# Clinical Prediction of Iron Deficiency at Age 2 Years: A National Cross-sectional Study in France

Claire Guivarch, Anne-Sylvia Sacri, Corinne Levy, Alain Bocquet, Nathanaël Lapidus, Serge Hercberg, Pascale Hebel, Anne Chevé, Christian Copin, Morched Zouari, et al.

**Clinical prediction of iron deficiency at 2 years-old: a national cross-sectional study in France**

**Authors' full name and academic degrees**

Claire Guivarch, MSc [1]; Anne-Sylvia Sacri, MD, PhD [1,2]; Corinne Levy, MD [3,4,5]; Alain Bocquet, MD [3,6]; Nathanaël Lapidus, MD, PhD [7]; Serge Hercberg, MD, PhD [8,9]; Pascale Hebel, PhD [10]; Anne Chevé, MD [11]; Christian Copin, MD [12]; Morched Zouari, MD [13]; Laurent Gouya, MD, PhD [14]; Mariane de Montalembert, MD, PhD [1,2]; Jérémie F. Cohen, MD, PhD [1,2]; Martin Chalumeau MD, PhD [1,2]

**Authors' affiliations**

[1] Centre of Research in Epidemiology and Statistics, Obstetrical, Perinatal and Pediatric Epidemiology Research team (EPOPé), INSERM UMR 1153, Université de Paris, 75004 Paris, France

[2] Department of General Pediatrics and Pediatric Infectious Diseases, AP-HP, Necker-Enfants malades hospital, Université de Paris, 75015 Paris, France

[3] Association Française de Pédiatrie Ambulatoire (AFPA), Talence, France

[4] Association Clinique et Thérapeutique Infantile du Val-de-Marne (ACTIV), Saint-Maur des Fossés, France

[5] Université Paris Est, IMRB- GRC GEMINI, 94000 Créteil, France

[6] Comité de nutrition de la Société Française de Pédiatrie

[7] Sorbonne University, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique IPLESP, Public Health Department, Saint-Antoine Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France

[8] Centre of Research in Epidemiology and Statistics, Nutritional Epidemiology Research Team (EREN), INSERM UMR 1153, Université de Paris, Sorbonne Paris Cité, France

[9] Département de Santé Publique, AP-HP, Hôpital Avicenne, Bobigny

[10] Centre de Recherche pour l'Etude et l'Observation des Conditions de vie (CREDOC), Paris, France

[11] AFPA, Brest, France.

[12] AFPA, Provins, France

[13] AFPA, Etampes, France

[14] Centre de recherche sur l'inflammation, INSERM UMR 1149, Université Paris Diderot; ERL CNRS 8252, Faculté de Médecine site Bichat, Paris, France

**Corresponding author: Claire Guivarch**

Maternité de Port-Royal, 53 Avenue de l'Observatoire, 75014 Paris

Phone: +33 1 42 34 55 80

E-mail: claire.guivarch@inserm.fr

**Short title**

Clinical prediction of iron deficiency at 2 years-old

**Conflict of interest statement**

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**Clinical Trial Registration**

Iron deficiency (ID) in infants, NCT02484274.

Deidentified individual participant data (including data dictionaries) will be made available, in addition to study protocols, the statistical analysis plan, and the informed consent form. The data will be made available upon publication to researchers who provide a methodologically sound proposal for use in achieving the goals of the approved proposal. Proposals should be submitted to martin.chalumeau@inserm.fr

**Abbreviations**

AAP: American Academy of Pediatrics

AUROC: Area under the receiver operating characteristic curve

CI: Confidence interval

ID: Iron deficiency

SF: Serum ferritin

TRIPOD: Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis

WG: Weeks of gestation

**Keywords**

Screening; prediction tools; nationwide cohort study

**Abstract**

**Objective(s):** The American Academy of Pediatrics (AAP) recommends iron deficiency (ID) screening in at-risk infants, based on several clinical criteria which diagnostic accuracy has never been evaluated. We aimed to assess this diagnostic accuracy and to develop prediction tools for ID in 2-years-old infants.

**Study design:** In a national cross-sectional study conducted in primary care pediatricians' practices throughout France, 2-years-old infants were consecutively included (2016-2017). Multivariable logistic regression modeling and bootstrapping were used to develop several clinical models to predict ID (serum ferritin <12 μg/L). These models used the best criteria and combinations among AAP criteria adapted to the European context (n=10), then all potential predictors (n=19). One model was then simplified into a simple prediction tool.

**Results:** Among 568 included infants, 38 had ID (6.7%). In univariable analyses, no significant association with ID was observed for 8 of the 10 adapted AAP criteria. Three criteria (both parents born outside the European Union, low weight at one year old, and weaning to cow's milk without supplemental iron) were retained in the "AAP model", which AUROC, sensitivity, and specificity were 0.62 (95% CI 0.58-0.67), 30% (22-39%) and 95% (92-97%), respectively. Four criteria were retained in a newly derived "simple prediction tool" (≥one criterion among the three previous plus duration of iron-rich formula consumption <12 months), which AUROC, sensitivity, and specificity were 0.72 (0.65-0.79), 63% (47-80%) and 81% (70-91%), respectively.

**Conclusion(s):** All prediction tools achieved acceptable diagnostic accuracy. The newly derived simple prediction tool offered potential ease of use.

## INTRODUCTION

Iron deficiency (ID) is the most prevalent micronutrient deficiency worldwide, affecting an estimated two billion people (1). Infants under two years are at risk of developing ID, because of the high iron requirements needed for their rapid growth. ID may be associated with short- and long-term adverse neurocognitive outcomes when it occurs in infants (2-4). Medical societies and public health authorities have developed primary prevention programs aiming at tackling ID in infants by recommending optimized iron intakes (5-7). Despite these preventive strategies, ID is estimated frequent among infants in industrialized countries (6-8), with prevalence oscillating between 3% and 33% in Europe and being much higher in deprived populations (5, 9, 10). This partial failure of preventive strategies raises the question of the relevance of adding validated screening strategies to reduce the consequences of ID.

There is no consensus between learned societies on the relevance of ID screening compared to no screening (6, 11). The US Preventive Services Task Force (11), and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (5) do not recommend screening for ID, based on the current insufficient evidence. On the contrary, the American Academy of Pediatrics (AAP) recommends universal screening for anemia at one-year-old and targeted screening when infants are considered at high risk of developing ID (6) (**Appendix 1**). The Canadian Paediatric Society (CPS) recommends risk assessment in the first 2 years and targeted screening for ID in high-risk infants (12) (**Appendix 1**). In these recommendations, there is no consensus on the relevance of universal screening of ID compared to targeted screening based on clinical predictors of ID (6, 11, 12). One of the most used reference standards for detecting ID is a low serum ferritin level (5-7, 13), but its measurement requires an invasive blood sampling and would be costly if measured for an entire population. Clinical signs of ID, such as pallor of the skin and fatigue, are neither sensitive nor specific (14). Predictors of ID are well known, including low socio-economic

status, poor perinatal iron stock and imbalance between requirements and dietary intake (1, 5-7). A reason for such variability in guidance at national and international levels could be the lack of formal assessment of the diagnostic accuracy of the proposed criteria (11), taken individually or combined in prediction tools. As a consequence, iron status is not routinely examined in infancy (15), and primary healthcare providers are left with various potential screening strategies ranging from no screening to universal screening.

Our objectives were to assess the diagnostic accuracy of AAP criteria and to develop and validate new prediction tools for detecting ID in 2-years-old infants, using data from a national cross-sectional study in France.

## MATERIAL AND METHODS

### Study design and setting

The present study is a planned ancillary analysis of a French cross-sectional observational study aiming at evaluating the effectiveness of a national ID prevention strategy based on using iron-fortified formula at weaning (16). The study protocol was approved by local ethics and administrative authorities (CPP IDF III no. 3295) and registered (ClinicalTrials.gov identifier: NCT02484274). Signed consent of at least one parent was obtained before inclusion. This study is reported according to the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) statement (17). We used Stata/SE 13.1 (StataCorp, College Station, Texas) for data analysis and R 3.6.2 software for internal validation. Sample size calculation was performed for the primary objective of the study (16), and no a posteriori calculation was performed for this ancillary analysis.

**Study participants**

From January 2016 to December 2017, 58 primary care pediatricians throughout 16 French regions were asked to include 10 consecutive patients (**Appendix 2**), aged from 22 to 26 months, living in France, with health insurance coverage (16). Children were excluded if they were known to have any chronic disease that may affect iron metabolism (e.g., celiac disease; complete list in **Appendix 3**).

**Clinical and laboratory data collection**

Socio-demographic data, medical history, and past and current dietary habits were collected by the investigator at inclusion (16). A fasting blood sample was taken in a nearby laboratory. Serum ferritin and CRP measurements were centralized in a unique laboratory (CERBA laboratory, Saint-Ouen l'Aumône, France) (16). In case of fever or any other condition that could alter the biological iron status (e.g., bronchiolitis, gastroenteritis), the blood sampling was postponed to 15 days after discontinuation of symptoms. Children for whom no blood sample was taken or who had a CRP level ≥ 10 mg/L were secondarily excluded (16) (flow chart in **Appendix 4**). Then, sensitivity analyses were performed by excluding infants who had a CRP level ≥ 5 mg/L.

**Iron deficiency and potential predictors definition**

ID was defined by a serum ferritin level of <12 µg/L, as in several previous studies (5, 6, 18, 19), and measured blinded to all clinical data.

AAP recommendations are reproduced verbatim in **Appendix 1** (6). Some AAP criteria required an adaptation to the European context (e.g., "children of Mexican American descent"), or clarification (e.g., "poor growth"). Some were not relevant to the study context (e.g., prevalence of lead poisoning which is almost null among French children (20)). Thus,

we studied the 10 following modified and dichotomized AAP criteria: parents' country of birth (both outside the European Union [EU] or not), parents' education level (both did not attend university or not), parents' professional status (both unemployed or not), family having a health coverage fully funded publicly, preterm birth (<37 or ≥ 37 weeks of gestation), small birth weight (<2500 or ≥ 2500 g), low weights for age at one and two year(s) old (WHO weight for age z-score <-2 SD), "exclusive" breastfeeding ("no other type of milk" but other liquids could be ingested) beyond four months without supplemental iron, and weaning to cow's milk without supplemental iron (**Appendix 1**).

We also used 9 other potential predictors of ID that were previously suggested in the literature, related to socio-economic status, perinatal and medical history, and nutrition (5-7): single motherhood (yes or no), rank among siblings (first/second child or ≥ third child), mother's ID during pregnancy (yes or no), iron supplementation since birth (yes or no), and durations of consumption of infant formula (<4 or ≥ 4 months), iron-rich formula [<12 or ≥ 12 months; a global variable that sums the durations of consumption of "follow-on formula" (recommended at age 6-12 months) and "young children formula" (recommended after 12 months of age)], milk protein hydrolysates (<6 or ≥ 6 months), cow's milk (<12 or ≥ 12 months) and iron-fortified cereals (<12 or ≥ 12 months) (**Appendix 1**).

We chose to a priori dichotomize all potential predictors of ID to take into account the results of a qualitative survey regarding clinical applicability of prediction tools conducted among a panel of ten primary care pediatricians. The panel stated that any clinical prediction tool aiming at a day-to-day use without computation should be based on less than 5 yes/no criteria. Dichotomization was performed after multiple imputations (see below). Sensitivity analyses were performed with different thresholds to test the robustness of our results. A target zone for acceptability of prediction models was also defined during this panel survey (see below).

**Statistical analyses**

We first described the general characteristics of study participants. Unadjusted associations between ID and each of the 19 dichotomized potential predictors were assessed by odds ratios (OR) with corresponding 95% confidence intervals (95% CI). Then, a backward stepwise selection of the 10 dichotomized adapted AAP criteria was performed in 5,000 bootstrap samples (*b*=200 bootstrap samples per multiple imputations dataset, *m* = 25, see below), using a *P*-value of 0.157 for removal, as suggested (21). The final model retained AAP criteria that were selected in at least 60% of the bootstrap models and is referred to as "*parsimonious AAP model*". One predictor (i.e., weaning to cow's milk without supplemental iron) that was not selected with this procedure, due to its low proportion in this study, was finally forced into prediction models because of its strong association with ID in univariable analysis and previous literature (22). Additional analyses were performed to study the diagnostic accuracy of AAP criteria according to how they were combined: (i) in a "*complete AAP model*" where all 10 dichotomized AAP were forced or (ii) in a "*simple additive AAP model*" where each of the 10 AAP criteria was assigned one point.

To develop new prediction models, all dichotomized candidate predictors (n=19; including the 10 adapted AAP criteria) were included in a multivariable logistic regression model, regardless of their association with ID in univariable analysis (23). The same above-described selection process of predictors was applied and provided a "*new model*". This "*new model*'' was further simplified into a "*simple prediction tool*" (binary tool: no criterion vs. ≥ 1) to fit with applicability constraints suggested by the panel of primary care pediatricians as described above. To assess all models' diagnostic accuracy, we used the area under the receiver operating characteristics curve (AUROC), sensitivities, specificities, and accuracies (correctly classified percentage). Internal validation was performed by bootstrap (*b*=500 per

multiple imputation dataset) (24). Calibration was studied graphically by plotting observed proportions of ID by deciles of predicted probabilities of ID (25).

Finally, to interpret the diagnostic accuracy of both individual predictors and prediction models, we used the target zone provided by the pediatricians panel, i.e., a specificity of at least 75%. This target zone was defined regarding the current situation in some European countries including France where no ID screening strategy is recommended, for a theoretical sensitivity of 0% and a theoretical specificity of 100%. Thus, clinical prediction models were dichotomized on the threshold providing a specificity of at least 75%, then sensitivity, specificity, accuracy, positive predictive value, negative predictive value, positive likelihood ratio (LR+) and negative likelihood ratio (LR-) were estimated. For the "*simple prediction tool*", theoretic positive predictive value and (1- negative predictive value) were estimated in settings with different prevalences of ID.

The number of missing data ranged from 0% to 12% per candidate predictor variable (**Appendix 5**). We used multiple imputations with chained equations ($m$ = 25) with predictive mean matching for continuous variables and logistic regression for categorical variables to generate values for missing data. Statistical analyses were performed separately in each imputed dataset; estimated parameters and corresponding variances were pooled using Rubin's rules (23, 26, 27).

## RESULTS

### Participants

As previously described (16), the mean age of the 568 infants included in the analysis was 24 months (SD, ±0.6), 49% were girls, 5.2% had both parents born outside the EU, 2.8% lived in

a single-motherhood family, 3.5% had both parents unemployed, 7.6% had health coverage fully funded publicly, and 38 (6.7%; 95% CI, 4.6-8.8%) had ID. Analyzed infants did not differ from excluded infants, except for some dietary variables (**Appendix 6**).

**Performance of AAP criteria alone or combined**

In univariable analyses, no significant association with ID was observed for 8 of the 10 adapted AAP criteria: parents' educational level, parents' professional status, having or not a health coverage fully funded publicly, birth term, birth weight, low weights at one and two years old, and "exclusive" breastfeeding beyond four months without iron supplementation. ID was significantly associated with two of the 10 adapted AAP criteria: both parents born outside the EU (OR 4.1; 95% CI, 1.6-10.7) and weaning to cow's milk without supplemental iron (OR 80.2; 95% CI, 9.1-706.0) (**Appendix 7**).

Two AAP predictors were selected in at least 60% of the backward stepwise selection models: both parents born outside the EU and low weight at one year old (**Appendix 8.A**). The logistic regression "*parsimonious AAP model*" was built with these two predictor variables plus weaning to cow's milk without supplemental iron that was forced into the model. Calibration plots of the "*parsimonious AAP model*" showed high agreement between predicted probabilities of ID and observed outcomes (**Figure 1**). After bootstrap internal validation, this model had an optimism-corrected AUROC of 0.62 (95% CI, 0.58 to 0.67). After dichotomization around an individual risk threshold of 0.18 (the first one providing a specificity >75%), its sensitivity was 30% (95% CI, 22-39%), its specificity 95% (95% CI, 92-97%) and its accuracy 90% (95% CI, 88-93%). Other prediction tools based on the 10 adapted AAP criteria (i.e., a "*simple additive AAP model*" and the "*complete AAP model*"), had similar diagnostic accuracy (**Appendix 9**). Estimations of the positive predictive value,

the negative predictive value, the LR+, the LR- and the 1/LR- of each developed prediction tools are reported in **Appendix 10.**

**New prediction models**

In univariable analyses, significant positive associations with ID were found for 3 of the 9 non-AAP criteria: mother's ID during pregnancy (OR 2.4; 95% CI, 1.2-4.7), duration of consumption of "iron-rich formula" <12 months (OR 7.2; 95% CI, 3.6-14.2), and duration of consumption of cow's milk ≥ 12 months (OR 4.0; 95% CI, 1.8-9.0) (**Appendix 7**).

Three among the 19 potential predictors were selected in at least 60% backward stepwise selection models performed on the bootstrap samples: both parents born outside the EU, low weight at one year old, and duration of consumption of "iron-rich formula" <12 months (**Appendix 8.B**). The "*new model*" was built with these three predictor variables plus weaning to cow's milk without supplemental iron. In logistic regression analysis, significant positive associations with ID were found with having both parents born outside the EU (adjusted OR 3.5; 95% CI, 1.8-10.3), low weight at one year (adjusted OR 5.9; 95% CI, 1.0-34.0), duration of consumption of "iron-rich formula" <12 months (adjusted OR 5.2; 95% CI, 2.5-11.0), and weaning to cow's milk without supplemental iron (adjusted OR 25.6; 95% CI, 2.7-239.2). Calibration plots of the "*new model*" showed high agreement between predicted probabilities of ID and observed outcomes (**Figure 1**). After bootstrap internal validation, this model had an optimism-corrected AUROC of 0.73 (95% CI, 0.66-0.80). After dichotomization around an individual risk threshold of 0.10, its sensitivity was 62% (95% CI, 49-78%), its specificity 81% (95% CI, 77-86%) and its accuracy 80% (95% CI, 76-84%). The "*new model*" was then simplified by dichotomization for a threshold of at least one criterion, providing the "*simple prediction tool*" (no criterion vs ≥ one criterion among the four above criteria), that had, after bootstrap internal validation, an optimism-corrected AUROC of 0.72

(95% CI, 0.65-0.79%). Its sensitivity was 63% (95% CI, 47-80%), its specificity 81% (95% CI, 70-91%) and its accuracy 80% (95% CI, 70-89%) (**Table 1**). For each developed prediction tool, estimations of the positive predictive value, the negative predictive value, the LR+, the LR- and the 1/LR- are reported in **Appendix 10**. For the "*simple prediction tool*", estimations of the positive predictive value and the (1 – negative predictive value) in settings with different prevalences of ID, but with the same sensitivity and specificity are reported in **Appendix 11**.

To test the robustness of these results, we first modified the thresholds used to dichotomize some potential predictors (durations of consumption of infant formula and iron-rich formula -**Appendix 12**-) and then excluded infants who had CRP level ≥ 5 mg/L, which led to a sample of 530 infants (**Appendix 13**). These sensitivity analyses yielded similar results to those of our main analyses.

## DISCUSSION

### Main results

This study is the first attempt to formally validate the AAP criteria for targeted screening ID in at-risk infants. We found that three AAP criteria were strong predictors of ID. Various combinations of AAP criteria provided clinically acceptable diagnostic accuracy, with a sensitivity varying from 30% (95% CI, 22-39%) to 41% (95% CI, 26-56%) for a specificity of at least 75%. We developed two new clinical prediction tools for ID that provided similar accuracy than tools based on AAP criteria. Notably, a simple tool in which high risk of ID was defined by the presence of at least one out of four simple criteria had an acceptable

AUROC of 0.72 (0.65 – 0.79) (28) and provided a sensitivity of 63% (95% CI, 47-80%) for a specificity of 81% (95% CI, 70-91%).

**Strengths and limitations**

Our study has several strengths. Measurements of serum ferritin levels were standardized and centralized, as recommended by the World Health Organization (29). Biological and clinical data were measured in a blinded way (16). We used recommended statistical methods for developing and validating clinical prediction tools, such as multiple imputations and bootstrap (21, 24), however we cannot exclude residual predictor selection bias.

There were several limitations. First, the interpretation and adaptation of some of the criteria of the AAP is arguable. For example, "poor growth" definitions in the literature (30) and other thresholds used to dichotomize the potential predictors are not consensual, leading to a potential non-differential misclassification bias. We applied AAP criteria to our study population of 2-years-old infants, while AAP seems to target preferably 1-year-old infants, which could explain why no association was found between ID and well-known risks factors of ID, such as history of low birth weight (31). We did not explore some risk factors of ID in the present study (e.g., special health care needs) (6, 31), because we found more relevant to target healthy infants seen in general medical practice. Second, to reach the pre-specified target zone for specificity of at least 75%, all prediction models finally had quite low sensitivities (between 30% and 63%) but good accuracies (between 78% and 90%). In the actual context of lack of international consensus about ID screening and as ID consequences are not fatal, these results were deemed acceptable from clinical and public health points of view. These conclusions may differ depending on the national epidemiology of ID and the local strategy to prevent its consequences. Third, the low number of infants with ID in our study (n=38, 6.7%) and the dichotomization of serum ferritin (<12 µg/L) and of potential

predictors may have been responsible for low statistical power (32). Indeed, in a study performed on the same dataset, history of prematurity was significantly associated with serum ferritin used as a continuous variable (16). Finally, the lowest prevalence of health coverage fully funded publicly (7.6%) than expected in the general French population points to a potential selection bias (33). An explanation could be that participants were recruited by pediatricians, while in France only 20% of infants are regularly followed by a pediatrician at 2 years of age (34). Children followed by pediatricians may have more favorable socio-economic characteristics and may be more likely to comply with nutritional recommendations, regardless of socio-economic level, than those followed by general practitioners. Thus, our results should be validated in a group of infants with a higher proportion of low socio-economic status families.

Our findings may not be generalizable to other high-income settings that have a higher prevalence of ID in infants. External validation of our screening tools is thus warranted (35, 36). The fact that dietary habits and ID prevention policies are different across countries also suggests the need for external validation studies. Our screening tools may only apply to countries where populations are used to consuming iron-rich formulas, such as France where respectively 65% of one year old and 43% of 2-years-old infant consume it regularly (37). Few data on iron-rich formulas consumption in toddlers is available in other high-income countries (37).

**Implications**

Primary healthcare providers were, until now, without clear recommendations regarding ID screening in infants, with guidance ranging from universal screening (6, 12) to no screening at all (5, 11). An intermediate solution would be to perform targeted screening based on validated combinations of ID predictors. The AAP suggested both universal screening and

targeted screening based on criteria which diagnostic accuracies have never been evaluated (6). In this first external validation study, two simple prediction tools had similar diagnostic accuracies than combinations of the AAP criteria. Thus, primary healthcare providers in contexts similar to the French one who wish to screen for ID in at-risk young children could rely on one of these new tools. The simple prediction tool, in which high risk of ID was defined by the presence of at least 1 criterion out of four (both parents born outside the EU, low weight at one year, duration of consumption of "iron-rich formula" <12 months and weaning to cow's milk without supplemental iron), offered potential ease of use, with the aim of limiting as much as possible additional costs and loss of time during the consultation. Nevertheless, impact analyses are necessary to assess the acceptability, usefulness and ease of use of our screening tools in primary healthcare providers' day-to-day practice (21, 38). Even if internal bootstrap validation of the prediction tools shown that the optimism bias in our study was low, these encouraging results need to be further validated in other age groups (closer to one year old) and in other settings to evaluate their transportability in different nutritional contexts and populations.

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**TABLES**

**Table 1**. Diagnostic accuracy of clinical prediction tools under evaluation (n=568)

**Figure 1**. Calibration plots of predicted probabilities of ID from the "*parsimonious AAP model*" (A) and the "*new model*" (B) and observed outcome

**Table 1**. Diagnostic accuracy of clinical prediction tools under evaluation (n=568) [a]

| Tool | Number of predictor variables | AUROC:<br>Estimate, (95% CI)<br>Validation [b], (95% CI) | Positivity threshold [c] | Sensitivity:<br>Estimate, % (95% CI)<br>Validation [b], % (95% CI) | Specificity:<br>Estimate, % (95% CI)<br>Validation [b], % (95% CI) | Accuracy [d]:<br>Estimate, % (95% CI)<br>Validation [b], % (95% CI) |
|---|---|---|---|---|---|---|
| "*Parsimonious AAP model*" | 3 | 0.63 (0.56 – 0.71)<br>0.62 (0.58 – 0.67) | Predicted probability of ID ≥ 0.18 | 32 (17 – 46)<br>30 (22 – 39) | 94 (92 – 96)<br>95 (92 – 97) | 90 (88 – 93)<br>90 (88 – 93) |
| "*New model*" | 4 | 0.74 (0.66 – 0.83)<br>0.73 (0.66 – 0.80) | Predicted probability of ID ≥ 0.10 | 63 (48 – 79)<br>62 (49 – 78) | 81 (77 – 86)<br>81 (77 – 86) | 80 (76 – 83)<br>80 (76 – 84) |
| *"Simple prediction tool"* | 4 | 0.72 (0.64 – 0.80)<br>0.72 (0.65 – 0.79) | ≥ 1 criterion | 63 (48 – 79)<br>63 (47 – 80) | 81 (77 – 84)<br>81 (70 – 91) | 80 (76 – 84)<br>80 (70 – 89) |

"*Parsimonious AAP model*" included the 3 AAP adapted criteria retained in at least 60% of the bootstrap models, i.e. both parents born outside the EU, low weight at one year old and weaning to cow's milk without supplemental iron (score range between 0 and 1, obtained as the individual risk prediction from a logistic regression model which included these 3 criteria).
"*New model"* included the 4 criteria retained in at least 60% of the bootstrap models, i.e. both parents born outside the EU, low weight at one year old, duration of consumption of "iron-rich formula" <12 months and weaning to cow's milk without supplemental iron (score range between 0 and 1, obtained as the individual risk prediction from a logistic regression model which included these 4 criteria)
*"Simple prediction tool"* included the 4 criteria mentioned in the "*New Model*" (binary score: no criterion vs at least 1 criterion)

[a] After performing statistical analyses separately in each imputed dataset (m=25); estimated parameters were pooled using Rubin's rules

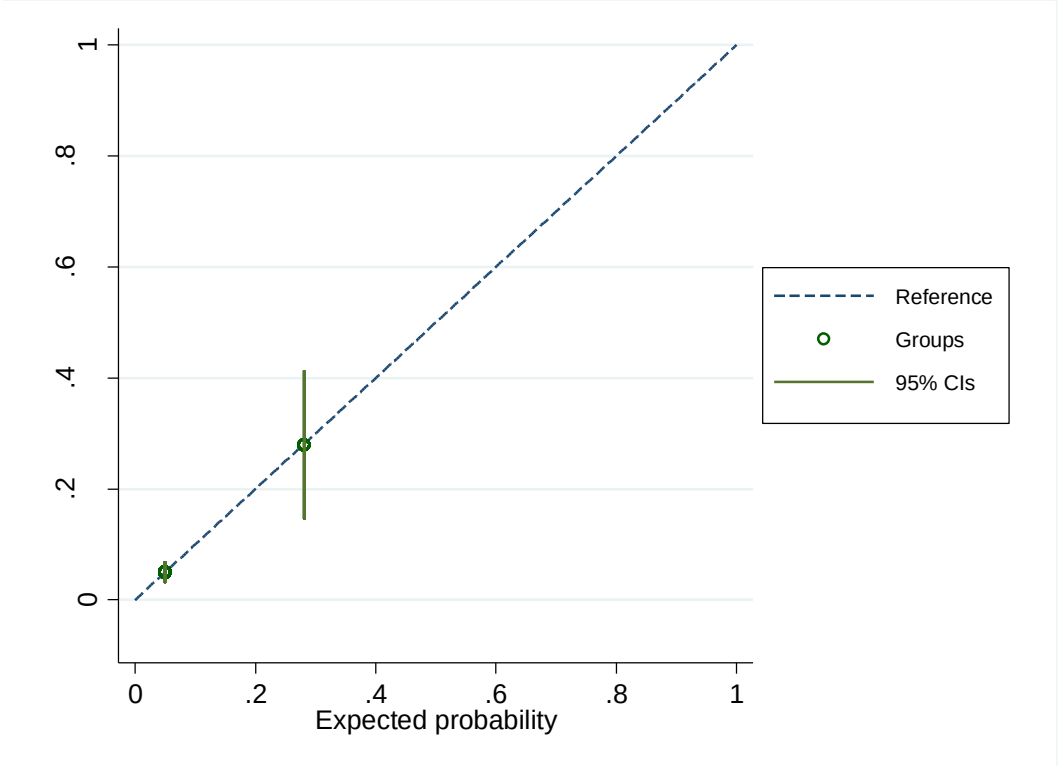
[b] Internal validation was performed by bootstrap (*b*=500 per multiple imputation dataset)

[c] The threshold was set to obtain a specificity of at least 75% (see explanations in the text)

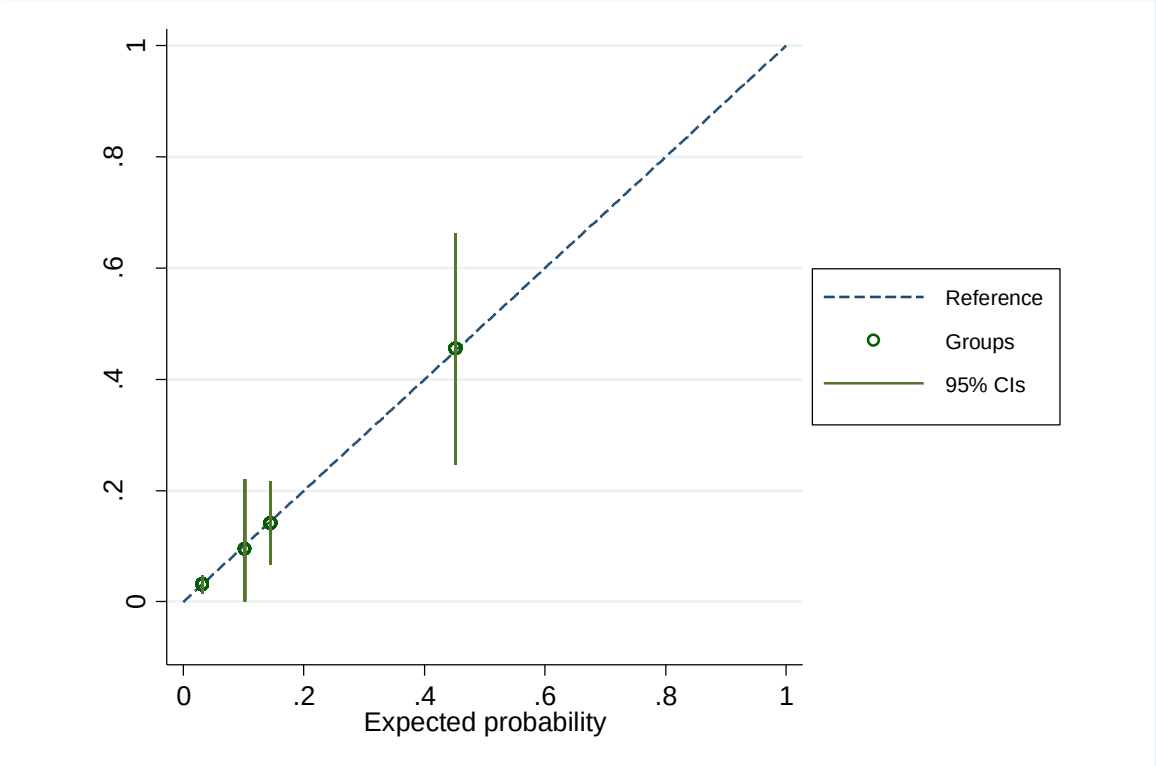
[d] Accuracy: correctly classified percentage

**Figure 1**. Calibration plots of predicted probabilities of ID from the "*parsimonious AAP model*" (A) and the "*new model*" (B) and observed outcome

**A.**




**B.**



Circles represent mean predicted probabilities versus observed proportions in subgroups defined by deciles of the predicted ID probabilities from the “complete” model and realized on the first imputed dataset. Vertical bars are 95% confidence intervals. Dashed diagonal line represents perfect calibration. Several infants have the same probability of having ID: eight (A) and six (B) “missing” deciles overlap with the first one.

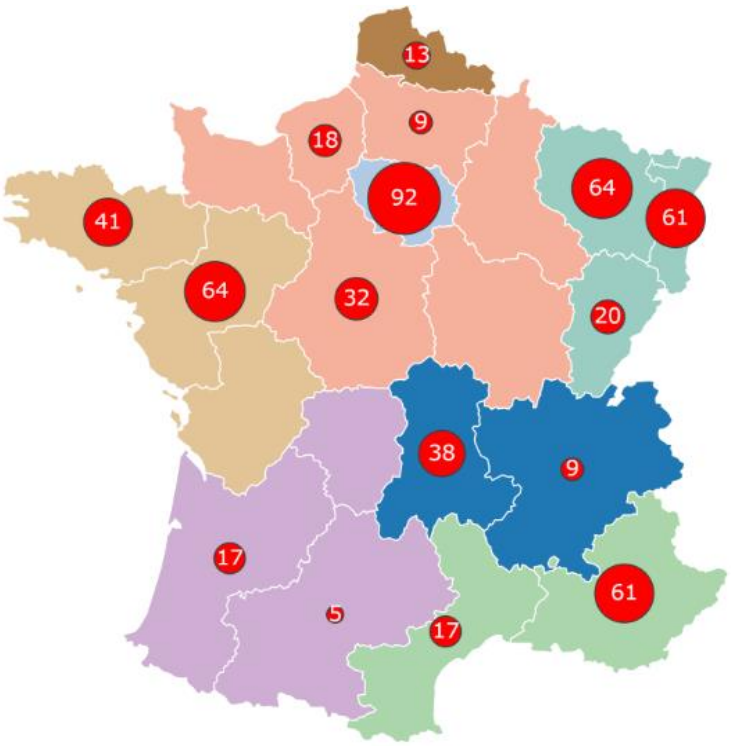
**Appendix 1**. Verbatim of the AAP and CPS recommendations for ID screening, AAP criteria adaptations needed for the present study, and list of other potential predictors of ID included in analyses

The verbatim of the recommendations of the AAP is as follow: "*Universal screening for anemia should be performed at approximately 12 months of age with determination of Hb concentration and an assessment of risk factors associated with ID/IDA. These risk factors would include low socio-economic status (especially children of Mexican American descent), a history of prematurity or low birth weight, exposure to lead, exclusive breastfeeding beyond 4 months of age without supplemental iron, and weaning to whole milk or complementary foods that do not include iron-fortified cereals or foods naturally rich in iron. Additional risk factors are the feeding problems, poor growth, and inadequate nutrition typically seen in infants with special health care needs*" (6).

| **AAP criterion (verbatim)** | **Adaptation needed** |
|---|---|
| History of prematurity or low birth weight | No adaptation needed |
| Exclusive breastfeeding beyond four months without supplemental iron | No adaptation needed |
| Weaning to whole milk or complementary foods that do not include iron-fortified cereals or foods naturally rich in iron | No adaptation needed for "weaning to whole milk".<br>Weaning "to complementary foods that do not include iron-fortified cereals or foods naturally rich in iron" could not be assessed in the present study due to lack of information on foods poor in iron used at weaning. |
| Low socio-economic status (especially children of Mexican American descent) | Needed an adaptation to the European context.<br>We decided to study low socio-economic status with 4 potential risk factors:<br>- parent's country of birth<br>- parent's education level<br>- parent's professional status<br>- health coverage fully funded publicly |
| Poor growth | Needed clarification. We decided to define "poor growth" with the WHO weight for age z-score at one and two years old |
| Exposure to lead | Not relevant in the French context (see text). We did not consider this variable in the present study |
| Feeding problems<br>Inadequate nutrition typically seen in infants with special health care needs | Not relevant in the study context that targeted apparently healthy infants. Infants with feeding problems or with special health care needs were excluded of the present study. We did not consider these variables in the present study. |
| **CPS criteria (verbatim)** | **Discussion** |

| | |
|---|---|
| Children living with chronic illness | Not relevant in the study context that targeted apparently healthy infants. Infants with chronic illness were excluded of the present analyses. We did not consider this variable in the present study. |
| Low socio-economic status | Common with AAP criteria |
| Suboptimal intake of iron-rich foods | We consider this variable by studying different nutritional variables (including those from AAP recommendations and other potential predictors from literature - see below). |
| Prolonged bottle feeding | This criterion was assessed by studying duration of consumption of infant formula, iron-rich formula (see below), milk protein hydrolysates, cow's milk iron-fortified infant cereals. |
| **Potential predictors from literature included in the present study** | **Additional information** |
| Single motherhood | (yes/no) |
| Rank among siblings | (<3 or ≥ 3rd child) |
| Mother's ID during pregnancy | (yes/no) |
| Iron supplementation since birth | (yes/no) |
| Infant formula: duration of consumption | (<4 or ≥ 4 months) – Infant formula is recommended for non-breastfed infants from birth to 6 months of age. In France, 4 months is the mean age of weaning from breastfeeding and the start of solid food. |
| "Iron-rich formula": duration of consumption | (<12 or ≥ 12 months) – Global variable that sums durations of consumption of "follow-on formula" (recommended from 6 to 10-12 months of age) and "young children formula" (recommended after 12 months of age). |
| Milk protein hydrolysates: duration of consumption | (<6 or ≥ 6 months) |
| Cow's milk: duration of consumption | (<12 or ≥ 12 months) |
| Iron-fortified infant cereals: duration of consumption | (<12 or ≥ 12 months) |

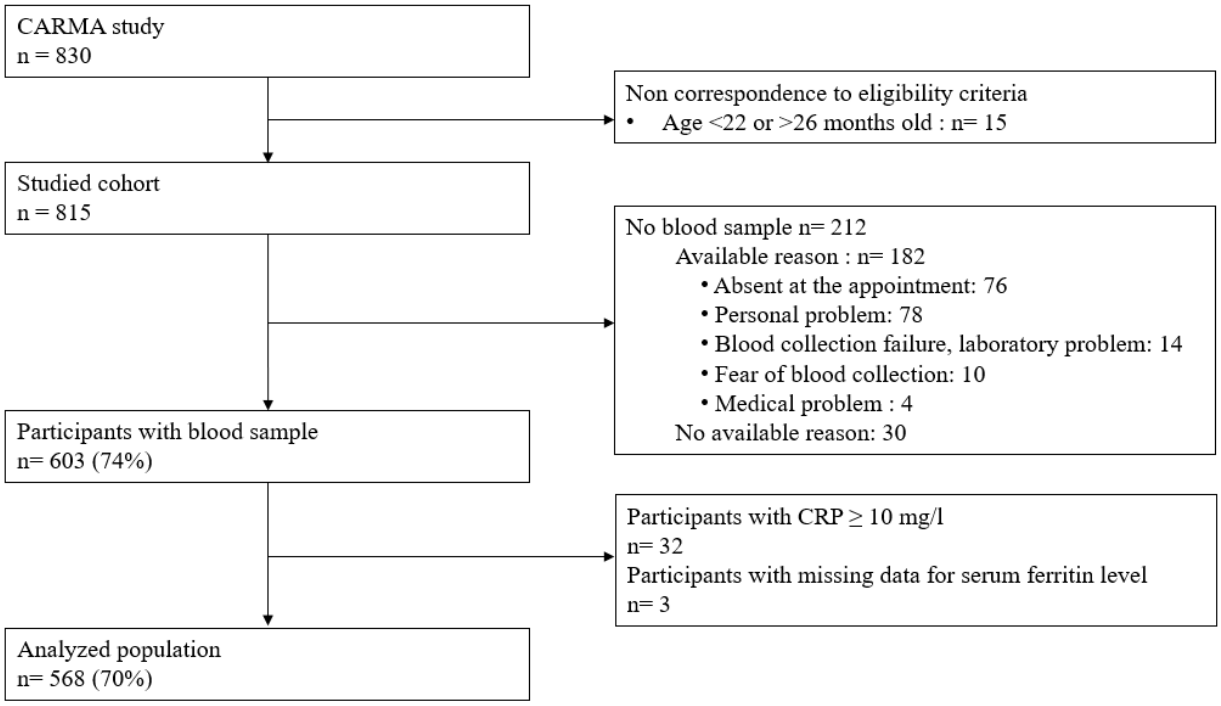
**Appendix 2**. Geographical distribution of children included, by French continental region



**Appendix 3**. Full list of the study exclusion criteria

| CARMA study exclusion criteria |
| --- |
| • Transfusion(s) since birth<br>• Celiac disease<br>• Inflammatory bowel disease<br>• Cystic fibrosis<br>• Other enteropathy (except allergy to cow's milk protein)<br>• Enteral nutrition for more than 15 days within the last six months<br>• Chronic hemolytic diseases (e.g. sickle cell disease)<br>• Chronic kidney disease<br>• Hemophilia<br>• Hemochromatosis<br>• Malignant diseases<br>• Lead poisoning |

**Appendix 4**. Flowchart of study participants




CRP: C-reactive protein

**Appendix 5**. Multiple imputations of missing data

**Patterns of missing values for candidate predictors of ID (n=568)**

Complete case analysis would have led to excluding 152/568 patients (26.8%)

| Candidate predictors of ID | Number of missing values | Percentage |
|---|---|---|
| **Sociodemographic characteristics** | | |
| Single motherhood | 1 | 0.18 |
| Both parents born outside the EU | 5 | 0.88 |
| Both parents who did not attend university | 4 | 0.70 |
| Both parents unemployed | 4 | 0.70 |
| **Perinatal and medical history** | | |
| Mother's ID during pregnancy | 32 | 5.63 |
| Iron supplementation since birth | 1 | 0.18 |
| Weight z-score at one year | 13 | 2.29 |
| **Feeding since birth** | | |
| Exclusive breastfeeding > 4 months of age without supplemental iron | 2 | 0.35 |
| Infant formula: duration of consumption (months) | 2 | 0.35 |
| Iron-rich formula: duration of consumption (months) [a] | 18 | 3.17 |
| Milk protein hydrolysates: duration of consumption (months) | 1 | 0.18 |
| Cow's milk: duration of consumption (months) | 43 | 7.57 |
| Iron-fortified infant cereals: duration of consumption (months) | 69 | 12.15 |

[a] Global variable that sums durations of consumption of "follow on formula" and "young children formula"

**Characteristics of patients with at least one missing value and complete cases**

| Candidate predictors of ID | Patients with at least one missing value (n=152) | Complete cases (n=416) | P[a] |
|---|---|---|---|
| ID [a] | 15/152 (9.9) | 23/416 (5.5) | 0.09 |
| **Socio-demographic characteristics** | | | |
| Single motherhood [a] | 10/151 (6.6) | 6/416 (1.4) | 0.002 |
| Both parents born outside the EU [a] | 8/147 (5.4) | 21/416 (5.1) | 0.853 |
| Both parents who did not attend university [a] | 26/148 (17.6) | 66/416 (15.9) | 0.630 |
| Both parents unemployed [a] | 0/148 (0.0) | 19/416 (4.6) | 0.008 |
| Health coverage fully funded publicly [a] | 15/152 (9.9) | 28/416 (6.7) | 0.211 |
| **Perinatal and medical history** | | | |
| Rank among siblings (≥ 3rd child) [a] | 29/152 (19.1) | 50/416 (12.0) | 0.031 |
| Mother's ID during pregnancy [a] | 51/120 (42.5) | 142/416 (34.1) | 0.093 |
| Iron supplementation since birth [a] | 16/151 (10.6) | 41/416 (9.9) | 0.796 |
| Term of birth (GW) [b] | 39.3 (1.7) | 39.1 (1.8) | 0.239 |
| Weight at birth (g) [b] | 3257.7 (528.4) | 3257.2 (519.4) | 0.992 |
| Weight z-score at one year [b] | 0.03 (0.99) | 0.04 (0.87) | 0.889 |
| Weight z-score at two years [b] | 0.06 (0.91) | 0.04 (0.89) | 0.818 |
| **Feeding since birth** | | | |
| Exclusive breastfeeding >4 months of age without supplemental iron [a] | 40/150 (26.7) | 83/416 (20.0) | 0.106 |
| Weaning to cow's milk without supplemental iron [a] | 2/152 (1.3) | 4/416 (1.0) | 0.661 |
| Infant formula: duration of consumption (months) [b] | 4.0 (2.9) | 4.2 (2.7) | 0.383 |
| Iron-rich formula: duration of consumption (months) [b, c] | 16.4 (5.4) | 16.1 (5.5) | 0.596 |
| Milk protein hydrolysates: duration of consumption (months) [b] | 0.8 (3.4) | 0.9 (3.7) | 0.780 |
| Cow's milk: duration of consumption (months) [b] | 2.6 (4.9) | 2.7 (2.3) | 0.821 |
| Iron-fortified infant cereals: duration of consumption (months) [b] | 8.3 (8.7) | 11.1 (8.3) | 0.006 |

Data are no./N of patients (%) or mean (SD).

[a] Chi-square or Fisher's exact test

[b] Student's *t* test

[c] Global variable that sums durations of consumption of "follow-on formula" and "young children formula"

GW: Weeks of gestation

**Multiple imputations of missing data**

We used multiple imputations with chained equations (MICE) because of missing values in almost all clinical potential predictors of ID we identified (26). MICE was performed in STATA 13/SE (*m*=25). Analyses were repeated in each dataset and estimates of interest were combined using Rubin's rules (27).

**Convergence of imputation diagnostics**

Convergence of the MICE algorithm was checked by graphically investigating the trends in the means and standard deviations of the imputed values over 100 iterations. The trace plots did not show apparent trends in the summaries of the imputed values, so the number of burn-in iterations was set to 25. The distribution of imputed and observed values were compared graphically. Distributional plots did not show significant departure between imputed values and observed values. We concluded that the fit of the imputation model was good.

**Appendix 6**. Characteristics of excluded and included patients

| Characteristics | Excluded (n=247) | Included (n=568) | P |
|---|---|---|---|
| Sex (boy) [a] | 129/247 (52%) | 291/568 (51%) | 0.79 |
| Age [b] | 24.26 (0.59) | 24.25 (0.60) | 0.79 |
| Socio-demographic characteristics | | | |
| Single motherhood [a] | 10/247 (4%) | 16/568 (3%) | 0.36 |
| Number of parents born outside the EU (one or both) [a] | 32/246 (13%) | 84/568 (15%) | 0.51 |
| Number of parents who did not go to the university (one or both) [a] | 117/243 (48%) | 268/568 (47%) | 0.80 |
| Number of parents unemployed (one or both) [a] | 68/242 (28%) | 140/568 (25%) | 0.30 |
| Health coverage fully funded publicly [a] | 22/247 (9%) | 43/568 (8%) | 0.52 |
| Perinatal and medical history | | | |
| Rank among siblings (≥ 3rd child) [a] | 35/247 (14%) | 79/568 (14%) | 0.92 |
| Mother's ID during pregnancy [a] | 96/237 (41%) | 206/568 (36%) | 0.26 |
| Iron supplementation since birth [a] | 19/246 (8%) | 58/568 (10%) | 0.27 |
| Term of birth (GW) [b] | 39.30 (1.68) | 39.11 (1.74) | 0.17 |
| Weight at birth (g) [b] | 3282.15 (489.03) | 3257.38 (521.40) | 0.53 |
| Feeding since birth | | | |
| Exclusive breastfeeding >4 months of age without supplemental iron [a] | 55/245 (22%) | 125/568 (22%) | 0.89 |
| Weaning to cow's milk without supplemental iron [a] | 4/247 (2%) | 6/568 (1%) | 0.50 |
| Duration of breastfeeding (months) [b] | 5.13 (6.23) | 4.58 (5.47) | 0.21 |
| Infant formula: duration of consumption (months) [b] | 4.32 (2.87) | 4.20 (2.74) | 0.58 |
| Iron-rich formula: duration of consumption (months) [b, c] | 14.52 (6.22) | 16.03 (5.52) | 0.004 |
| Milk protein hydrolysates: duration of consumption (months) [b] | 0.34 (2.05) | 0.84 (3.58) | 0.04 |
| Cow's milk: duration of consumption (months) [b] | 1.60 (3.97) | 3.00 (4.75) | <0.001 |
| Iron-fortified infant cereals: duration of consumption (months) [b] | 7.46 (7.87) | 11.01 (8.31) | <0.001 |

Data are no./N of patients (%) or mean (SD).

[a] Chi-square or Fisher's exact test

[b] Student's *t* test. The results presented in this table were obtained on the first imputed dataset;

[c] Global variable that sums durations of consumption of "follow-on formula" and "young children formula"

GW: Weeks of gestation

**Appendix 7.** Characteristics of participants (n=568), univariable associations with iron deficiency (serum ferritin, SF <12 µG/L) and diagnostic accuracy

| Characteristics | | Iron deficiency (SF <12 µg/l) | Diagnostic accuracy | |
|---|---|---|---|---|
| | Proportion [a] (%) | Unadjusted OR (95% CI) | Sensitivity, % (95% CI) | Specificity, % (95% CI) |
| **Sociodemographic characteristics** | | | | |
| Single motherhood | | | | |
| No | 97.2 | Ref. | | |
| Yes | 2.8 | 2.04 (0.45 – 9.31) | 5.3 (0.0 – 12.4) | 97.3 (96.0 – 98.7) |
| * Both parents born outside the EU | | | | |
| No | 94.8 | Ref. | | |
| Yes | 5.2 | 4.07 (1.55 – 10.69) | 15.8 (4.2 – 27.4) | 95.6 (93.8 – 97.3) |
| * Both parents who did not attend university | | | | |
| No | 83.4 | Ref. | | |
| Yes | 16.6 | 1.62 (0.74 – 3.54) | 23.7 (10.2 – 37.2) | 83.9 (80.8 – 87.0) |
| * Both parents unemployed | | | | |
| No | 96.5 | Ref. | | |
| Yes | 3.5 | 0.72 (0.09 – 5.55) | 2.6 (0.0 – 7.7) | 96.4 (94.8 – 98.0) |
| * Having a health coverage fully funded publicly [b] | | | | |
| No | 92.4 | Ref. | | |
| Yes | 7.6 | 1.48 (0.50 – 4.39) | 10.5 (0.8 – 20.3) | 92.6 (90.4 – 94.9) |
| **Perinatal and medical history** | | | | |
| Rank among siblings | | | | |
| 1st or 2nd child | 86.1 | Ref. | | |
| ≥ 3rd child | 13.9 | 2.04 (0.93 – 4.49) | 23.7 (10.2 – 37.2) | 86.8 (83.9 – 89.7) |
| Mother's ID during pregnancy | | | | |
| No | 63.8 | Ref. | | |
| Yes | 36.2 | 2.36 (1.19 – 4.68) | 55.8 (39.6 – 71.9) | 65.2 (61.0 – 69.3) |
| Iron supplementation since birth | | | | |
| Yes | 10.1 | Ref. | | |
| No | 89.9 | 2.10 (0.49 – 8.94) | 94.7 (87.6 – 100) | 10.4 (7.8 – 13.0) |
| * Term of birth (WG) | | | | |
| ≥ 37 | 92.3 | Ref. | | |
| <37 | 7.7 | 1.02 (0.30 – 3.47) | 7.9 (0.0 – 16.5) | 92.3 (90.0 – 94.5) |
| * Birth weight (g) | | | | |
| ≥ 2500 | 91.9 | Ref. | | |
| <2500 | 8.1 | 1.37 (0.46 – 4.04) | 10.5 (0.1 – 20.3) | 92.1 (89.8 – 94.4) |
| * Low weight at one year old (weight for age z-score <-2 SD) | | | | |
| No | 98.5 | Ref. | | |
| Yes | 1.5 | 4.67 (0.91 – 24.00) | 5.3 (0.0 – 12.4) | 98.8 (97.9 – 99.8) |
| * Low weight at two years old (weight for age z-score <-2 SD) | | | | |
| No | 98.8 | Ref. | | |
| Yes | 1.2 | 2.36 (0.28 – 20.12) | 2.6 (0.0 – 7.7) | 98.9 (98.0 – 99.8) |

**Appendix 7 (*continued*)**

| Characteristics | | Iron deficiency (SF <12 µg/l) | Diagnostic accuracy | |
|---|---|---|---|---|
| | Proportion [a] (%) | Unadjusted OR (95% CI) | Sensitivity, % (95% CI) | Specificity, % (95% CI) |
| **Feeding since birth** | | | | |
| * "Exclusive" breastfeeding beyond 4 months without iron supplementation | | | | |
| No | 78.1 | Ref. | | |
| Yes | 21.9 | 1.95 (0.97 – 3.93) | 34.2 (19.1 – 49.3) | 78.9 (75.5 – 82.4) |
| Infant formula: duration of consumption | | | | |
| ≥ 4 months | 53.5 | Ref. | | |
| <4 months | 46.5 | 1.30 (0.67 – 2.52) | 52.6 (36.8 – 68.5) | 54.0 (49.7 – 58.2) |
| "Iron-rich formula": duration of consumption [c] | | | | |
| ≥ 12 months | 82.5 | Ref. | | |
| <12 months | 17.5 | 7.17 (3.61 -14.23) | 55.4 (39.5 – 71.2) | 85.2 (82.2 – 88.3) |
| Milk protein hydrolysates: duration of consumption | | | | |
| ≥ 6 months | 5.5 | Ref. | | |
| <6 months | 94.5 | 1.03 (0.24 – 4.47) | 5.3 (0.0 – 12.4) | 94.5 (92.6 – 96.5) |
| Cow's milk: duration of consumption | | | | |
| <12 months | 90.0 | Ref. | | |
| ≥ 12 months | 10.0 | 4.02 (1.79 – 9.03) | 28.0 (12.9 – 42.9) | 91.2 (88.6 – 93.8) |
| Iron-fortified infant cereals: duration of consumption | | | | |
| ≥ 12 months | 52.7 | Ref | | |
| <12 months | 47.3 | 1.95 (0.95 – 4.01) | 62.6 (44.6 – 78.9) | 53.8 (49.3 – 58.3) |
| * Weaning to cow's milk without supplemental iron | | | | |
| No | 98.9 | Ref. | | |
| Yes | 1.1 | 80.15 (9.10 – 705.96) | 13.2 (0.2 – 23.9) | 99.8 (99.4 – 100) |

[a] After performing statistical analyses separately in each imputed dataset (m=25); estimated parameters were pooled using Rubin's rules. It explains why we presented proportions instead of number of participants

[*] Criteria recommended by AAP are marked with an asterisk to differentiate them from those suggested by the literature

[b] Health coverage fully funded publicly; an indicator strongly linked to poverty in France

[c] Global variable that sums durations of consumption of "follow-on formula" (recommended at age 6-12 months) and "young children formula" (recommended after 12 months of age)

WG: weeks of gestation

**Appendix 8**. Selection of predictor variables in 200 bootstrap backwards stepwise selection procedures across 25 imputed datasets (N=568)

**A.** Selection among all AAP potential predictor variables (binary) adapted to the European context: implementation of the *"parsimonious AAP model"*

| | **Number of times each predictor was selected in the 200 bootstraps by the 25 imputed datasets** | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Candidate predictor** | **Imputed dataset** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** | **Total (%)** | **In final model** |
| Both parents born outside the EU | 169 | 173 | 172 | 174 | 184 | 168 | 168 | 168 | 171 | 184 | 168 | 177 | 163 | 174 | 172 | 173 | 180 | 173 | 163 | 176 | 163 | 159 | 177 | 167 | 167 | 4283 (85.7) | **Yes** |
| Both parents unemployed | 34 | 30 | 31 | 26 | 33 | 38 | 26 | 30 | 42 | 33 | 30 | 33 | 27 | 38 | 42 | 31 | 33 | 31 | 33 | 31 | 32 | 32 | 26 | 29 | 26 | 797 (15.9) | No |
| Both parents who did not attend university | 76 | 80 | 69 | 73 | 76 | 67 | 76 | 74 | 84 | 67 | 72 | 70 | 64 | 68 | 72 | 74 | 63 | 64 | 68 | 67 | 74 | 81 | 64 | 84 | 73 | 1800 (36.0) | No |
| Health coverage fully funded publicly [a] | 53 | 49 | 43 | 49 | 52 | 35 | 49 | 48 | 53 | 50 | 49 | 45 | 49 | 58 | 36 | 51 | 46 | 38 | 51 | 54 | 46 | 45 | 44 | 49 | 45 | 1187 (23.7) | No |
| Prematurity [b] | 38 | 27 | 34 | 32 | 38 | 38 | 34 | 36 | 39 | 40 | 33 | 32 | 38 | 36 | 35 | 33 | 38 | 36 | 38 | 36 | 28 | 28 | 40 | 40 | 38 | 885 (17.7) | No |
| Low birth weight [c] | 29 | 21 | 35 | 19 | 30 | 24 | 17 | 33 | 26 | 28 | 33 | 24 | 20 | 29 | 26 | 27 | 28 | 26 | 28 | 24 | 24 | 27 | 20 | 29 | 28 | 655 (13.1) | No |
| Low weight at one year old [d] | 134 | 124 | 126 | 122 | 123 | 115 | 111 | 114 | 111 | 124 | 114 | 116 | 115 | 127 | 132 | 123 | 116 | 114 | 125 | 132 | 123 | 120 | 123 | 133 | 117 | 3024 (60.5) | **Yes** |
| Low weight at two years old [d] | 31 | 32 | 35 | 27 | 36 | 35 | 41 | 38 | 40 | 37 | 43 | 25 | 41 | 33 | 38 | 30 | 22 | 31 | 35 | 34 | 36 | 41 | 29 | 34 | 29 | 853 (17.1) | No |
| Breastfeeding > 4 months | 36 | 34 | 38 | 46 | 35 | 37 | 32 | 43 | 35 | 39 | 40 | 30 | 43 | 32 | 34 | 35 | 35 | 45 | 36 | 41 | 36 | 33 | 33 | 40 | 42 | 930 (18.6) | No |
| Weaning to cow's milk without supplemental iron | 71 | 79 | 83 | 72 | 83 | 81 | 64 | 71 | 89 | 76 | 72 | 73 | 58 | 72 | 70 | 90 | 78 | 68 | 73 | 81 | 74 | 76 | 77 | 72 | 73 | 1876 (37.5) | **Yes** [e] |

[a] Health coverage publicly, an indicator strongly linked to poverty in France

[b] Prematurity, defined as <37 weeks of gestation in this study

[c] Low birth weight, defined as <2500 g

[d] Low weight at one and two year(s) old, defined as weight for age z-score <-2

[e] Weaning to cow's milk without supplemental iron was forced in the model

**B.** Selection among all binary predictor variables: implementation of the "*new model*".

| | **Number of times each predictor was selected in the 200 bootstraps by the 25 imputed datasets** | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Candidate predictor** | **Imputed dataset** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** | **Total (%)** | **In final model** |
| Single motherhood | 84 | 71 | 84 | 83 | 86 | 80 | 84 | 93 | 74 | 66 | 62 | 53 | 87 | 70 | 78 | 72 | 83 | 77 | 90 | 68 | 91 | 83 | 71 | 74 | 65 | 1929 (38.6) | No |
| Both parents born outside the EU | 164 | 160 | 155 | 160 | 152 | 149 | 158 | 153 | 150 | 156 | 152 | 156 | 159 | 154 | 151 | 158 | 155 | 155 | 152 | 168 | 159 | 151 | 155 | 144 | 158 | 3884 (77.7) | **Yes** |
| Both parents unemployed | 35 | 33 | 24 | 14 | 28 | 24 | 21 | 20 | 27 | 19 | 25 | 26 | 39 | 18 | 26 | 20 | 24 | 25 | 27 | 35 | 22 | 21 | 24 | 27 | 27 | 631 (12.6) | No |
| Both parents who did not attend university | 45 | 46 | 53 | 43 | 44 | 42 | 49 | 38 | 57 | 58 | 48 | 38 | 38 | 41 | 43 | 50 | 41 | 50 | 46 | 55 | 41 | 47 | 57 | 39 | 38 | 1147 (22.9) | No |
| Health coverage fully funded publicly [a] | 95 | 94 | 107 | 88 | 87 | 102 | 107 | 104 | 107 | 113 | 102 | 76 | 83 | 91 | 83 | 94 | 99 | 89 | 94 | 94 | 97 | 79 | 97 | 80 | 76 | 2338 (46.8) | No |
| Rank among siblings: ≥ 3rd child | 60 | 71 | 60 | 67 | 62 | 78 | 58 | 59 | 52 | 59 | 79 | 71 | 64 | 64 | 59 | 64 | 63 | 63 | 66 | 57 | 68 | 80 | 63 | 65 | 65 | 1617 (32.3) | No |
| Mother's ID during pregnancy | 86 | 84 | 93 | 79 | 80 | 77 | 113 | 66 | 127 | 98 | 71 | 110 | 91 | 84 | 100 | 108 | 75 | 109 | 92 | 88 | 93 | 84 | 68 | 106 | 118 | 2300 (46.0) | No |
| Iron supplementation since birth | 27 | 23 | 23 | 24 | 38 | 18 | 26 | 18 | 20 | 15 | 19 | 28 | 22 | 18 | 21 | 24 | 14 | 16 | 24 | 18 | 21 | 21 | 16 | 23 | 25 | 542 (10.8) | No |
| Prematurity [b] | 37 | 40 | 39 | 41 | 50 | 34 | 37 | 33 | 38 | 32 | 36 | 47 | 48 | 42 | 41 | 41 | 46 | 42 | 38 | 39 | 38 | 41 | 35 | 40 | 43 | 998 (20.0) | No |
| Low birth weight [c] | 46 | 39 | 32 | 30 | 38 | 34 | 42 | 39 | 34 | 33 | 46 | 34 | 41 | 24 | 32 | 39 | 29 | 37 | 40 | 40 | 47 | 28 | 44 | 46 | 30 | 924 (18.5) | No |
| Low weight at one year old [d] | 124 | 129 | 134 | 126 | 135 | 124 | 127 | 150 | 125 | 120 | 113 | 115 | 126 | 121 | 123 | 109 | 127 | 121 | 131 | 125 | 130 | 123 | 138 | 116 | 120 | 3132 (62.6) | **Yes** |
| Low weight at two years old [d] | 30 | 46 | 28 | 40 | 22 | 34 | 54 | 36 | 34 | 40 | 37 | 28 | 29 | 29 | 37 | 38 | 38 | 49 | 27 | 25 | 29 | 46 | 38 | 53 | 44 | 911 (18.2) | No |
| Breastfeeding > 4 months | 51 | 57 | 38 | 52 | 46 | 51 | 56 | 41 | 41 | 51 | 59 | 60 | 57 | 59 | 61 | 55 | 52 | 62 | 52 | 54 | 60 | 56 | 56 | 57 | 48 | 1332 (26.6) | No |
| Consumption of "infant formula" <4 months | 69 | 45 | 59 | 71 | 68 | 51 | 52 | 59 | 63 | 60 | 58 | 71 | 58 | 62 | 58 | 55 | 57 | 66 | 65 | 67 | 59 | 63 | 65 | 64 | 69 | 1534 (30.7) | No |
| Consumption of "iron-rich formula" <12 months [e] | 199 | 196 | 195 | 199 | 192 | 197 | 192 | 195 | 197 | 198 | 198 | 198 | 199 | 198 | 198 | 194 | 199 | 195 | 199 | 199 | 198 | 197 | 199 | 196 | 199 | 4926 (98.5) | **Yes** |
| Consumption of milk protein hydrolysates <6 months | 48 | 88 | 56 | 72 | 49 | 63 | 45 | 55 | 61 | 75 | 76 | 60 | 71 | 64 | 82 | 58 | 83 | 63 | 75 | 83 | 62 | 59 | 63 | 62 | 67 | 1640 (32.8) | No |
| Consumption of cow's milk ≥ 12 months | 61 | 49 | 108 | 51 | 81 | 60 | 123 | 89 | 85 | 71 | 54 | 43 | 38 | 48 | 42 | 68 | 42 | 48 | 46 | 40 | 55 | 39 | 46 | 62 | 43 | 1492 (29.8) | No |
| Consumption of cereals <12 months | 73 | 61 | 52 | 44 | 37 | 58 | 60 | 37 | 65 | 85 | 40 | 42 | 50 | 55 | 39 | 49 | 57 | 45 | 45 | 35 | 37 | 46 | 47 | 39 | 35 | 1233 (24.7) | No |
| Weaning to cow's milk without supplemental iron | 80 | 74 | 67 | 69 | 76 | 75 | 74 | 69 | 66 | 79 | 80 | 79 | 83 | 61 | 70 | 71 | 69 | 72 | 80 | 74 | 67 | 60 | 63 | 71 | 75 | 1804 (36.1) | **Yes** [f] |

[a] Health coverage publicly, an indicator strongly linked to poverty in France

[b] Prematurity, defined as <37 weeks of gestation in this study

[c] Low birth weight, defined as <2500 g

[d] Low weight at one and two year(s) old, defined as weight for age z-score <-2

[e] Global variable that sums durations of consumption of "follow on formula" and "young children formula"

[f] Weaning to cow's milk without supplemental iron was forced in the model

**Appendix 9**. Sensitivity analyses: diagnostic accuracy of AAP criteria using additive or complete combination (N=568) [a]

| Tool | Number of predictor variables | AUROC: Estimate, (95% CI) Validation [b], (95% CI) | Positivity threshold [c] | Sensitivity: Estimate, % (95% CI) Validation [b], % (95% CI) | Specificity: Estimate, % (95% CI) Validation [b], % (95% CI) | Accuracy [d]: Estimate, % (95% CI) Validation [b], % (95% CI) |
|---|---|---|---|---|---|---|
| *"Simple additive AAP model"* | 10 | 0.62 (0.53 – 0.72)<br>0.58 (0.52 – 0.65) | ≥ 2 criteria | 34 (19 – 49)<br>34 (21 – 47) | 83 (80 – 86)<br>83 (78 – 88) | 80 (76 – 83)<br>80 (75 – 85) |
| *"Complete AAP model"* | 10 | 0.66 (0.57 – 0.76)<br>0.63 (0.54 – 0.71) | Predicted probability of ID ≥ 0.05 | 53 (37 – 69)<br>41 (26 – 56) | 77 (73 – 80)<br>81 (72 – 90) | 75 (72 – 79)<br>78 (66 – 90) |

"*Simple additive AAP model*", combination of the 10 adapted AAP criteria (score range between 0 and 10, assigning one point for each AAP adapted criterion).
"*Complete AAP model*" included all of the 10 AAP adapted criteria (score range between 0 and 1, obtained as the individual risk prediction from a logistic regression model where all AAP criteria were forced to enter the model).

[a] After performing statistical analyses separately in each imputed dataset (m=25); estimated parameters were pooled using Rubin's rules

[b] Internal validation was performed by bootstrap (*b*=500 per multiple imputation dataset)

[c] The threshold was set to obtain a specificity of at least 75%

[d] Accuracy: correctly classified percentage

**Appendix 10**. Additional diagnostic accuracy of clinical prediction tools under evaluation (n=568)

**A.** Positive predictive value and negative predictive value of clinical prediction tools under evaluation

| **Tool** | **Number of predictor variables** | **Positivity threshold** [a] | **PPV** [b]**, % (95% CI)** | **NPV** [c]**, % (95% CI)** |
|---|---|---|---|---|
| *"Parsimonious AAP model"* | 3 | Predicted probability of ID ≥ 0.18 | 28 (15 – 42) | 95 (93 – 97) |
| *"New model"* | 4 | Predicted probability of ID ≥ 0.10 | 19 (12 – 26) | 97 (95 – 98) |
| *"Simple prediction tool"* | 4 | ≥ 1 criterion | 19 (12 – 26) | 97 (95 – 98) |
| *"Simple additive AAP model"* | 10 | ≥ 2 criteria | 12 (6 – 19) | 95 (93 – 97) |
| *"Complete AAP model"* | 10 | Predicted probability of ID ≥ 0.05 | 14 (8 – 20) | 96 (94 – 98) |

"*Parsimonious AAP model*" included the 3 AAP adapted criteria retained in at least 60% of the bootstrap models, i.e. both parents born outside the EU, low weight at one year old and weaning to cow's milk without supplemental iron (score range between 0 and 1, obtained as the individual risk prediction from a logistic regression model which included these 3 criteria).

"*New model*" included the 4 criteria retained in at least 60% of the bootstrap models, i.e. both parents born outside the EU, low weight at one year old, duration of consumption of "iron-rich formula" <12 months and weaning to cow's milk without supplemental iron (score range between 0 and 1, obtained as the individual risk prediction from a logistic regression model which included these 4 criteria)

"*Simple prediction tool*" included the 4 criteria mentioned in the "*New Model*" (binary score: no criterion vs at least 1 criterion)

"*Simple additive AAP model*", combination of the 10 adapted AAP criteria (score range between 0 and 10, assigning one point for each AAP adapted criterion).

"*Complete AAP model*" included all of the 10 AAP adapted criteria (score range between 0 and 1, obtained as the individual risk prediction from a logistic regression model where all AAP criteria were forced to enter the model).

[a] The threshold was set to obtain a specificity of at least 75%

[b] PPV: positive predictive value

[c] NPV: negative predictive value

**B.** Positive likelihood ratio and negative likelihood ratio of clinical prediction tools under evaluation

| Tool | Number of predictor variables | Positivity threshold [a] | LR+ [b] (95% CI) | LR- [c] (95% CI) | 1/LR- |
|---|---|---|---|---|---|
| *"Parsimonious AAP model"* | 3 | Predicted probability of ID ≥ 0.18 | 6.00 (3.26 – 11.06) | 0.74 (0.60 – 0.91) | 1.35 |
| *"New model"* | 4 | Predicted probability of ID ≥ 0.10 | 3.27 (2.41 – 4.43) | 0.47 (0.31 – 0.70) | 2.13 |
| *"Simple prediction tool"* | 4 | ≥ 1 criterion | 3.31 (2.45 – 4.47) | 0.46 (0.30 – 0.69) | 2.17 |
| *"Simple additive AAP model"* | 10 | ≥ 2 criteria | 2.00 (1.23 – 3.23) | 0.80 (0.63 – 1.00) | 1.25 |
| *"Complete AAP model"* | 10 | Predicted probability of ID ≥ 0.05 | 2.16 (1.42 – 3.29) | 0.73 (0.56 – 0.95) | 1.37 |

"*Parsimonious AAP model*" included the 3 AAP adapted criteria retained in at least 60% of the bootstrap models, i.e. both parents born outside the EU, low weight at one year old and weaning to cow's milk without supplemental iron (score range between 0 and 1, obtained as the individual risk prediction from a logistic regression model which included these 3 criteria).

"*New model*" included the 4 criteria retained in at least 60% of the bootstrap models, i.e. both parents born outside the EU, low weight at one year old, duration of consumption of "iron-rich formula" <12 months and weaning to cow's milk without supplemental iron (score range between 0 and 1, obtained as the individual risk prediction from a logistic regression model which included these 4 criteria)

"*Simple prediction tool*" included the 4 criteria mentioned in the "*New Model*" (binary score: no criterion vs at least 1 criterion)

"*Simple additive AAP model*", combination of the 10 adapted AAP criteria (score range between 0 and 10, assigning one point for each AAP adapted criterion).

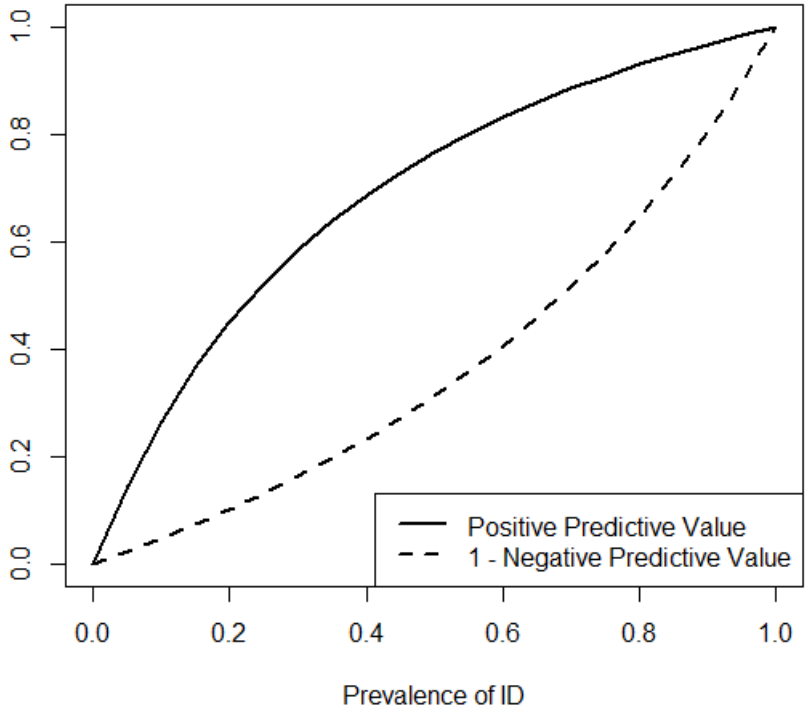
"*Complete AAP model*" included all of the 10 AAP adapted criteria (score range between 0 and 1, obtained as the individual risk prediction from a logistic regression model where all AAP criteria were forced to enter the model).

[a] The threshold was set to obtain a specificity of at least 75%

[b] LR+: positive likelihood ratio

[c] LR-: negative likelihood ratio

**Appendix 11**. *Simple prediction tool*: estimations of the predictive positive value and of the (1 – negative predictive value) for settings with different prevalences of ID, a sensitivity of 63% and a specificity of 81%



**Appendix 12**. Sensitivity analyses: using different thresholds to dichotomize durations of consumption of infant formula and iron rich formula

**Appendix 12.1.** Characteristics of participants (n=568), univariable associations with ID (serum ferritin, SF <12 µg/L) and diagnostic accuracy

| Characteristics | | Iron deficiency (SF <12 µg/l) | Diagnostic accuracy | |
|---|---|---|---|---|
| | Proportion [a] (%) | Unadjusted OR (95% CI) | Sensitivity, % (95% CI) | Specificity, % (95% CI) |
| Infant formula: duration of consumption | | | | |
| ≥ 3 months | 64.1 | Ref. | | |
| <3 months | 35.9 | 1.48 (0.76 – 2.88) | 44.7 (28.9 – 60.5) | 64.7 (60.6 – 68.8) |
| Infant formula: duration of consumption | | | | |
| ≥ 4 months | 53.5 | Ref. | | |
| <4 months | 46.5 | 1.30 (0.67 – 2.52) | 52.6 (36.8 – 68.5) | 54.0 (49.7 – 58.2) |
| Infant formula: duration of consumption | | | | |
| ≥ 5 months | 45.1 | Ref. | | |
| <5 months | 54.9 | 1.14 (0.58 – 2.21) | 58.0 (42.2 – 73.6) | 45.3 (41.0 – 49.5) |
| "Iron-rich formula": duration of consumption [b] | | | | |
| ≥ 11 months | 83.1 | Ref. | | |
| <11 months | 16.9 | 7.50 (3.78 – 14.89) | 55.3 (39.4 – 71.1) | 85.9 (82.9 – 88.9) |
| "Iron-rich formula": duration of consumption [b] | | | | |
| ≥ 12 months | 82.5 | Ref. | | |
| <12 months | 17.5 | 7.17 (3.61 – 14.23) | 55.4 (39.5 – 71.2) | 85.2 (82.2 – 88.3) |
| "Iron-rich formula": duration of consumption [b] | | | | |
| ≥ 13 months | 76.0 | Ref. | | |
| <13 months | 24.0 | 6.40 (3.20 – 12.81) | 63.3 (47.9 – 78.6) | 78.8 (75.3 – 82.3) |

[a] After performing statistical analyses separately in each imputed dataset (m=25); estimated parameters were pooled using Rubin's rules. It explains why we presented proportions instead of number of participants

[b] Global variable that sums durations of consumption of "follow-on formula" (recommended between age 6-12 months) and "young children formula" (recommended after 12 months of age)

**Appendix 12.2**. Selection of predictor variables in 200 bootstrap backwards stepwise selection procedures across 25 imputed datasets (N=568), among all binary predictor variables: implementation of the "*new model*"

**A.** Infant formula – duration of consumption: dichotomized at 3 months

| | **Number of times each predictor was selected in the 200 bootstraps by the 25 imputed datasets** | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Candidate predictor** | **Imputed dataset** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** | **Total (%)** | **In final model** |
| Single motherhood | 79 | 72 | 79 | 81 | 83 | 84 | 81 | 88 | 75 | 63 | 71 | 70 | 66 | 65 | 75 | 87 | 82 | 67 | 80 | 93 | 82 | 70 | 66 | 76 | 70 | 1905 (38.1%) | No |
| Both parents born outside the EU | 162 | 161 | 154 | 166 | 146 | 153 | 150 | 157 | 145 | 161 | 147 | 153 | 148 | 160 | 157 | 150 | 155 | 151 | 156 | 165 | 156 | 148 | 162 | 155 | 152 | 3870 (77.4%) | **Yes** |
| Both parents unemployed | 30 | 24 | 19 | 36 | 20 | 29 | 18 | 22 | 24 | 21 | 23 | 13 | 23 | 25 | 19 | 18 | 32 | 26 | 28 | 22 | 23 | 27 | 26 | 13 | 33 | 594 (11.9%) | No |
| Both parents who did not attend university | 56 | 60 | 59 | 46 | 40 | 53 | 43 | 47 | 50 | 33 | 43 | 43 | 38 | 42 | 57 | 43 | 46 | 47 | 45 | 52 | 50 | 43 | 41 | 39 | 50 | 1166 (23.3%) | No |
| Health coverage fully funded publicly [a] | 112 | 87 | 114 | 97 | 104 | 91 | 118 | 96 | 104 | 112 | 71 | 96 | 91 | 85 | 92 | 91 | 97 | 82 | 92 | 116 | 98 | 85 | 90 | 106 | 85 | 2412 (48.2%) | No |
| Rank among siblings: ≥3rd child | 66 | 73 | 72 | 65 | 67 | 66 | 65 | 81 | 71 | 61 | 76 | 64 | 71 | 69 | 57 | 64 | 72 | 68 | 74 | 61 | 69 | 74 | 70 | 74 | 63 | 1713 (34.3%) | No |
| Mother's ID during pregnancy | 107 | 78 | 97 | 82 | 96 | 82 | 101 | 60 | 117 | 98 | 81 | 108 | 89 | 79 | 96 | 123 | 74 | 104 | 103 | 98 | 92 | 83 | 75 | 109 | 102 | 2334 (46.7%) | No |
| Iron supplementation since birth | 16 | 22 | 20 | 23 | 18 | 17 | 23 | 20 | 18 | 25 | 14 | 13 | 17 | 24 | 26 | 17 | 15 | 16 | 22 | 25 | 27 | 19 | 22 | 25 | 20 | 504 (10.1%) | No |
| Prematurity [b] | 34 | 36 | 37 | 34 | 30 | 40 | 35 | 38 | 49 | 28 | 37 | 49 | 42 | 31 | 39 | 41 | 36 | 30 | 29 | 34 | 41 | 32 | 39 | 42 | 32 | 915 (18.3%) | No |
| Low birth weight [c] | 38 | 41 | 37 | 31 | 45 | 43 | 39 | 40 | 39 | 48 | 34 | 36 | 47 | 33 | 31 | 35 | 32 | 29 | 33 | 45 | 36 | 50 | 38 | 40 | 36 | 956 (19.1%) | No |
| Low weight at one year old [d] | 128 | 130 | 126 | 142 | 129 | 122 | 117 | 137 | 113 | 132 | 122 | 126 | 117 | 115 | 127 | 105 | 129 | 104 | 137 | 131 | 136 | 121 | 126 | 114 | 142 | 3128 (62.6%) | **Yes** |
| Low weight at two years old [d] | 31 | 30 | 42 | 43 | 44 | 48 | 56 | 26 | 52 | 37 | 40 | 42 | 35 | 34 | 45 | 40 | 47 | 53 | 30 | 44 | 36 | 46 | 27 | 42 | 37 | 1007 (20.1%) | No |
| Breastfeeding >4 months | 48 | 43 | 46 | 40 | 43 | 39 | 41 | 41 | 51 | 33 | 51 | 48 | 58 | 48 | 53 | 57 | 44 | 44 | 37 | 54 | 56 | 42 | 52 | 58 | 41 | 1168 (23.4%) | No |
| Consumption of "infant formula" <3 months | 83 | 67 | 74 | 70 | 62 | 73 | 75 | 69 | 73 | 37 | 68 | 74 | 73 | 70 | 72 | 77 | 81 | 81 | 72 | 76 | 77 | 79 | 73 | 88 | 61 | 1805 (36.1%) | No |
| Consumption of "iron-rich formula" <12 months [e] | 196 | 198 | 196 | 198 | 200 | 196 | 185 | 194 | 198 | 194 | 199 | 196 | 198 | 200 | 200 | 199 | 199 | 197 | 198 | 199 | 198 | 195 | 196 | 197 | 200 | 4926 (98.5%) | **Yes** |
| Consumption of milk protein hydrolysates <6 months | 47 | 62 | 49 | 67 | 60 | 56 | 40 | 53 | 50 | 57 | 74 | 57 | 64 | 65 | 64 | 58 | 62 | 54 | 74 | 58 | 68 | 52 | 65 | 58 | 62 | 1476 (29.5%) | No |
| Consumption of cow's milk <12 months | 56 | 51 | 98 | 47 | 72 | 60 | 122 | 84 | 77 | 78 | 44 | 49 | 38 | 45 | 57 | 63 | 48 | 41 | 36 | 45 | 45 | 55 | 53 | 63 | 50 | 1477 (29.5%) | No |

| Candidate predictor | Number of times each predictor was selected in the 200 bootstraps by the 25 imputed datasets | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Imputed dataset | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | Total (%) | In final model |
| Consumption of cereals <12 months | 87 | 67 | 49 | 37 | 32 | 62 | 34 | 48 | 63 | 91 | 38 | 44 | 54 | 54 | 47 | 34 | 49 | 41 | 37 | 43 | 26 | 52 | 35 | 37 | 43 | 1204 (24.1%) | No |
| Weaning to cow's milk without supplemental iron | 81 | 61 | 68 | 70 | 72 | 74 | 73 | 68 | 70 | 81 | 70 | 65 | 72 | 76 | 71 | 68 | 77 | 70 | 73 | 79 | 74 | 78 | 68 | 72 | 71 | 1802 (36.0%) | **Yes** [f] |

[a] Health coverage publicly, an indicator strongly linked to poverty in France

[b] Prematurity, defined as <37 weeks of gestation in this study

[c] Low birth weight, defined as <2500 g

[d] Low weight at one and two year(s) old, defined as weight for age z-score <-2

[e] Global variable that sums durations of consumption of "follow on formula" and "young children formula"

[f] Weaning to cow's milk without supplemental iron was forced in the model

**B.** Infant formula – duration of consumption: dichotomized at 5 months

| Candidate predictor | Number of times each predictor was selected in the 200 bootstraps by the 25 imputed datasets | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Imputed dataset | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** | **Total (%)** | **In final model** |
| Single motherhood | 82 | 55 | 84 | 71 | 82 | 79 | 85 | 90 | 73 | 58 | 65 | 74 | 65 | 68 | 77 | 70 | 73 | 63 | 70 | 72 | 92 | 73 | 81 | 66 | 59 | 1827 (36.5%) | No |
| Both parents born outside the EU | 162 | 155 | 151 | 157 | 154 | 162 | 149 | 152 | 164 | 158 | 153 | 148 | 163 | 169 | 149 | 146 | 163 | 147 | 163 | 157 | 156 | 154 | 163 | 139 | 148 | 3882 (77.6%) | **Yes** |
| Both parents unemployed | 24 | 34 | 17 | 23 | 25 | 27 | 19 | 28 | 17 | 16 | 27 | 32 | 31 | 37 | 23 | 23 | 23 | 22 | 31 | 15 | 28 | 24 | 25 | 23 | 26 | 620 (12.4%) | No |
| Both parents who did not attend university | 46 | 49 | 49 | 57 | 38 | 49 | 45 | 41 | 37 | 37 | 43 | 42 | 45 | 43 | 42 | 42 | 45 | 34 | 56 | 53 | 34 | 46 | 50 | 36 | 50 | 1109 (22.2%) | No |
| Health coverage fully funded publicly [a] | 109 | 88 | 108 | 92 | 83 | 94 | 120 | 83 | 117 | 107 | 90 | 92 | 90 | 90 | 98 | 109 | 80 | 91 | 107 | 105 | 90 | 97 | 83 | 80 | 85 | 2388 (47.8%) | No |
| Rank among siblings: ≥3rd child | 55 | 68 | 51 | 60 | 78 | 71 | 55 | 60 | 63 | 34 | 67 | 68 | 62 | 73 | 69 | 73 | 77 | 69 | 71 | 70 | 65 | 73 | 64 | 63 | 58 | 1617 (32.3%) | No |
| Mother's ID during pregnancy | 103 | 87 | 84 | 83 | 98 | 69 | 107 | 63 | 117 | 98 | 75 | 110 | 81 | 68 | 97 | 111 | 67 | 101 | 75 | 89 | 81 | 76 | 62 | 93 | 104 | 2199 (44.0%) | No |
| Iron supplementation since birth | 16 | 26 | 21 | 30 | 23 | 20 | 18 | 31 | 24 | 21 | 21 | 18 | 15 | 18 | 13 | 24 | 22 | 14 | 15 | 18 | 17 | 10 | 28 | 22 | 24 | 509 (10.2%) | No |
| Prematurity [b] | 44 | 34 | 33 | 46 | 46 | 45 | 43 | 40 | 44 | 35 | 35 | 44 | 38 | 36 | 37 | 42 | 39 | 37 | 41 | 31 | 42 | 39 | 34 | 40 | 35 | 980 (19.6%) | No |
| Low birth weight [c] | 40 | 35 | 38 | 43 | 3 | 36 | 43 | 35 | 39 | 35 | 38 | 37 | 39 | 25 | 39 | 36 | 31 | 29 | 32 | 34 | 33 | 39 | 29 | 37 | 31 | 856 (17.1%) | No |
| Low weight at one year old [d] | 128 | 125 | 131 | 123 | 118 | 127 | 111 | 128 | 121 | 136 | 117 | 113 | 129 | 131 | 124 | 100 | 130 | 105 | 126 | 128 | 123 | 118 | 138 | 115 | 129 | 3074 (61.5%) | **Yes** |
| Low weight at two years old [d] | 30 | 29 | 35 | 23 | 31 | 36 | 44 | 28 | 34 | 37 | 32 | 28 | 22 | 30 | 26 | 35 | 39 | 42 | 35 | 24 | 31 | 38 | 33 | 50 | 22 | 814 (16.2%) | No |
| Breastfeeding >4 months | 51 | 58 | 44 | 58 | 49 | 59 | 59 | 35 | 57 | 55 | 68 | 50 | 65 | 54 | 65 | 54 | 48 | 60 | 47 | 51 | 69 | 48 | 54 | 53 | 54 | 1365 (27.3%) | No |
| Consumption of "infant formula" <5 months | 55 | 77 | 55 | 71 | 77 | 71 | 75 | 64 | 69 | 60 | 32 | 81 | 53 | 76 | 66 | 70 | 73 | 71 | 64 | 71 | 53 | 66 | 71 | 65 | 79 | 1665 (33.3%) | No |
| Consumption of "iron-rich formula" <12 months [e] | 197 | 200 | 198 | 199 | 199 | 198 | 195 | 196 | 196 | 194 | 196 | 198 | 199 | 200 | 200 | 199 | 200 | 199 | 199 | 197 | 199 | 197 | 196 | 197 | 198 | 4946 (98.9%) | **Yes** |
| Consumption of milk protein hydrolysates <6 months | 57 | 64 | 55 | 77 | 61 | 70 | 42 | 56 | 62 | 58 | 71 | 59 | 88 | 70 | 76 | 56 | 37 | 57 | 65 | 67 | 79 | 72 | 59 | 63 | 88 | 1609 (32.2%) | No |
| Consumption of cow's milk <12 months | 59 | 36 | 105 | 49 | 80 | 46 | 125 | 87 | 78 | 67 | 43 | 47 | 56 | 47 | 58 | 66 | 41 | 48 | 47 | 49 | 48 | 41 | 42 | 52 | 45 | 1462 (29.2%) | No |
| Consumption of cereals <12 months | 70 | 62 | 45 | 41 | 42 | 65 | 55 | 43 | 67 | 80 | 34 | 35 | 51 | 46 | 49 | 54 | 53 | 48 | 44 | 41 | 35 | 44 | 46 | 39 | 38 | 1277 (25.5%) | No |
| Weaning to cow's milk without supplemental iron | 71 | 77 | 73 | 67 | 70 | 77 | 69 | 74 | 67 | 75 | 70 | 79 | 69 | 78 | 78 | 71 | 59 | 71 | 78 | 78 | 76 | 62 | 71 | 68 | 57 | 1785 (35.7%) | **Yes** [f] |

[a] Health coverage publicly, an indicator strongly linked to poverty in France

[b] Prematurity, defined as <37 weeks of gestation in this study

[c] Low birth weight, defined as <2500 g

[d] Low weight at one and two year(s) old, defined as weight for age z-score <-2

[e] Global variable that sums durations of consumption of “follow on formula” and “young children formula”

[f] Weaning to cow’s milk without supplemental iron was forced in the model

**Appendix 12.3**. Selection of predictor variables in 200 bootstrap backwards stepwise selection procedures across 25 imputed datasets (N=568), among all binary predictor variables: implementation of the "*new model*"

**A.** Iron rich formula – duration of consumption: dichotomized at 11 months

| | **Number of times each predictor was selected in the 200 bootstraps by the 25 imputed datasets** | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Candidate predictor** | **Imputed dataset** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** | **Total (%)** | **In final model** |
| Single motherhood | 79 | 68 | 88 | 67 | 77 | 81 | 85 | 90 | 75 | 66 | 75 | 73 | 63 | 61 | 74 | 77 | 83 | 74 | 85 | 65 | 85 | 71 | 77 | 65 | 68 | 1872 (37.4%) | No |
| Both parents born outside the EU | 150 | 153 | 144 | 157 | 157 | 157 | 142 | 150 | 153 | 167 | 150 | 153 | 158 | 154 | 159 | 161 | 159 | 156 | 155 | 154 | 160 | 161 | 162 | 145 | 149 | 3866 (77.3%) | **Yes** |
| Both parents unemployed | 20 | 22 | 19 | 23 | 24 | 21 | 15 | 20 | 15 | 17 | 21 | 27 | 34 | 26 | 23 | 25 | 20 | 26 | 25 | 24 | 21 | 19 | 26 | 22 | 30 | 565 (11.3%) | No |
| Both parents who did not attend university | 39 | 52 | 55 | 42 | 44 | 53 | 47 | 43 | 46 | 55 | 37 | 43 | 41 | 36 | 45 | 58 | 43 | 45 | 48 | 57 | 53 | 51 | 52 | 41 | 45 | 1171 (23.4%) | No |
| Health coverage fully funded publicly [a] | 100 | 92 | 101 | 99 | 94 | 92 | 108 | 84 | 107 | 112 | 97 | 74 | 71 | 82 | 82 | 104 | 90 | 97 | 112 | 94 | 100 | 92 | 104 | 85 | 77 | 2350 (47.0%) | No |
| Rank among siblings: ≥3rd child | 68 | 85 | 68 | 63 | 68 | 60 | 57 | 57 | 54 | 63 | 77 | 73 | 69 | 63 | 69 | 59 | 79 | 67 | 83 | 66 | 81 | 73 | 67 | 66 | 65 | 1700 (34.0%) | No |
| Mother's ID during pregnancy | 95 | 88 | 99 | 81 | 96 | 91 | 11 | 71 | 117 | 94 | 87 | 111 | 88 | 83 | 96 | 112 | 74 | 107 | 112 | 94 | 101 | 88 | 65 | 103 | 114 | 2278 (45.6%) | No |
| Iron supplementation since birth | 17 | 21 | 23 | 16 | 20 | 16 | 23 | 28 | 24 | 23 | 21 | 113 | 23 | 17 | 22 | 18 | 27 | 15 | 22 | 15 | 20 | 20 | 24 | 17 | 16 | 601 (12.0%) | No |
| Prematurity [b] | 47 | 43 | 42 | 33 | 34 | 36 | 44 | 37 | 27 | 38 | 36 | 41 | 37 | 38 | 45 | 34 | 38 | 39 | 36 | 37 | 34 | 39 | 31 | 29 | 39 | 934 (18.7%) | No |
| Low birth weight [c] | 29 | 32 | 33 | 41 | 34 | 39 | 45 | 33 | 45 | 32 | 37 | 33 | 32 | 36 | 32 | 36 | 32 | 34 | 35 | 40 | 44 | 44 | 40 | 34 | 42 | 914 (18.3%) | No |
| Low weight at one year old [d] | 126 | 129 | 127 | 134 | 126 | 107 | 117 | 148 | 137 | 133 | 125 | 125 | 123 | 137 | 113 | 91 | 120 | 106 | 139 | 125 | 128 | 119 | 121 | 128 | 127 | 3111 (62.2%) | **Yes** |
| Low weight at two years old [d] | 32 | 39 | 33 | 28 | 29 | 30 | 44 | 32 | 42 | 37 | 32 | 38 | 31 | 30 | 38 | 56 | 41 | 49 | 36 | 30 | 27 | 43 | 25 | 48 | 43 | 913 (18.3%) | No |
| Breastfeeding >4 months | 55 | 62 | 44 | 54 | 55 | 63 | 50 | 51 | 46 | 65 | 61 | 63 | 61 | 74 | 55 | 57 | 28 | 58 | 54 | 67 | 67 | 66 | 60 | 43 | 61 | 1420 (28.4%) | No |
| Consumption of "infant formula" <4 months | 64 | 65 | 63 | 50 | 50 | 63 | 68 | 55 | 60 | 54 | 55 | 65 | 62 | 62 | 62 | 63 | 47 | 71 | 62 | 56 | 61 | 57 | 44 | 65 | 60 | 1484 (29.6%) | No |
| Consumption of "iron-rich formula" <11 months [e] | 194 | 197 | 193 | 200 | 196 | 197 | 196 | 197 | 190 | 195 | 198 | 197 | 199 | 199 | 197 | 199 | 197 | 198 | 200 | 199 | 200 | 200 | 197 | 199 | 199 | 4933 (98.7%) | **Yes** |
| Consumption of milk protein hydrolysates <6 months | 52 | 96 | 56 | 75 | 67 | 69 | 45 | 61 | 45 | 61 | 71 | 70 | 76 | 78 | 61 | 63 | 79 | 80 | 81 | 74 | 90 | 81 | 75 | 69 | 78 | 1753 (35.1%) | No |
| Consumption of cow's milk <12 months | 71 | 53 | 95 | 49 | 65 | 52 | 123 | 83 | 115 | 67 | 55 | 48 | 41 | 41 | 49 | 55 | 55 | 41 | 41 | 52 | 44 | 43 | 48 | 49 | 59 | 1494 (29.9%) | No |

| Candidate predictor | Number of times each predictor was selected in the 200 bootstraps by the 25 imputed datasets | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Imputed dataset | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** | **Total (%)** | **In final model** |
| Consumption of cereals <12 months | 74 | 49 | 61 | 32 | 35 | 63 | 62 | 32 | 37 | 80 | 45 | 48 | 58 | 51 | 53 | 44 | 41 | 50 | 34 | 37 | 31 | 44 | 35 | 35 | 42 | 1173 (23.5%) | No |
| Weaning to cow's milk without supplemental iron | 77 | 74 | 74 | 70 | 71 | 66 | 77 | 64 | 76 | 73 | 84 | 67 | 76 | 65 | 65 | 76 | 63 | 78 | 78 | 68 | 73 | 69 | 78 | 74 | 69 | 1805 (36.1%) | **Yes** [f] |

[a] Health coverage publicly, an indicator strongly linked to poverty in France

[b] Prematurity, defined as <37 weeks of gestation in this study

[c] Low birth weight, defined as <2500 g

[d] Low weight at one and two year(s) old, defined as weight for age z-score <-2

[e] Global variable that sums durations of consumption of "follow on formula" and "young children formula"

[f] Weaning to cow's milk without supplemental iron was forced in the model

**B.** Iron rich formula – duration of consumption: dichotomized at 13 months

| Candidate predictor | Number of times each predictor was selected in the 200 bootstraps by the 25 imputed datasets | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Imputed dataset | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** | **Total (%)** | **In final model** |
| Single motherhood | 96 | 80 | 102 | 83 | 102 | 98 | 101 | 99 | 101 | 83 | 81 | 97 | 94 | 75 | 93 | 98 | 82 | 92 | 97 | 88 | 86 | 80 | 75 | 81 | 85 | 2249 (45.0%) | No |
| Both parents born outside the EU | 147 | 139 | 147 | 139 | 143 | 169 | 145 | 140 | 140 | 141 | 132 | 144 | 149 | 158 | 137 | 138 | 147 | 144 | 138 | 141 | 145 | 152 | 145 | 137 | 142 | 3599 (72.0%) | **Yes** |
| Both parents unemployed | 17 | 26 | 16 | 21 | 16 | 31 | 10 | 30 | 13 | 14 | 27 | 21 | 24 | 34 | 29 | 21 | 24 | 29 | 18 | 28 | 30 | 25 | 25 | 22 | 27 | 578 (11.6%) | No |
| Both parents who did not attend university | 48 | 51 | 49 | 43 | 42 | 52 | 48 | 45 | 48 | 40 | 46 | 50 | 46 | 49 | 47 | 49 | 44 | 42 | 35 | 48 | 43 | 38 | 50 | 45 | 43 | 1141 (22.8%) | No |
| Health coverage fully funded publicly [a] | 73 | 68 | 82 | 66 | 78 | 80 | 88 | 77 | 86 | 92 | 75 | 69 | 66 | 64 | 69 | 82 | 60 | 73 | 61 | 81 | 72 | 65 | 80 | 71 | 58 | 1836 (36.7%) | No |
| Rank among siblings: ≥3rd child | 66 | 63 | 54 | 61 | 69 | 77 | 56 | 66 | 69 | 60 | 69 | 65 | 65 | 67 | 46 | 69 | 72 | 63 | 71 | 53 | 64 | 66 | 64 | 58 | 74 | 1607 (32.1%) | No |
| Mother's ID during pregnancy | 96 | 58 | 72 | 80 | 81 | 60 | 107 | 57 | 98 | 93 | 67 | 86 | 65 | 51 | 84 | 109 | 71 | 97 | 76 | 74 | 86 | 71 | 57 | 89 | 102 | 1987 (39.7%) | No |
| Iron supplementation since birth | 19 | 17 | 15 | 13 | 29 | 17 | 21 | 23 | 25 | 26 | 15 | 15 | 18 | 17 | 17 | 17 | 20 | 20 | 14 | 18 | 20 | 21 | 19 | 21 | 23 | 480 (9.6%) | No |
| Prematurity [b] | 60 | 56 | 48 | 53 | 60 | 45 | 56 | 58 | 58 | 61 | 50 | 63 | 62 | 53 | 40 | 55 | 68 | 51 | 51 | 54 | 44 | 49 | 55 | 52 | 47 | 1349 (27.0%) | No |
| Low birth weight [c] | 40 | 27 | 43 | 33 | 37 | 41 | 58 | 36 | 36 | 39 | 32 | 27 | 41 | 29 | 29 | 34 | 30 | 36 | 32 | 28 | 30 | 48 | 28 | 35 | 37 | 886 (17.7%) | No |
| Low weight at one year old [d] | 129 | 133 | 124 | 136 | 141 | 141 | 126 | 124 | 127 | 134 | 124 | 133 | 136 | 142 | 143 | 127 | 142 | 113 | 129 | 132 | 138 | 113 | 131 | 130 | 132 | 3280 (65.6%) | **Yes** |
| Low weight at two years old [d] | 26 | 23 | 22 | 3 | 23 | 33 | 37 | 17 | 35 | 24 | 24 | 23 | 26 | 27 | 35 | 37 | 19 | 29 | 21 | 13 | 27 | 36 | 21 | 33 | 21 | 635 (12.7%) | No |
| Breastfeeding >4 months | 56 | 54 | 48 | 57 | 62 | 52 | 53 | 47 | 45 | 51 | 53 | 50 | 46 | 52 | 58 | 55 | 45 | 58 | 45 | 42 | 43 | 62 | 47 | 48 | 47 | 1276 (25.5%) | No |
| Consumption of "infant formula" <4 months | 62 | 69 | 60 | 66 | 60 | 55 | 53 | 59 | 55 | 65 | 53 | 54 | 49 | 62 | 64 | 61 | 63 | 49 | 60 | 50 | 48 | 60 | 31 | 59 | 62 | 1429 (28.6%) | No |
| Consumption of "iron-rich formula" <13 months [e] | 194 | 200 | 194 | 198 | 197 | 197 | 191 | 196 | 198 | 194 | 198 | 200 | 200 | 200 | 195 | 196 | 200 | 199 | 199 | 200 | 198 | 199 | 196 | 194 | 200 | 4933 (98.7%) | **Yes** |
| Consumption of milk protein hydrolysates <6 months | 46 | 62 | 35 | 41 | 58 | 55 | 39 | 37 | 38 | 54 | 58 | 62 | 54 | 66 | 40 | 44 | 65 | 47 | 51 | 44 | 51 | 36 | 51 | 47 | 52 | 1233 (24.7%) | No |
| Consumption of cow's milk <12 months | 65 | 42 | 109 | 55 | 82 | 51 | 129 | 88 | 112 | 84 | 33 | 42 | 42 | 42 | 52 | 80 | 28 | 37 | 49 | 29 | 45 | 62 | 37 | 65 | 35 | 1495 (29.9%) | No |
| Consumption of cereals <12 months | 73 | 64 | 65 | 37 | 33 | 79 | 67 | 47 | 75 | 84 | 48 | 48 | 48 | 55 | 56 | 34 | 60 | 61 | 54 | 52 | 42 | 59 | 52 | 36 | 44 | 1373 (27.5%) | No |
| Weaning to cow's milk without supplemental iron | 69 | 71 | 81 | 66 | 73 | 73 | 62 | 70 | 67 | 69 | 70 | 68 | 71 | 84 | 85 | 73 | 73 | 76 | 62 | 70 | 69 | 82 | 72 | 74 | 73 | 1803 (36.1%) | **Yes** [f] |

[a] Health coverage publicly, an indicator strongly linked to poverty in France

[b] Prematurity, defined as <37 weeks of gestation in this study

[c] Low birth weight, defined as <2500 g

[d] Low weight at one and two year(s) old, defined as weight for age z-score <-2

[e] Global variable that sums durations of consumption of “follow on formula” and “young children formula”

[f] Weaning to cow’s milk without supplemental iron was forced in the model

**Appendix 12.4**. Sensitivity analyses: additional diagnostic accuracy of clinical prediction tools under evaluation (n=568)

**A.** Sensitivity, specificity, positive predictive value and negative predictive value

| Tool | Number of predictor variables | AUROC (95% CI) | Positivity threshold [b] | Sensitivity, % (95% CI) | Specificity, % (95% CI) | Accuracy [c], % (95% CI) | PPV [d], % (95% CI) | NPV [e], % (95% CI) |
|---|---|---|---|---|---|---|---|---|
| **Iron rich formula – duration of consumption: cut off value of 11 months** | | | | | | | | |
| "*New model*" | 4 | 0.74 (0.66 – 0.83) | Predicted probability of ID ≥ 0.10 | 63 (48 – 78) | 81 (78 – 85) | 80 (77 – 83) | 20 (13 – 27) | 97 (95 – 98) |
| *"Simple prediction tool"* | 4 | 0.72 (0.64 – 0.80) | ≥ 1 criterion | 63 (48 – 78) | 81 (78 – 85) | 80 (77 – 83) | 20 (13 – 27) | 97 (95 – 98) |
| **Iron rich formula – duration of consumption: cut off value of 13 months** | | | | | | | | |
| *"New model"* | 4 | 0.76 (0.67 – 0.84) | Predicted probability of ID ≥ 0.12 | 66 (51 – 81) | 78 (74 – 81) | 77 (74 – 81) | 18 (11 – 24) | 97 (95 – 99) |
| *"Simple prediction tool"* | 4 | 0.73 (0.66 – 0.81) | ≥ 1 criterion | 71 (57 – 86) | 75 (71 – 79) | 75 (71 – 78) | 17 (11 – 23) | 97 (96 – 99) |

"*New model*" included the 4 criteria retained in at least 60% of the bootstrap models, i.e., both parents born outside the EU, low weight at one year old, duration of consumption of "iron-rich formula" <11 months and weaning to cow's milk without supplemental iron (score range between 0 and 1, obtained as the individual risk prediction from a logistic regression model which included these 4 criteria)

"*Simple prediction tool*" included the 4 criteria mentioned in the "*New Model*" (binary score: no criterion vs at least 1 criterion)

[a] After performing statistical analyses separately in each imputed dataset (m=25); estimated parameters were pooled using Rubin's rules

[b] The threshold was set to obtain a specificity of at least 75%

[c] Accuracy: correctly classified percentage

[d] PPV: positive predictive value

[e] NPV: negative predictive value

**B.** Positive likelihood ratio and negative likelihood ratio

| Tool | Number of predictor variables | AUROC (95% CI) | Positivity threshold [a] | LR+ [b] (95% CI) | LR- [c] (95% CI) | 1/LR- |
|---|---|---|---|---|---|---|
| **Iron rich formula – duration of consumption: cut off value of 11 months** | | | | | | |
| *"New model"* | 4 | 0.74 (0.66 – 0.83) | Predicted probability of ID ≥ 0.10 | 3.31 (2.45 – 4.47) | 0.46 (0.30 – 0.69) | 2.17 |
| *"Simple prediction tool"* | 4 | 0.72 (0.64 – 0.80) | ≥ 1 criterion | 3.31 (2.45 – 4.47) | 0.46 (0.30 – 0.69) | 2.17 |
| **Iron rich formula – duration of consumption: cut off value of 13 months** | | | | | | |
| *"New model"* | 4 | 0.76 (0.67 – 0.84) | Predicted probability of ID ≥ 0.12 | 3.00 (2.27 – 3.97) | 0.44 (0.28 – 0.68) | 2.27 |
| *"Simple prediction tool"* | 4 | 0.73 (0.66 – 0.81) | ≥ 1 criterion | 2.84 (2.21 – 3.65) | 0.39 (0.23 – 0.64) | 2.5 |

"*New model*" included the 4 criteria retained in at least 60% of the bootstrap models, i.e., both parents born outside the EU, low weight at one year old, duration of consumption of "iron-rich formula" <11 months and weaning to cow's milk without supplemental iron (score range between 0 and 1, obtained as the individual risk prediction from a logistic regression model which included these 4 criteria)

"*Simple prediction tool*" included the 4 criteria mentioned in the "*New Model*" (binary score: no criterion vs at least 1 criterion)

[a] The threshold was set to obtain a specificity of at least 75%

[b] LR+: positive likelihood ratio

[c] LR-: negative likelihood ratio

**Appendix 13**. Sensitivity analyses: diagnostic accuracy of clinical prediction tools under evaluation (n=530), after excluding infants with a CRP > 5 mg/L [a]

**A.** Sensitivity, specificity, accuracy, positive predictive value and negative predictive value

| **Tool** | **Number of predictor variables** | **AUROC (95% CI)** | **Positivity threshold [b]** | **Sensitivity, % (95% CI)** | **Specificity, % (95% CI)** | **Accuracy [c], % (95% CI)** | **PPV [d], % (95% CI)** | **NPV [e], % (95% CI)** |
|---|---|---|---|---|---|---|---|---|
| *"Parsimonious AAP model"* | 3 | 0.63 (0.55 – 0.71) | Predicted probability of ID ≥ 0.18 | 32 (17 – 46) | 94 (92 – 96) | 89 (86 – 92) | 29 (15 – 43) | 95 (93 – 97) |
| *"New model"* | 4 | 0.74 (0.66 – 0.83) | Predicted probability of ID ≥ 0.11 | 63 (48 – 79) | 81 (77 – 84) | 80 (76 – 83) | 20 (13 – 28) | 97 (95 – 98) |
| *"Simple prediction tool"* | 4 | 0.72 (0.64 – 0.80) | ≥ 1 criterion | 63 (48 – 79) | 81 (77 – 84) | 80 (76 – 83) | 20 (13 – 28) | 97 (95 – 98) |
| *"Simple additive AAP model"* | 10 | 0.62 (0.52 – 0.71) | ≥ 2 criteria | 34 (19 – 49) | 82 (78 – 85) | 78 (75 – 82) | 13 (6 – 19) | 94 (92 – 96) |
| *"Complete AAP model"* | 10 | 0.66 (0.57 – 0.76) | Predicted probability of ID ≥ 0.05 | 50 (34 – 66) | 75 (71 – 79) | 73 (70 – 77) | 13 (8 – 19) | 95 (93 – 97) |

"*Parsimonious AAP model*" included the 3 AAP adapted criteria retained in at least 60% of the bootstrap models, i.e. both parents born outside the EU, low weight at one year old and weaning to cow's milk without supplemental iron (score range between 0 and 1, obtained as the individual risk prediction from a logistic regression model which included these 3 criteria).

"*New model*" included the 4 criteria retained in at least 60% of the bootstrap models, i.e. both parents born outside the EU, low weight at one year old, duration of consumption of "iron-rich formula" <12 months and weaning to cow's milk without supplemental iron (score range between 0 and 1, obtained as the individual risk prediction from a logistic regression model which included these 4 criteria)

"*Simple prediction tool*" included the 4 criteria mentioned in the "*New Model*" (binary score: no criterion vs at least 1 criterion)

"*Simple additive AAP model*", combination of the 10 adapted AAP criteria (score range between 0 and 10, assigning one point for each AAP adapted criterion).

"*Complete AAP model*" included all of the 10 AAP adapted criteria (score range between 0 and 1, obtained as the individual risk prediction from a logistic regression model where all AAP criteria were forced to enter the model).

[a] After performing statistical analyses separately in each imputed dataset (m=25); estimated parameters were pooled using Rubin's rules

[b] The threshold was set to obtain a specificity of at least 75%

[c] Accuracy: correctly classified percentage

[d] PPV: positive predictive value

[e] NPV: negative predictive value

**B.** Positive likelihood ratio and negative likelihood ratio

| Tool | Number of predictor variables | AUROC (95% CI) | Positivity threshold [a] | LR+ [b] (95% CI) | LR- [c] (95% CI) | 1/LR- |
|---|---|---|---|---|---|---|
| *"Parsimonious AAP model"* | 3 | 0.63 (0.55 – 0.71) | Predicted probability of ID ≥ 0.18 | 5.35 (3.02 – 9.48) | 0.72 (0.58 – 0.90) | 1.39 |
| *"New model"* | 4 | 0.74 (0.66 – 0.83) | Predicted probability of ID ≥ 0.11 | 3.31 (2.45 – 4.37) | 0.46 (0.30 – 0.69) | 2.17 |
| *"Simple prediction tool"* | 4 | 0.72 (0.64 – 0.80) | ≥ 1 criterion | 3.31 (2.45 – 4.37) | 0.46 (0.30 – 0.69) | 2.17 |
| *"Simple additive AAP model"* | 10 | 0.62 (0.52 – 0.71) | ≥ 2 criteria | 1.89 (1.17 – 3.05) | 0.81 (0.64 – 1.00) | 1.25 |
| *"Complete AAP model"* | 10 | 0.66 (0.57 – 0.76) | Predicted probability of ID ≥ 0.05 | 2.00 (1.41 – 2.84) | 0.67 (0.48 – 0.92) | 1.49 |

"*Parsimonious AAP model*" included the 3 AAP adapted criteria retained in at least 60% of the bootstrap models, i.e. both parents born outside the EU, low weight at one year old and weaning to cow's milk without supplemental iron (score range between 0 and 1, obtained as the individual risk prediction from a logistic regression model which included these 3 criteria).

"*New model*" included the 4 criteria retained in at least 60% of the bootstrap models, i.e. both parents born outside the EU, low weight at one year old, duration of consumption of "iron-rich formula" <12 months and weaning to cow's milk without supplemental iron (score range between 0 and 1, obtained as the individual risk prediction from a logistic regression model which included these 4 criteria)

"*Simple prediction tool*" included the 4 criteria mentioned in the "*New Model*" (binary score: no criterion vs at least 1 criterion)

"*Simple additive AAP model*", combination of the 10 adapted AAP criteria (score range between 0 and 10, assigning one point for each AAP adapted criterion).

"*Complete AAP model*" included all of the 10 AAP adapted criteria (score range between 0 and 1, obtained as the individual risk prediction from a logistic regression model where all AAP criteria were forced to enter the model).

[a] The threshold was set to obtain a specificity of at least 75%

[b] LR+: positive likelihood ratio

[c] LR-: negative likelihood ratio