Re-Evaluation of Serum Ferritin Cut-Off Values for the Diagnosis of Iron Deficiency in Children Aged 12-36 Months

Kawsari Abdullah, PhD, MBBS[1], Catherine S. Birken, MD, MSc, FRCPC[1,2,3,4,5], Jonathon L. Maguire, MD, MSc, FRCPC[2,3,4,5,6,7], Darcy Fehlings, MD, MSc, FRCPC[2,3,5,8], Anthony J. Hanley, PhD[3,9,10], Kevin E. Thorpe, MMath[3,6], and Patricia C. Parkin, MD, FRCPC[1,2,3,4,5]

An ongoing challenge has been determining clinically relevant serum ferritin cut-offs in the diagnosis of iron deficiency in children aged 1-3 years. We identified 2 potential clinically relevant serum ferritin cut-off values through their association with clinically important cut-off of hemoglobin as the indicator of anemia. *(J Pediatr 2017;188:287-90).*

See editorial, p 8

Iron deficiency is the most common nutritional deficiency found in young children, peaking in prevalence between 1 and 3 years of age. It may lead to irreversible neurocognitive impairment.[1,2] The iron status of children can be assessed with hematologic tests (ie, hemoglobin [Hb] concentration, hematocrit, mean cell volume, and red blood cell distribution width) and iron-specific biomarkers (ie, serum ferritin concentration, serum iron, erythrocyte protoporphyrin concentration, total iron-binding capacity, transferrin saturation, and serum transferrin receptors).[3,4]

Because changes in Hb concentration occur only at the late stage of iron deficiency, it is considered a late indicator of iron deficiency. The American Academy of Pediatrics recommends universal screening for anemia with determination of Hb concentration with a cut-off of <110 g/L for children aged 1-3 years.[2] Strong evidence has been found showing this Hb cut-off for anemia to be associated with delayed neurodevelopment in young children.[5,6] In addition, the American Academy of Pediatrics, Centers for Disease Control and Prevention, and a Cochrane Systematic Review recommend clinical management in young children whose Hb level is less than this level.[2,4,7] Hence, this cut-off (<110 g/L) has clinical importance for both practitioners and iron deficiency researchers.

Serum ferritin is one of the most widely used and specific biomarkers of iron status in young children,[3,4,8] reflecting body iron stores.[9] Currently recommended serum ferritin cut-off values for identifying iron deficiency in children are between <10 and 12 µg/L.[2,3] This cut-off is recommended in a review by Dallman et al published in 1980, which cites an original study by Siimes et al published in 1974.[10,11] In this study, children aged 0-15 years with iron deficiency anemia (n = 13) were identified to have a serum ferritin range of 1.5-9 ng/mL. Two other studies evaluated the cut-off of serum ferritin in young children (0-5 years) using the fifth percentile of the distribution of serum ferritin as the cut-off for defining iron deficiency. One study identified the fifth percentile to correspond to a serum ferritin of <10 µg/L, whereas the other found this value to be 12 µg/L.[12,13] A study targeting a younger age group of children (9-12 months) has suggested 2 SD cut-off values and has reported values as low as 5 µg/L for serum ferritin in this age group.[14]

The evidence behind these values is weak. They were not developed based on clinical relevance and are not specific for infants 12-36 months of age (the age of peak prevalence). In 2015, the National Institutes of Health established an iron initiative to prioritize research gaps, including current challenges in measuring and screening for iron status in young children, highlighting the importance of re-evaluating serum ferritin cut-off values.[15]

It also is important to understand the relationship between serum ferritin cut-offs and important child health outcomes such as neurodevelopment. Some evidence suggests that low serum ferritin alone may have significant impact on children's neurodevelopment[5,16,17]; however, this evidence is not conclusive and further research is needed.[18]

Considering the extended time course for development of long-term health outcomes that may be related to iron deficiency in children, identifying clinically relevant cut-off values

CRP	C-reactive protein
Hb	Hemoglobin
RCS	Restricted cubic spline

From the [1]Child Health Evaluative Sciences, The Hospital for Sick Children Research Institute; [2]Institute of Health Policy, Management and Evaluation, University of Toronto; [3]Dalla Lana School of Public Health, University of Toronto; [4]Pediatrics Outcomes Research Team, Division of Pediatric Medicine, Department of Pediatrics, The Hospital for Sick Children; [5]Department of Pediatrics, Faculty of Medicine, University of Toronto, Toronto, Canada; [6]Applied Health Research Centre, Li Ka Shing Knowledge Institute of St. Michael's Hospital, Toronto, Canada; [7]Department of Pediatrics, St. Michael's Hospital, University of Toronto; [8]Division of Developmental Pediatrics, Holland Bloorview Kids Rehabilitation Hospital, Bloorview Research Institute, Toronto; [9]Department of Nutritional Sciences, University of Toronto, Toronto, Canada; and [10]Division of Endocrinology, Department of Medicine, University of Toronto, Toronto, Canada

Supported by The Hospital for Sick Children Foundation ([SP05-602] with a grant to the Pediatric Outcomes Research Team), St Michael's Hospital Foundation (2012-0051-GF), and Canadian Institutes for Health Research (CIHR; FRN-115059). The authors declare no conflicts of interest.

0022-3476/$ - see front matter.
http://dx.doi.org10.1016/j.jpeds.2017.03.028

for serum ferritin will enhance the laboratory diagnosis of iron deficiency in young children. The objective of this study was to identify clinically relevant serum ferritin cut-off values for the diagnosis of iron deficiency in children aged 12-36 months by examining its relationship with Hb concentration.

Methods

This was a cross-sectional study of healthy, urban children living in Toronto, Canada. Data were collected from May 2010 to July 2014. Study participants included children aged 12-36 months recruited during a scheduled health supervision visit with a physician participating in the TARGet Kids! primary care practice based research network (www.targetkids.ca).[19] Excluded children were those diagnosed with anemia other than iron deficiency, having C-reactive protein (CRP) ≥10 mg/L, previously diagnosed with a hematologic disorder (thalassemia and other disorders of Hb), developmental disorder, genetic, chromosomal, or syndromic condition, and chronic medical conditions (except asthma and allergy). Serum ferritin is an acute-phase reactant, and concentrations of serum ferritin may be elevated in the presence of chronic inflammation, infection, malignancy, or liver disease.[2] In this study we have combined serum ferritin concentration with determination of another acute-phase reactant (CRP) to exclude children whose serum ferritin may have been elevated due to inflammation or infection.

Data on children's health, nutrition, and sociodemographic characteristics were collected prospectively with a standardized parent-completed survey instrument. A sample of blood also was collected and analyzed to determine children's iron status by the use of iron specific indicators—Hb, serum ferritin, and CRP. Serum ferritin was measured with a Roche Modular platform (Roche Diagnostics Limited, Rotkreuz, Switzerland), and Hb was measured using Sysmex platform (Sysmex Canada, Mississuaga, ON, Canada).[20,21] All diagnostic assessments were performed at the Mount Sinai Services Laboratory (www.mountsinaiservices.com). Approval for data collection was received from the Hospital for Sick Children and St Michael's Hospital research ethics boards, and informed consent was received from parents of participating children.

Statistical Analyses

Descriptive statistics were used to describe the distribution of the variables: child age and sex, Hb, and serum ferritin. Nonlinearity assumptions were tested by visual inspection and by performing a likelihood ratio test to compare the nested univariate models with linear predictors vs restricted cubic spline (RCS) regression models, which included nonlinear predictors.[22]

Some variables have a curvilinear relationship (nonlinear) with each other. Increases in "X" variable initially produce increases in "Y," but after a while subsequent increases in "X" produce declines in "Y." RCS regression analysis can estimate such nonlinear relationships. The graph of the relationship between "X" and "Y" consists of a curve with one or more bends, termed "knots," at which the slope of the curve changes signs. If nonlinearity assumptions were satisfied, we planned to apply RCS regression analysis to characterize the associations between serum ferritin (independent variable) and Hb (dependent variable). We intended to add children's age and sex to the model as potential confounders[13,23-25]; however, the spline function would be performed only for the continuous variable serum ferritin.

By applying the RCS regression analysis (using 4 or 5 knots) we planned to identify 2 specific serum ferritin values. First, a serum ferritin value that predicted a maximum increase in Hb as a function of serum ferritin was calculated by finding the root of the derivative of the predicted mean Hb. This maximum Hb level is known as the "Hb plateau point." Second, because a Hb cut-off of 110 g/L is used to define anemia, we intended to find the corresponding serum ferritin value that predicted a Hb of 110 g/L from the RCS regression models.

It has been recommended that for a large sample ($n > 100$), 4 or 5 knots is a good choice and provides enough flexibility for a reasonable loss of precision caused by overfitting the data.[22,26] Therefore, we performed the analysis with both 4 and 5 knots.

Data analyses were performed with SAS, version 9.1 (SAS institute Inc, Cary, North Carolina). R version 3.0.1 (http://www.r-project.org/, Vienna, Austria) was used for RCS analysis.[27] Alpha (2-tailed) was set at values less than .05.

Results

Blood samples of 1257 children (12-36 months of age) were analyzed for Hb and serum ferritin. The mean (±SD) age of the children was 18.9 (5.9) months, and 53.5% were male. Mean (±SD) serum ferritin and Hb were 27.7 (19.7) µg/L and 119.3 (8.8) g/L, respectively. Children's health, nutrition, and sociodemographic characteristics have been reported previously in the cohort profile.[28] Compared with nested univariate models of linear predictors, the model with RCS showed a likelihood ratio χ^2 value of 91.07 ($P < .001$), indicating that a nonlinear relationship (between serum ferritin and Hb) had a better fit to the data.

Using RCS regression analysis, we found serum ferritin to be significantly associated with Hb ($P \leq .001$), age ($P \leq .001$), and sex ($P = .004$) in the 4-knots model and with Hb ($P \leq .001$) and age ($P = .003$) in the 5-knots model. From the model with 4 knots, we identified a serum ferritin value of 23.7 µg/L corresponding to the "Hb plateau point" of 121.2 g/L (see Methods for definition). **Figure 1** shows the plot of the RCS regression model with 4 knots, which depicts a 2-phase association between serum ferritin and Hb with a strong increase up to the "Hb plateau point," followed by a much milder increase afterwards. The serum ferritin value of 2.4 µg/L corresponded to the Hb value of 110 g/L (mean age 18.9 months and sex = male). Similarly, the model with 5 knots (**Figure 2**) identified a serum ferritin value of 17.9 µg/L corresponding to the "Hb plateau point" of 121.0 g/L; and a serum ferritin value of 4.6 µg/L corresponded to the Hb value of 110 g/L (mean age 18.9 months and sex = male).