritin cannot be used to determine iron status. If iron stores are sufficient for haemoglobin synthesis, ret-Hb remains within normal limits, but with lower iron stores, ret-Hb decreases.⁷ Even though iron deficiency has been causally related to impaired neurodevelopment,⁹ there are no studies on the relation of iron deficiency on motor development in infants younger than 6 months of age,¹⁰ nor on the association between ret-Hb and infant development.¹¹

The aims of this prospective cohort study were to investigate associations between iron status and gross motor scores in infants aged 3–7 months and to evaluate their psychomotor function at 2 years of age.

2 | MATERIALS AND METHODS

2.1 | Study population

We invited infants born at gestational age ≥ 32 weeks, without identified perinatal neurological disease.^{12–14} All infants were recruited at Vestfold Hospital Trust and were examined by a paediatrician at invitation. Small for gestational age was defined as birth weight below the 10th percentile for gestational age. Infants with a birth weight below 2500 g were advised to be supplemented with 9 mg iron daily for the first year of life starting at 4 weeks of age according to local procedures, based on national recommendations. The mothers completed a non-standardised questionnaire on their use of vitamin supplements during pregnancy and lactation. The study was approved by the Norwegian Regional Committee for Medical and Health Research Ethics (179/2018) and conducted according to the Declaration of Helsinki. Written informed consent was provided by all participants.

2.2 | Assessment of gross motor skills

A single study visit per infant was scheduled consecutively to cover the age span between 3 and 7 months. The examiners were blinded to biochemical test results, clinical and perinatal history, and were only given information of the infants' age corrected for term date prior to clinical testing. The infants were examined using the Alberta Infant Motor Scale (AIMS), an observational assessment scale to assess gross motor development from birth until the acquisition of independent walking.¹⁵ The infant is scored from 0 to 60 with a recommended cut-off for suboptimal development set to <10th percentile for age.¹⁶ Age corrected for term was used for infants born before 37 weeks gestational age, and AIMS z-scores for age were calculated by using mean and standard deviations published from the reference material.¹⁵

Key notes

- Reticulocyte haemoglobin of <28 pg and ferritin <51 μ g/L were associated with suboptimal gross motor scores in infants 3–7 months.
- Reticulocyte haemoglobin displayed less biological variation with age than ferritin in infants at 3–7 months of age.
- Consider including iron status when evaluating infants at risk of iron deficiency or delayed motor development.

2.3 | Biochemical analyses

Venous blood samples were collected non-fasting in 4-ml Vacuette serum tubes with serum separator and clot activator (Greiner Bio-One) and analysed at the Department of Medical Biochemistry at Vestfold Hospital Trust. The laboratory is accredited in accordance with NS-EN ISO 15189. To obtain serum for analyses of ferritin and CRP, the blood samples were left at room temperature for a minimum of 30 min to allow for coagulation and centrifuged within 2 h. Haematology samples were analysed using XN-9000 analysers (Sysmex Co.). To ensure optimal use of low sample volumes of analyses that cannot be retrieved in case of a new request, our laboratory performs per default a full haematological panel when either <1 ml sample material is received or Hb <9.5 g/dl. Ret-Hb was retrieved from the full haematological panel. Reference intervals according to chronological age were used. Reference values for ret-Hb in our laboratory are 28–39 pg for 0 to <6 months and 29–36 pg for 6 to <24 months.¹⁷ Ferritin was measured on Cobas e801 (Roche Diagnostics) with reagents from the supplier. Reference values for ferritin in our laboratory are 50–200 μ g/L for 1 to <6 months and 10–150 μ g/L for 6 months to 15 years.¹⁸ Reference values for haemoglobin in our laboratory are 8.9–12.7 g/100 ml for 1 to <6 months and 10.7–13.4 g/100 ml for 6 months to 8 years.¹⁹ The parents were informed about test results and were given nutritional advice or recommended iron supplementation when appropriate.

2.4 | Ages and Stages Questionnaire (ASQ)

The parents were invited to complete the validated Norwegian translation²⁰ of the Ages and Stages Questionnaire second version (ASQ-2) at 24 months of age. The questionnaire is designed to be answered by the caregivers and contains 30 developmental items divided into five domains: communication, gross motor, fine motor, problem-solving and personal-social. Each item is given a score according to the child's conduct with the item. The possible score range for each domain is 0–60. According to the manual, we categorised the ASQ-2 scores as suboptimal if at or below the cut-off according to the frequency distribution on the scoring sheet.²⁰

2.5 | Statistical analysis

Data were registered in EpiData version 4.4 (EpiData Association). Continuous variables were presented as mean and standard deviation or if skewed, as median and interquartile range (IQR). Categorical variables were given as proportions and percentages and compared between groups using the chi-square test of proportions or Fisher's exact test for small samples. Differences between independent groups were analysed with the Mann-Whitney *U* test because of skewness in the data. The strength of association between variables was measured using Pearson's or Spearman's correlation coefficient (ρ) where appropriate. We used receiver operator characteristics (ROC) curves with AIMS z-score <10th percentile as outcome variable to test the ret-Hb and ferritin performance as classifiers and Youden's index to find optimal cut-off values. Logistic regression was applied to evaluate significant covariates for AIMS score <10th percentile. All statistical tests were two-sided, and a *p*-value <0.05 was considered statistically significant. Regression models were significant with *p* <0.05, and all independent variables were entered. Analyses were performed in IBM SPSS Statistics version 28 (IBM Inc), and graphs were created in NCSS 2021 Statistical Software (NCSS LLC).

3 | RESULTS

3.1 | Characteristics of population

We invited 327 infants between May 2018 and March 2019, of which 61 did not meet to the appointment. Moreover, 12 infants were undergoing work-up for suspected disease before study start and were, therefore, excluded. We included 254 presumably healthy infants in total. Two infants were withdrawn from the study by their parents after inclusion, leaving 252 infants for analyses. Details on inclusion, background characteristics and clinical and biochemical findings have been published elsewhere.¹²⁻¹⁴ The infants were categorised into three groups: 170 born at gestational age ≥ 37 weeks and appropriate weight for gestational age, 39 born at a gestational age of ≥ 37 weeks and 43 born preterm at gestational age 32-36+ 6 weeks. The 7/43 (16%) infants born preterm and small for gestational age were categorised as preterm. Venepuncture failed in two infants, haematology status was not analysable in one infant, and ferritin was missing in six infants due to haemolysis. A full haematological panel, including ret-Hb, was performed in 71 infants. We completed AIMS for 232 infants, analysed ferritin in 244 infants and ret-Hb in 71 infants, of whom 51 were term infants, nine term small for gestational age

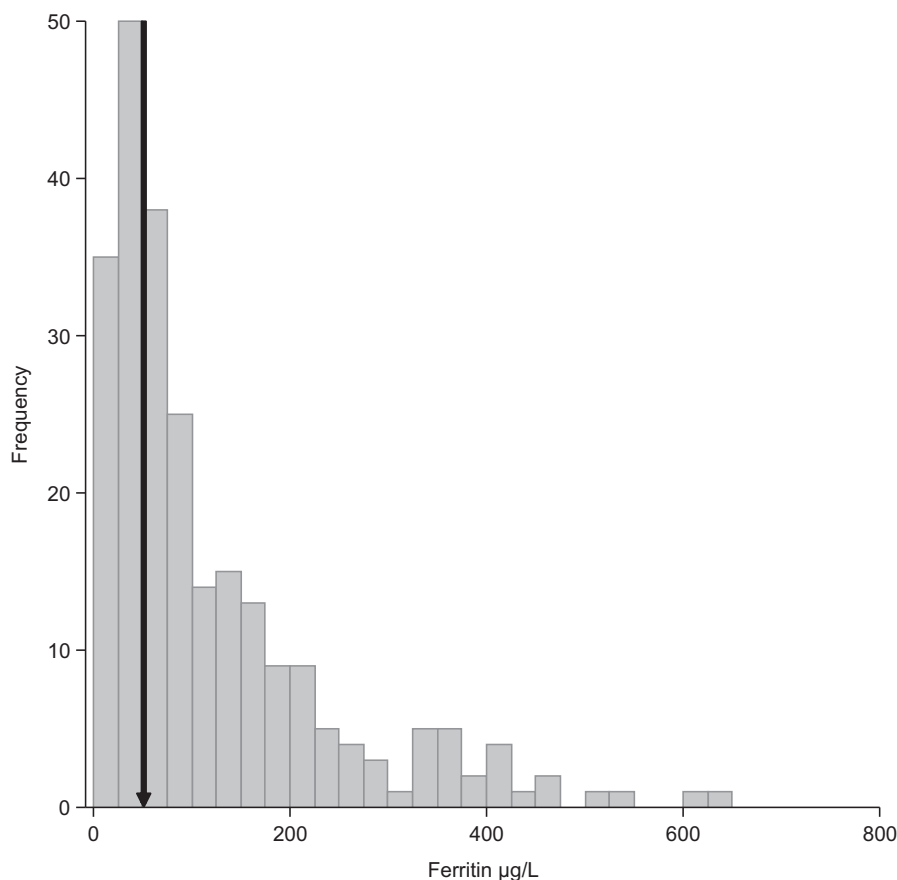


FIGURE 1 Frequency distribution of ferritin ($n = 244$) with vertical arrow at ferritin 51 $\mu\text{g/L}$

infants and 11 preterm infants. Insufficient state resulted in 20 missing AIMS scores. We obtained combined AIMS and ferritin results for 226 infants and AIMS and ret-Hb results for 61 infants. None of the infants had increased creatinine as a sign of kidney failure. Low haemoglobin was measured in 2/205 (1%) infants <6 months and in 6/44 (14%) infants ≥6 months. Ferritin (Figure 1) decreased with increasing age, but with a large variation at all time points (Figure 2). Ferritin was <10 µmol/L in 2/244 (0.8%) and <15 µmol/L in 6/244 (2.5%). Ferritin was below our laboratory reference ranges in 60/244 (25%), all of them <6 months of age (Table 1). Consequently, 32/244 (13%) were given nutritional advice and 23/244 (9.4%) were advised iron supplementation. Ferritin was associated with ret-Hb ($\rho = 0.306$, $p = 0.010$). Ret-Hb (Figure 3) showed no variation with age (Figure 4). Median [IQR] ferritin for infants after cord clamping ≥1 min was 86 [40–179] µg/L and 56 [35–86] µg/L in infants after cord clamping <1 min ($p = 0.025$, Mann-Whitney, $n = 229$). Median [IQR] ferritin for infants after cord clamping ≥3 and <3 min was 87 [43–190] µg/L and 64 [37–132] µg/L, respectively ($p = 0.035$, Mann-Whitney U , $n = 229$). None of the 26 infants receiving iron supplementation had ret-Hb <28 pg. We found no associations between cord clamping ≥3 min and ret-Hb ($\rho = -0.036$, $p = 0.772$) or ret-Hb ≥28 pg (chi-square, $p = 0.259$, $n = 66$). Median [IQR] ret-Hb after cord clamping ≥1 min versus <1 min was 30 [29–32] and 30 [30–31], respectively. We found no associations between the use of iron supplementation during pregnancy and infant iron status (data not shown).

3.2 | Associations between ferritin and AIMS

Ferritin correlated with AIMS z-score ($r = 0.207$, 95% CI 0.079–0.329, $n = 226$, $p = 0.002$). Infants scoring <10th percentile on AIMS had lower ferritin (median [IQR] = 44 [23–64]) compared with infants scoring ≥10th percentile on AIMS (median [IQR] = 86 [43–170] $p < 0.001$, Mann-Whitney U , $n = 226$). The 15/74 (20%) of infants with ferritin <51 µg/L scored <10th percentile on AIMS, compared to 11/152 (7.2%) of infants with ferritin ≥51 µg/L (chi-square, $p = 0.004$). Ferritin <51 µg/L predicted an AIMS z-score <10th percentile in a logistic regression (OR 3.3, 95% CI 1.4–7.5, $p = 0.006$, $n = 226$).

3.3 | Associations between ret-Hb and AIMS

Ret-Hb correlated with AIMS z-score ($r = 0.320$, 95% CI 0.074–0.529, $n = 61$, $p = 0.012$). Infants scoring <10th percentile on AIMS had lower ret-Hb (median [IQR] = 26 [22–27]) compared with infants scoring ≥10th percentile on AIMS (median [IQR] = 30 [29–32], $p < 0.001$, Mann-Whitney U , $n = 61$). The 6/14 (43%) of infants with ret-Hb <28 pg scored <10th percentile on AIMS compared to 1/47 (2.1%) of infants with ret-Hb ≥28 µg/L (Exact, $p < 0.001$). The 12/14 (86%) infants with ret-Hb <28 pg scored below mean on AIMS (Exact, $p = 0.013$).

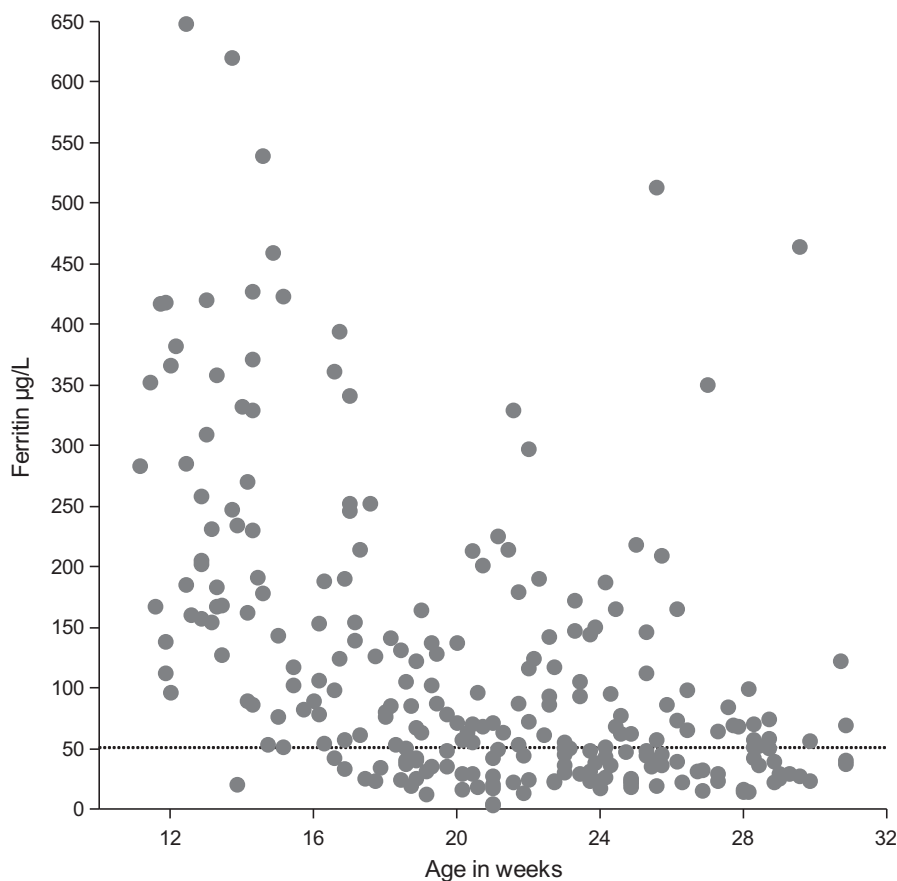


FIGURE 2 Relationship between ferritin and age ($n = 244$) with dashed line at ferritin 51 µg/L

3.4 | ROC of ferritin and ret-Hb

In a comparison of ROC of ferritin and ret-Hb with AIMS z-score <10th percentile as outcome measure, we found the optimal cut-off value for ferritin at 51 µg/L (Figure 5) with sensitivity = 86% and specificity = 81% and for ret-Hb at 28 pg with a sensitivity = 86% and specificity = 85%. The area under the curve for ferritin and ret-Hb was 0.886 and 0.896, respectively ($n = 61$). In a ROC of ferritin using all 226 available observations and with AIMS z-score <10th percentile as outcome measure, we found the optimal cut-off value for ferritin at 69 µg/L with a sensitivity of 81% and a specificity of 60%, AUC = 0.729 ($n = 226$).

3.5 | Analyses of potential confounders

In a logistic regression analysis, with AIMS z-score <10th percentile as dependent variable, and ferritin <51 µg/L, prematurity, and maternal university education as independent variables, only ferritin <51 µg/L predicted AIMS z-score <10th percentile (OR = 3.7,

95% CI 1.6–8.8, $p = 0.003$, $n = 223$). In a logistic regression analysis, with AIMS z-score <10th percentile as dependent variable, ret-Hb <28 pg, prematurity and maternal university education as independent variables, only ret-Hb <28 pg predicted AIMS z-score <10th percentile (OR = 39, 95% CI 3.7–397, $p = 0.002$, $n = 60$).

3.6 | Ages and Stages Questionnaire at 2 years

At 24 months, 3/13 (23%) of the infants with a ret-Hb <28 pg at 3–7 months of age scored below cut-off for problem-solving on ASQ-2 compared to 2/43 (4.6%) of infants with a ret-Hb ≥28 pg at 3–7 months of age (chi-square, $p = 0.041$) (Table 2).

4 | DISCUSSION

In this study, we found that ret-Hb <28 pg and ferritin <51 µg/L were associated with suboptimal gross motor scores in 3–7-month-old infants. Ferritin had a large age-dependent variation which is in

TABLE 1 Descriptive characteristics, mean (SD), median [interquartile range] or ratio (per cent)

	Term infants ($n = 170$)	Term infants, small for gestational age ($n = 39$)	Preterm infants ($n = 43$)	All infants ($n = 252$)
Ferritin (µg/L)	95 [50–191]	70 [36–102]	39 [24–56]	74 [39–163]
Reticulocyte haemoglobin (pg) ^a	30 [29–31]	29 [29–32]	31 [28–32]	30 [29–32]
Haemoglobin (g/100 ml)	11.6 [11.0–12.1]	11.4 [11.0–11.9]	11.6 [11.2–12.5]	11.5 [11.0–12.1]
Mean corpuscular volume (fl)	79 [77–82]	80 [77–84]	77 [76–79]	79 [77–82]
Mean corpuscular haemoglobin (pg)	27 [26–28]	27 [26–29]	26 [26–28]	27 [26–28]
Creatinine (mmol/L)	18 [15–20]	17 [15–20]	17 [14–20]	17 [15–20]
C-reactive protein (mmol/L)	0.9 [0.9–0.9]	0.9 [0.9–0.9]	0.9 [0.9–0.9]	0.9 [0.9–0.9]
University education	118/169 (70%)	20/38 (53%)	31/39 (79%)	169/246 (69%)
Maternal iron supplementation during pregnancy	37/170 (22%)	13/39 (33%)	8/41 (20%)	58/250 (23%)
Gestational age weeks	40.1 [39.3–40.9]	39.0 [38.0–39.9]	35.4 [34.0–36.4]	39.7 [37.9–40.6]
Caesarean section	24/170 (14%)	10/39 (26%)	22/43 (51%)	56/252 (22%)
Cord clamping >1 min	144/161 (90%)	32/37 (87%)	22/38 (58%)	198/236 (84%)
Cord clamping >3 min	106/161 (66%)	21/37 (57%)	10/38 (26%)	137/236 (58%)
Female sex	84/170 (49%)	23/39 (59%)	17/43 (40%)	124/252 (49%)
Birthweight (g)	3652 (433)	2648 (304)	2458 (462)	3293 (668)
Exclusively breastmilk	63/170 (37%)	11/38 (29%)	8/41 (20%)	82/249 (33%)
Maternal iron supplementation during breastfeeding	21/170 (12%)	5/39 (13%)	2/40 (5.0%)	28/249 (11%)
Infant iron supplementation	0/167 (0%)	7/38 (18%)	19/38 (50%)	26/243 (11%)
Infant age in weeks	20.5 (5.4)	19.7 (5.0)	23.0 (3.7)	20.8 (5.2)
Weight z-score ^b	0.30 (0.90)	-1.06 (0.79)	-0.77 (0.96)	-0.09 (1.06)
Ferritin low ^c	26/141 (16%)	11/37 (30%)	23/40 (58%)	60/244 (25%)
Reticulocyte haemoglobin <28 pg	12/51 (24%)	1/9 (11%)	1/11 (9.1%)	14/71 (20%)
AIMS <10 percentile	18/163 (11%)	5/34 (15%)	3/35 (8.6%)	26/232 (11%)

Abbreviation: AIMS, Alberta Infant Motor Scale.

^aTerm infants = 51, term small for gestational age infants = 9 and preterm infants = 11.

^bNorwegian growth charts.

^cBelow cut-off 50 µg/L if <6 months, <10 µg/L if ≥6 months.

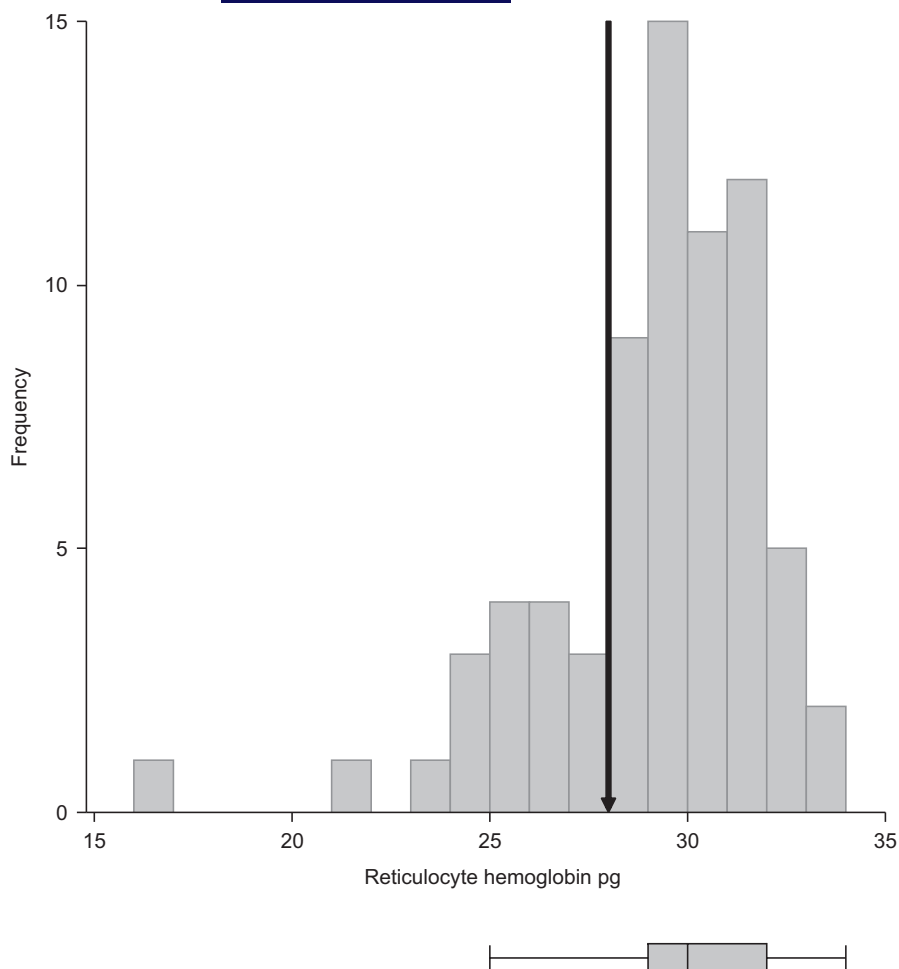


FIGURE 3 Frequency distribution of ret-Hb ($n = 71$) with vertical arrow at ret-Hb 28 pg

line with current knowledge,⁶ but ret-Hb was found to be age-independent between 3 and 7 months, in accordance with another study including both preterm and term-born infant.²¹ Both ferritin and ret-Hb levels were found to correlate positively with gross motor scores. We believe ret-Hb is a better biomarker due to substantially lower inter-individual biological variation and age variation. In addition, when available, ret-Hb can be analysed on the same blood sample as the haematology panel at no extra cost, in contrast to ferritin, which needs an extra serum sample. Different cut-offs for ret-Hb have been proposed but have relied on biochemical data only. In a recently published reference material for ret-Hb, the 2.5th percentile was used as the lower limit, equivalent to a ret-Hb of 25 pg with 90% confidence interval of 21–25 pg.²² In the present study, we combined biochemical data with AIMS, a validated assessment of gross motor development, commonly used in research and clinical settings. Even with our small sample size, we clearly demonstrated that a ret-Hb <28 pg was associated with suboptimal gross motor scores. In a ROC analysis, we found an optimal ret-Hb cut-off at 28 pg for an AIMS z-score <10th percentile. Our results showed that none of the infants supplemented with iron had ret-Hb <28 pg. In addition, the literature supports a ret-Hb cut-off at 28 pg: Torsvik et al.²³ demonstrated a breakpoint at ret-Hb of 28.2 pg in a dose-response curve for 4-month-old infants when predicting haemoglobin at 6 months of age. Pomrop et al.²⁴ found an optimal cut-off of

<29 pg when screening preterm infants for iron deficiency. Ullrich et al.²⁵ argued that ret-Hb <27.5 pg could be used when screening healthy 9–12-month-old infants for iron deficiency. Together, all these findings support the use of 28 pg as a cut-off for optimal ret-Hb in 3–7-month-old infants.

It is challenging to set the lower limits of reference intervals for ferritin for compound age groups since ferritin is varying considerably with age,⁶ and is generally based on an iron deficiency anaemia diagnosis rather than iron deficiency and clinical outcome.²⁶ There is a physiologic decline in ferritin level during the first 6 months due to consumption of iron store supplied prenatally. We argue that only a relevant clinical outcome can decide at what level this physiological decline becomes pathological. Reference intervals solely based on biochemical data may set the limits unnaturally low from inclusion of infants with suboptimal clinical outcome due to deficiency. Voluntary and goal-directed movements start from the age of 3–4 months²⁷ while ferritin declined between 4 and 6 months in our study. We speculate that humans evolutionary were introduced to tasting of raw meat²⁸ once infants started to reach for food: By grasping and tasting different food, they started to refill micronutrient stores from around 4 months of age. In a position paper, Fewtrell et al.²⁹ found data to support the introduction of iron-containing complementary food alongside breastfeeding from 4 months also to infants with a low risk of iron deficiency. Our findings of optimal

FIGURE 4 Relation between ret-Hb and age ($n = 71$) with dashed line at ret-Hb 28 pg

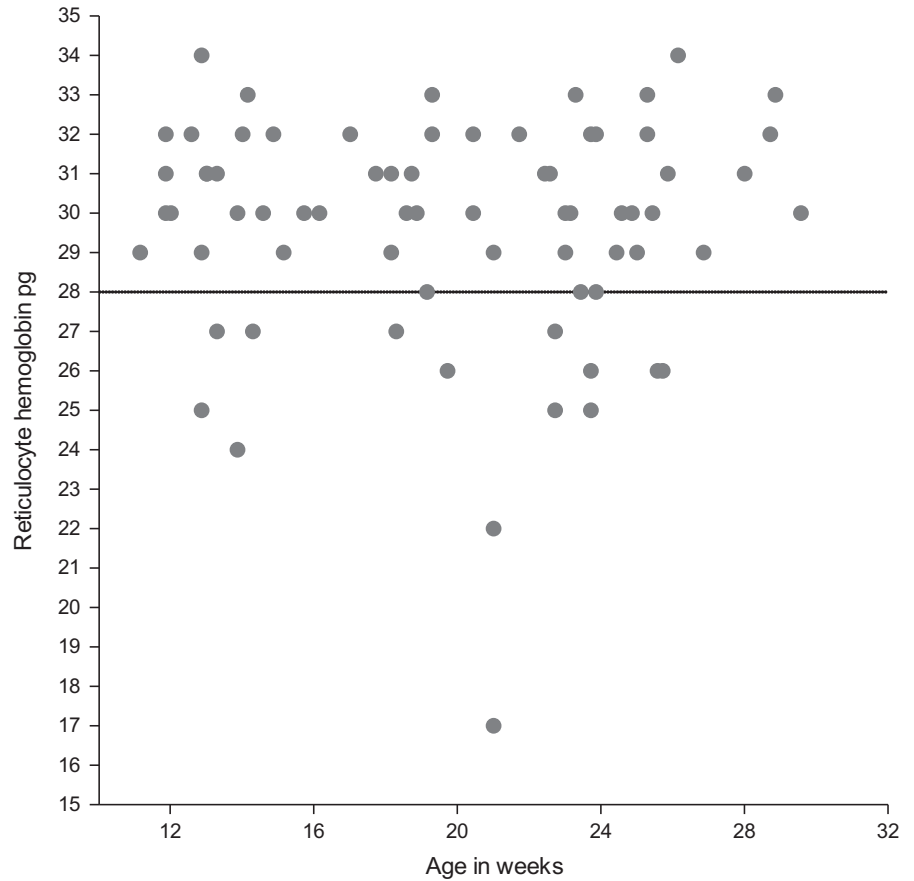


FIGURE 5 Comparison of ROC of ferritin and ret-Hb with AIMS z-score <10th percentile as outcome variable ($n = 61$)

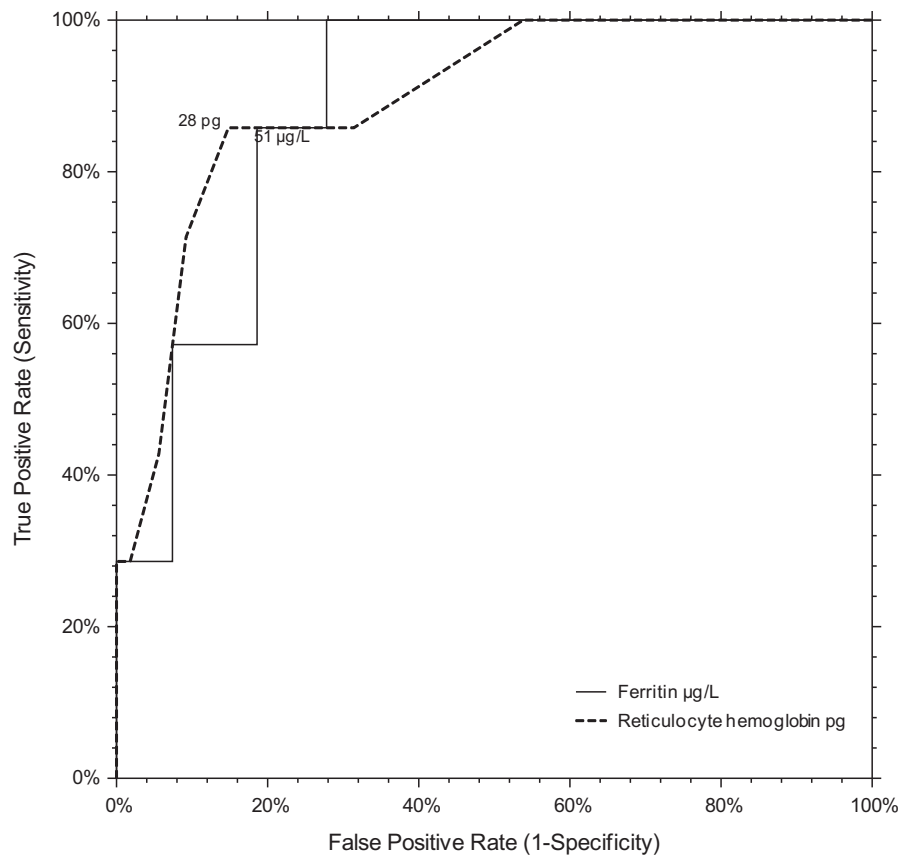


TABLE 2 Comparison of rate of infants scoring below cut-off on Ages and Stages Questionnaire (ASQ) subscales at 24 months between infants with a reticulocyte haemoglobin <28 and ≥28 pg at 3–7 months of age

ASQ subscale	Reticulocyte haemoglobin		Chi-square
	<28 pg	≥28 pg	<i>p</i>
Communication	3/13	6/43	0.433
Gross motor	0/13	1/43	0.579
Fine motor	1/13	2/43	0.670
Problem-solving	3/13	2/43	0.041
Personal-social	0/13	4/43	0.254

cut-offs for ferritin between 51 and 69 µg/L in relation to optimal gross motor scores support a higher physiological lower ferritin level in the age group 3–7 months than the lower limit 10–20 µg/L derived from biochemical reference intervals.⁶ This is in line with the recommendations to use a decision limit of ferritin of 30 µg/L for children 4 months to <13 years of age for optimal erythropoiesis.³⁰

When assessed with ASQ-2 at 24 months, 23% of infants with ret-Hb <28 pg scored below cut-off on problem-solving compared to 4.7% of infants with a ret-Hb ≥28 pg. This difference was only borderline significant and should be interpreted with caution due to the small numbers. We found no differences in the domains of communication, gross motor, fine motor and social-personal subscales when comparing infants with ret-Hb below and above 28 pg.

According to Norwegian national recommendations, all infants with a birthweight below 2500 g are advised a daily iron supplementation. In the present study, only half of the preterm infants received iron supplementation. In infants born small for gestational age, the fraction was 18%, indicating suboptimal compliance with the recommendations. Fewer infants in the latter two groups were exclusively breastfed, and the formula is supplemented with iron. The combination of more frequent use of formula and iron supplementation possibly explained the lower frequency of ret-Hb <28 pg in the latter two groups compared with term infants with appropriate weight for gestational age. The higher proportion of low ferritin for the preterm infants may partly be explained by a mean age of 23 weeks compared with 20 weeks for the other groups, since ferritin levels decrease with age. None of the infants receiving iron supplementation prior to the visit had ret-Hb <28 pg and this reinforces the importance of substituting breastfed infants at risk of iron deficiency to optimise development. Our laboratory's lower limit for ferritin of 50 µg/L for infants <6 months of age¹⁸ is higher compared with other recommendations.³¹ In our study population, we accordingly found a high proportion with ferritin <50 µg/L, encompassing 16% of term-born infants with appropriate birth weight. Our study was not designed to estimate the prevalence of iron deficiency. Nevertheless, our findings of 0.8% infants 3–7 months old with ferritin <10 µg/L and 2.5% with <15 µg/L were within the same range as in a previous Norwegian study,³² where 2% and 6% of 6-month-old infants had ferritin <10 and <15 µg/L, respectively.

We cannot exclude the possibility of a selection bias in our study as 19% of invited infants did not show up for examination. However, 11% of infants scored <10th percentile compared to 10% in the normative material for AIMS. Hence, our study population was also representative in terms of the outcome measure motor scores. Therefore, we assume that the missing values of the different biochemical analyses were random. Six women did not report their level of education and that may have introduced some selection bias. The main study was designed with the primary aim of evaluating infant vitamin B12 status in relation to neurological and motor development, and a secondary aim was to study iron status. Thus, the design ensured that blood was drawn before testing the infants, a strength to our study, but ferritin was chosen as a marker for iron status when the study was planned, limiting the number of infants available for ret-Hb analysis. We cannot conclude that the associations we found were causal effects due to the observational design, and there may be important confounders not adjusted for. We can only speculate on the generalisability of our results to other populations, but it is plausible to assume that the importance of iron for development is generic.

In conclusion, we observed an association between lower iron stores and suboptimal gross motor scores in infants 3–7 months, possibly related to iron-deficient neurodevelopment. Iron status should be considered in the work-up for infants at risk of iron deficiency or delayed motor development. In the present study, ret-Hb of <28 pg and ferritin <51 µg/L were associated with lower AIMS score.

ACKNOWLEDGEMENTS

We thank the children and families who participated in the study. The co-author Trine Tangeraas is a health care representative on behalf of Oslo University Hospital, member of the European Reference Network for Rare Hereditary Metabolic Disorders (MetabERN).

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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REFERENCES

- Pallone LV, de Jesus FA, Gonçalves GA, et al. Effects of intrauterine latent iron deficiency on auditory neural maturation in full-term newborns. *J Pediatr (Rio J)*. 2020;96(2):202-209. doi:10.1016/J.JPED.2018.08.007
- Angulo-Barroso RM, Li M, Santos DCC, et al. Iron supplementation in pregnancy or infancy and motor development: a randomized controlled trial. *Pediatrics*. 2016;137(4):e20153547. doi:10.1542/peds.2015-3547
- Moffatt ME, Longstaffe S, Besant J, Dureski C. Prevention of iron deficiency and psychomotor decline in high-risk infants through use of iron-fortified infant formula: a randomized clinical trial. *J Pediatr*. 1994;125(4):527-534. doi:10.1016/s0022-3476(94)70003-6
- Friel JK, Aziz K, Andrews WL, Harding SV, Courage ML, Adams RJ. A double-masked, randomized control trial of iron supplementation

- in early infancy in healthy term breast-fed infants. *J Pediatr*. 2003;143(5):582-586. doi:10.1067/S0022-3476(03)00301-9
5. Mei Z, Cogswell ME, Parvanta I, et al. Hemoglobin and ferritin are currently the most efficient indicators of population response to iron interventions: an analysis of nine randomized controlled trials. *J Nutr*. 2005;135(8):1974-1980. doi:10.1093/jn/135.8.1974
 6. Larsson SM, Hillarp A, Hellström-Westas L, Domellöf M, Lundahl T, Andersson O. When age really matters; ferritin reference intervals during infancy revisited. *Scand J Clin Lab Invest*. 2019;79(8):590-594. doi:10.1080/00365513.2019.1681028
 7. Ogawa C, Tsuchiya K, Maeda K. Reticulocyte hemoglobin content. *Clin Chim Acta*. 2020;504:138-145. doi:10.1016/j.cca.2020.01.032
 8. Mast AE, Blinder MA, Lu Q, Flax S, Dietzen DJ. Clinical utility of the reticulocyte hemoglobin content in the diagnosis of iron deficiency. *Blood*. 2002;99(4):1489-1491. doi:10.1182/blood.v99.4.1489
 9. Berglund SK, Domellöf M. Iron deficiency in infancy: current insights. *Curr Opin Clin Nutr Metab Care*. 2021;24(3):240-245. doi:10.1097/MCO.0000000000000749
 10. McCann S, Perapoch Amadó M, Moore SE. The role of iron in brain development: a systematic review. *Nutrients*. 2020;12(7):2001. doi:10.3390/nu12072001
 11. German KR, Juul SE. Iron and neurodevelopment in preterm infants: a narrative review. *Nutrients*. 2021;13(11):3737. doi:10.3390/NU13113737
 12. Ljungblad UW, Paulsen H, Mørkrid L, et al. The prevalence and clinical relevance of hyperhomocysteinemia suggesting vitamin B12 deficiency in presumed healthy infants. *Eur J Paediatr Neurol*. 2021;35:137-146. doi:10.1016/j.ejpn.2021.10.008
 13. Ljungblad UW, Paulsen H, Tangeraas T, Evensen KAI. Reference material for Hammersmith Infant Neurologic Examination scores based on healthy, term infants age 3-7 months. *J Pediatr*. 2022;244:79-85.e12. doi:10.1016/j.jpeds.2022.01.032
 14. Ljungblad UW, Astrup H, Mørkrid L, et al. Breastfed infants with spells, tremor, or irritability rule out vitamin B12 deficiency. *Pediatr Neurol*. 2022;131:4-12. doi:10.1016/j.pediatrneurol.2022.03.003
 15. Piper MC, Johanna D. *Alberta Infant Motor Scale (AIMS): Motor Assessment of the Developing Infant*. WB Saunders Company; 1994.
 16. Darrach J, Piper M, Watt MJ. Assessment of gross motor skills of at-risk infants: predictive validity of the Alberta Infant Motor Scale. *Dev Med Child Neurol*. 1998;40(7):485-491.
 17. Soldin SJ, Wong EC, Brugnara C, Soldin OP. *Pediatric Reference Intervals*. 7th ed. AACC Press; 2011.
 18. Wu A. *Tietz Clinical Guide to Laboratory Tests Fourth Edition*. 6th ed. Saunders Elsevier; 2006.
 19. Aldrimer M, Ridefelt P, Rödö P, Niklasson F, Gustafsson J, Hellberg D. Population-based pediatric reference intervals for hematology, iron and transferrin. *Scand J Clin Lab Invest*. 2013;73(3):253-261. doi:10.3109/00365513.2013.769625
 20. Janson H, Squires J. Parent-completed developmental screening in a Norwegian population sample: a comparison with US normative data. *Acta Paediatr*. 2004;93(11):1525-1529. doi:10.1111/j.1651-2227.2004.tb02641.x
 21. Takala TI, Mäkelä E, Suominen P, et al. Blood cell and iron status analytes of preterm and full-term infants from 20 weeks onwards during the first year of life. *Clin Chem Lab Med*. 2010;48(9):1295-1301. doi:10.1515/CCLM.2010.242
 22. Larsson SM, Hellström-Westas L, Hillarp A, et al. Haemoglobin and red blood cell reference intervals during infancy. *Arch Dis Child*. 2021;107(4):351-358. doi:10.1136/archdischild-2021-321672
 23. Torsvik IK, Markestad T, Ueland PM, Nilsen RM, Midttun Ø, Bjørke Monsen AL. Evaluating iron status and the risk of anemia in young infants using erythrocyte parameters. *Pediatr Res*. 2013;73(2):214-220. doi:10.1038/PR.2012.162
 24. Pomrop M, Manopunya S, Tantiprabha W, Khuwuthyakorn V, Kosarat S, Natesirinilkul R. Reticulocyte hemoglobin concentration for screening iron deficiency in very low birth weight preterm neonates. *J Matern Fetal Neonatal Med*. 2020;1-5. doi:10.1080/14767058.2020.1818216
 25. Ullrich C, Wu A, Armsby C, et al. Screening healthy infants for iron deficiency using reticulocyte hemoglobin content. *Jama*. 2005;294(8):924-930. doi:10.1001/jama.294.8.924
 26. Domellöf M, Dewey KG, Lönnnerdal B, Cohen RJ, Hernell O. The diagnostic criteria for iron deficiency in infants should be reevaluated. *J Nutr*. 2002;132(12):3680-3686. doi:10.1093/jn/132.12.3680
 27. Hadders-Algra M. Early human motor development: from variation to the ability to vary and adapt. *Neurosci Biobehav Rev*. 2018;90:411-427. doi:10.1016/j.neubiorev.2018.05.009
 28. Andrews P, Johnson RJ. Evolutionary basis for the human diet: consequences for human health. *J Intern Med*. 2020;287(3):226-237. doi:10.1111/JOIM.13011
 29. Fewtrell M, Bronsky J, Campoy C, et al. Complementary feeding: a position paper by the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) committee on nutrition. *J Pediatr Gastroenterol Nutr*. 2017;64(1):119-132. doi:10.1097/MPG.0000000000001454
 30. Markus C, Saxon B, Metz M. Ferritin as a functional biomarker of iron status in children and young adults. *Br J Haematol*. 2019;184(4):640-642. doi:10.1111/bjh.15127
 31. Magnus Domellöf Å, Braegger C, Campoy C, et al. Iron requirements of infants and toddlers. *J Pediatr Gastroenterol Nutr*. 2014;58(1):119-129. doi:10.1097/MPG.0000000000000206
 32. Hay G, Sandstad B, Whitelaw A, Borch-Johnsen B. Iron status in a group of Norwegian children aged 6-24 months. *Acta Paediatr*. 2004;93(5):592-598. doi:10.1111/j.1651-2227.2004.tb02983.x

How to cite this article: Ljungblad UW, Tangeraas T, Paulsen H, Lindberg M. Lower iron stores were associated with suboptimal gross motor scores in infants at 3-7 months. *Acta Paediatr*. 2022;111:1941-1949. <https://doi.org/10.1111/apa.16469>