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## Detection of iron deficiency in children with Down syndrome

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### Abstract

**Purpose:** Current American Academy of Pediatrics guidelines for children with Down syndrome (DS) recommend a complete blood count (CBC) at birth and hemoglobin annually to screen for iron deficiency (ID) and ID anemia (IDA) in low-risk children. We aimed to determine if macrocytosis masks the diagnosis of ID/IDA and to evaluate the utility of biochemical and red blood cell indices for detecting ID/IDA in DS.

**Methods:** We reviewed data from 856 individuals from five DS specialty clinics. Data included hemoglobin, mean corpuscular volume, red cell distribution width (RDW), percent transferrin saturation (TS), ferritin, and c-reactive protein. Receiver operating characteristic curves were calculated.

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#### SUPPLEMENTARY INFORMATION

The online version of this article (<https://doi.org/10.1038/s41436-019-0637-4>) contains supplementary material, which is available to authorized users.

#### DISCLOSURE

The other authors declare no conflicts of interest.

**Results:** Macrocytosis was found in 32% of the sample. If hemoglobin alone was used for screening, all individuals with IDA would have been identified, but ID would have been missed in all subjects. RDW had the highest discriminability of any single test for ID/IDA. The combination of RDW with ferritin or TS led to 100% sensitivity, and RDW combined with ferritin showed the highest discriminability for ID/IDA.

**Conclusion:** We provide evidence to support that a CBC and ferritin be obtained routinely for children over 1 year old with DS rather than hemoglobin alone for detection of ID.

### Keywords

Down syndrome; iron deficiency; iron deficiency anemia; trisomy 21; macrocytosis

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## INTRODUCTION

Down syndrome (DS; OMIM 190685) is associated with hematological differences, including a relatively high prevalence of macrocytosis (large mean corpuscular volume of red blood cells) compared with individuals without DS.<sup>1-4</sup> While macrocytosis has not been linked to health problems in DS, it may contribute to difficulties in the diagnosis of iron deficiency using red blood cell indices in this population.<sup>1,2</sup> A prior prospective, single-site study by Dixon and colleagues demonstrated that while the prevalence of iron deficiency/iron deficiency anemia (ID/IDA) in DS was found to be similar to the general population, the diagnosis of ID/IDA was missed in a majority of individuals when red blood cell indices alone were used for screening.<sup>2</sup>

Iron deficiency with or without anemia early in life has been associated with adverse long-term outcomes.<sup>5</sup> Studies in both humans and animals have demonstrated that early iron deficiency affects neuronal function and myelination and is associated with behavioral abnormalities.<sup>6</sup> Long-term follow-up studies have additionally shown that iron deficiency in childhood is associated with poorer cognitive function and alterations in behavior and mood through adolescence and adulthood.<sup>5-7</sup> Therefore, it is critical to identify and treat iron deficiency for all children at an early stage of brain development.

IDA is typically detected by screening hemoglobin or hematocrit in the general population, with IDA defined by hemoglobin values less than 11.0 g/dL (110 g/L).<sup>8</sup> Hemoglobin has good sensitivity but poor specificity for detection of IDA in the general population, requiring further evaluation with serum ferritin and c-reactive protein (CRP) or reticulocyte hemoglobin concentration to determine if ID is the underlying cause.<sup>9</sup> For children with DS, the current health-care guidelines recommend a complete blood count (CBC) at birth and screening hemoglobin levels for children without additional risk factors for ID/IDA (e.g., low dietary iron intake), with further evaluation of ferritin and CRP recommended when risk factors are present or hemoglobin levels are less than 11.0 g/dL.<sup>10</sup>

The goal of the current study was to evaluate the utility of screening measures for ID/IDA in DS in a relatively larger sample than the prior study by Dixon and colleagues.<sup>2</sup> We evaluated the utility of both individual and combined biochemical measures and red blood cell indices for detection of ID/IDA. Based on prior findings from Dixon and colleagues showing poor

utility of hemoglobin and high prevalence of macrocytosis,<sup>2</sup> we hypothesized that the currently recommended guidelines to use hemoglobin alone for screening in low-risk children annually would be inadequate for diagnosing ID/IDA in a larger sample of children with DS.

## MATERIALS AND METHODS

Participants provided informed consent to participate in the International Down Syndrome Patient Database,<sup>11</sup> a multicenter registry of provider-entered data. Five sites with dedicated DS clinics participated in this particular study including Bambino Gesù Children's Hospital and Research Institute (Rome, Italy), Duke University Medical Center (Durham, NC), Massachusetts General Hospital (Boston, MA), Nationwide Children's Hospital (Columbus, OH), and University of Pittsburgh Medical Center Children's Hospital of Pittsburgh (Pittsburgh, PA). Approval was obtained from the institutional review boards at each center. Consent was obtained from participants or their legal guardians to extract data from medical records and enter information into a shared database. All sites agreed on the clinical need for the laboratory measures, and some sites modified their routine clinical practice for all patients with DS based on prior published evidence recommending a CBC, percent transferrin saturation (TS), and ferritin for screening of ID/IDA for children with DS.<sup>2</sup>

The inclusion criterion for participation was any individual with DS who had not been previously treated for ID/IDA. For patients who had data from multiple clinic visits as part of the registry, data from the first clinic visit were chosen for inclusion in the study unless the individual was under 1 year of age at that visit. In this case, data from the next visit where the participant was over 1 year of age were chosen for inclusion. These criteria were based on the higher risk for ID/IDA in toddlers who have transitioned from iron-fortified formula and infant foods to cow's milk.<sup>9</sup>

Data collection included demographic variables, red blood cell indices including hemoglobin, mean corpuscular volume (MCV), and red cell distribution width (RDW), and biochemical measures including serum iron, total iron binding capacity (TIBC), ferritin, and CRP. TS was calculated as the serum iron divided by TIBC. As normal reference ranges varied across sites based on individual laboratory instruments and reagents, inclusive minimum and maximum values were used to determine normal ranges across all sites (lowest minimum and highest maximum). Data was also collected from a subset of participants who had data available on general dietary information (breast milk, formula, or cow's milk) to include as secondary variables in the analysis.

Definitions of ID/IDA were the same as in the prior study by Dixon and colleagues.<sup>2</sup> ID was defined as two abnormal biochemical markers of iron (low ferritin and low TS), or one abnormal biochemical marker plus elevated RDW. IDA was defined as ID plus a low hemoglobin concentration as defined by inclusive reference ranges across institutions (Table S1). Those individuals identified as having ID did not include those who met criteria for IDA.

Statistical analyses included Fisher exact tests or Kruskal–Wallis tests where appropriate. Receiver operating characteristic (ROC) curves were calculated to determine discriminability for each measure in detecting ID/IDA. Test characteristics were calculated including sensitivity (proportion correctly identified as having ID/IDA), specificity (proportion correctly identified as not having ID/IDA), positive predictive value (PPV, probability that those with abnormal values truly have ID/IDA), negative predictive value (NPV, probability that those with normal values truly do not have ID/IDA), and ROC (ability to distinguish between the presence and absence of ID/IDA). To assess for effects of potential inflammation on serum ferritin and TS levels, the utility of these measures were assessed both in isolation and in the setting of a normal CRP. Additionally, R squared was calculated for the relationships between CRP and ferritin and between CRP and TS. Finally, based on a study that compared ferritin with the gold standard of bone marrow iron stores, a supplementary analysis was performed to explore the effects of an alternative cutoff (under 18 ng/mL or 40.45 pmol/Lng/mL) for ferritin in detecting ID/IDA.<sup>12</sup>

## RESULTS

Data were collected from a total of 856 participants (Table 1). ID was relatively more common in children under 36 months of age (present in 18/178, or 10%) than those over 36 months (present in 28/678, or 4%) ( $p = 0.005$ ). No significant differences were found in the prevalence of ID/IDA by sex, race, or ethnicity. The prevalence of ID overall was 6.66% and prevalence of IDA was 1.19% for this clinic-based cohort. There was a significant overall impact of site on the prevalence of ID/IDA ( $p < 0.0001$ ) (Table 2). The site with highest prevalence of ID/IDA (Nationwide Children's) also had a relatively younger population with more children under 36 months of age ( $p < 0.0001$ ) than other sites. In children over age 3 years, IDA was more common in males than females ( $p = 0.05$ ). Within the group of females, the prevalence of IDA was higher in those age 13 or older (5.5%) compared with females under age 13 years (0.7%) ( $p = 0.03$ ).

Average laboratory values for individuals with no ID/IDA, ID, and IDA are presented in Table 3. Macrocytosis (defined as MCV greater than the normal reference range) was present in 31.9% of the population overall. The prevalence was 41.9% in those under 36 months and 29.3% in those over 36 months.

The diagnostic utility of individual and combined measures for detecting ID/IDA is presented in Tables 4 and 5. Hemoglobin measures alone were normal for 46/56 subjects (82%) with ID/IDA, corresponding to normal hemoglobin values in all individuals with ID only ( $n = 46$ ) and low hemoglobin in all individuals with IDA ( $n = 10$ ) (per our predefined criteria for IDA). High RDW alone had the highest discriminability of the individual tests for ID. The combination of high RDW with low ferritin or low TS led to 100% sensitivity for detecting ID/IDA, with the combination of high RDW or low ferritin showing the highest discriminability of the combined tests. The supplementary analysis of ferritin utility using a cutoff of under 18 ng/mL showed higher discriminability for detecting ID/IDA than with the institution-based reference ranges (Table S2). Statistically significant associations ( $p < 0.0001$ ) were found when correlating CRP with ferritin and CRP with TS values, but both

relationships were associated with low R-squared values of 4.9% and 4.7%, respectively (Figs S1 and S2).

For a subset of 88 individuals with available dietary information (breast milk, formula, or cow's milk), the prevalence of ID/IDA was significantly different between groups ( $p = 0.006$ ). ID was present in 6 of 60 individuals taking cow's milk (10%), 4 of 6 individuals taking breast milk (66.7%), and 1 of 22 individuals taking formula (4.6%). IDA was present in 3.3% of those on cow's milk (2/60), with no individuals taking breast milk or formula having IDA.

## DISCUSSION

The results of this study indicate that the use of hemoglobin as a solitary screening measure likely leads to missed diagnoses of ID in individuals with DS. Hemoglobin measures are currently recommended by the American Academy of Pediatrics (AAP) to screen for ID/IDA in both the general population<sup>9,13</sup> and in individuals with DS with no additional risk factors at 1 year of age, repeated annually for children with DS.<sup>10</sup> However, our results indicated a high prevalence of macrocytosis with poor discriminability for both hemoglobin and MCV values for identifying ID/IDA in DS, supporting the role of macrocytosis (high MCV) in masking the diagnosis.

Our data indicated that isolated elevation in RDW values had the highest discriminability (based on the ROC value) of the individual tests for detecting ID/IDA in DS. While RDW had a relatively low PPV of 38, it is notable that PPV is not the ideal predictor of utility of screening measures for a low-prevalence condition, as PPV values decrease with prevalence.<sup>14</sup> However, RDW had the highest ability to discriminate between the presence and absence of ID/IDA because of the tradeoff between sensitivity and specificity. Elevated RDW is the earliest hematological indicator of ID as iron begins to be depleted from bone marrow.<sup>15</sup> The discriminability of RDW for identifying ID/IDA in the current data was found to be higher than in the prior study by Dixon and colleagues,<sup>2</sup> which found that RDW had a sensitivity of 62% (compared with 93% for ID/IDA combined in the current study). This may be related to differences in the sample size, characteristics of the cohorts, or variation in normal reference ranges between studies.<sup>2</sup> Our study used inclusive reference ranges across multiple institutions, as these ranges have some variation between laboratories. A study of the ability of RDW to predict ID/IDA in typically developing children showed limited utility as a primary biomarker, but direct comparison to our data set is confounded by differences in reference ranges and the definitions of ID/IDA.<sup>16</sup>

According to our a priori definitions of ID/IDA, the combination of high RDW *and* an abnormal biochemical measure (ferritin or TS) would have perfect discriminability. We aimed to clarify the relative utility of panels of measures where at least one abnormal value would be required (e.g., high RDW *or* an abnormal biochemical measure). While the measures are inherently correlated with one another, the goal of these analyses was to determine which measures account for the most variability and provide the most clinical utility. Of the combined tests, the combination of either high RDW or low ferritin values had 100% sensitivity with the highest discriminability for detecting ID/IDA in DS. The addition

of TS to RDW measures also led to improved sensitivity to 100% as defined by our criteria, but this combination was also associated with more false positives according to our definitions of ID/IDA (i.e., cases where TS was low but criteria for ID/IDA were not met because ferritin and RDW were normal). The combination of low ferritin or low TS (without including RDW) led to a reduction in sensitivity for ID/IDA, suggesting that inclusion of RDW has particular utility.

Macrocytosis was found at a total prevalence of 31.9% in our clinic-based sample of individuals with DS and at 41.9% in the younger children with DS, compared with the prevalence of 1.7–3.6% in the general population.<sup>17–20</sup> While the etiology of macrocytosis in DS is unknown, it has been previously demonstrated that MCV values are significantly higher in children with DS than in typically developing children.<sup>1–4</sup> Based on the increased MCV values, the current AAP guidelines specify that MCV is not useful as a screening tool for ID in children with DS.<sup>10</sup> Multiple studies have found no significant relationships between macrocytosis and other comorbid health issues in DS, including hypothyroidism, megaloblastic anemia, or congenital heart disease.<sup>1,2,4</sup> While our sample was not population-based, the prevalence of macrocytosis in the current study was consistent with prior reports in DS, which have ranged from 22% to 66% with greater prevalence in younger age ranges.<sup>1–3</sup> This suggests that the typical reference ranges used in the assessment of ID are misleading in DS, as MCV levels in a child with DS and ID/IDA may be within the “normal” range, and yet reflect a relative microcytosis for the DS population.<sup>1</sup>

In general, there have been few new studies on screening for iron deficiency since publication of the AAP guidelines for the general population in 2010.<sup>8,9</sup> Hemoglobin is typically used as a first-line screen in the general population because of its low cost and convenience for measurement, but it is a late indicator of ID that is found to be low after lack of iron impacts circulating hemoglobin levels and is a poor predictor of ID without IDA in typically developing children.<sup>9,13,21</sup> The current AAP guidelines for screening ID/IDA in both the general population and in individuals with DS recommend screening with additional laboratory measures beyond hemoglobin for children with risk factors, including history of prematurity or low birth weight, lead exposure, and dietary factors including a lack of iron-rich foods and weaning to whole milk.<sup>9,10</sup> Low socioeconomic status and having Mexican American ancestry have also been identified as risk factors in the general population.<sup>9</sup> Studies investigating utility of screening dietary factors (low intake of meat, bread, cereals, fruits, and vegetables and high intake of dairy, fried foods, and sweets) have shown variable estimates of sensitivity (ranging from 71%–95%) and specificity (15%–95%) for ID/IDA in high-risk populations.<sup>22–24</sup> While dietary information is likely to be useful for identifying additional risk for ID/IDA, the data suggest that screening by definitive laboratory measures is still necessary to identify ID before IDA develops.<sup>22</sup> In cases where a comprehensive dietary history cannot be obtained, the use of laboratory measures is likely to be especially important in screening for ID/IDA.

The current view by the US Preventive Services Task Force is that there is insufficient evidence to recommend screening of ID/IDA in the general population of children due to lack of evidence that screening or iron supplementation improves neurocognitive outcomes.<sup>8</sup> While additional data are needed, multiple human and animal studies have supported the

role of ID/IDA in neurodevelopment and cognitive and behavioral deficits.<sup>5,7,25–27</sup> The view of the AAP is that screening young children to minimize ID/IDA is worthwhile even if criteria establishing causality between ID/IDA and adverse outcomes have not yet been established.<sup>9</sup> Screening children with DS using accurate laboratory measures can allow for prevention of IDA and the potential associated neurocognitive sequelae, especially important given that developmental delay and intellectual disability are universally present to some degree in DS. Further, children with DS are more likely to have associated health conditions increasing risk for ID/IDA including surgeries during infancy, feeding problems/food selectivity, gastroesophageal reflux, thyroid disease, and celiac disease.<sup>2</sup>

While ferritin measures alone have been found to have promise for detecting ID/IDA in typically developing children at low risk for acute or chronic inflammation,<sup>28</sup> these findings may not apply to the DS population, which has been found to have differences in inflammatory pathways and immune dysregulation.<sup>29</sup> While our data did not show a strong relationship between ferritin and CRP, it is generally recommended to obtain CRP measures if ferritin alone is used to test for ID/IDA because ferritin is known to be an acute-phase reactant and may be elevated in the presence of inflammation, infection, or other disease.<sup>9,10</sup> If ferritin is elevated in a setting of suspicious ID/IDA, examining other parameters such as RDW and TS would be helpful, in addition to review of a blood smear by a provider with expertise in hematology.

An additional factor influencing the utility of ferritin for detecting ID/IDA is the optimal reference range. One study has investigated the association between ferritin levels and bone marrow iron (the gold standard for detecting ID) in a sample of 87 children from Malawi, and found that a cutoff of less than 18 ng/mL was optimal for the studied population.<sup>12</sup> As reference ranges differ across institutions based on the local population for each institution, it is not surprising that cutoff values would vary in a region where infection is endemic. However, our supplementary analyses indicated that changing the cutoff value to less than 18 ng/mL led to higher discriminability of ferritin for ID/IDA, suggesting that consideration of alternative cutoff values may be useful for interpretation of low ferritin as a solitary measure for ID/IDA. The data in the current study indicated that RDW and TS measures had higher discriminability for ID/IDA than ferritin even with this higher cutoff value, suggesting that RDW and TS provide additional utility for screening for ID/IDA.

Based on the poor utility of hemoglobin as a screening measure for children with DS, challenges with screening based on parental report of dietary history, and high prevalence of macrocytosis in DS, our approach to screening for ID/IDA in DS is to annually obtain a CBC, with additional periodic measurements of TS (by obtaining serum iron and TIBC) and serum ferritin. ID would be considered likely in individuals with abnormal RDW, TS, or ferritin. This approach follows the rationale of White,<sup>13</sup> who has argued that hemoglobin measures provide false reassurance of iron sufficiency in many children who are at risk for ID/IDA. Costs of these measures vary between institutions, but anecdotal review of cost at our institutions suggests that obtaining ferritin and CRP may be more costly than TS (by obtaining serum iron and TIBC). For example, at one US institution in 2019 the costs of a CBC, TS, and ferritin with CRP were around US\$90, \$210, and \$260, respectively. While there are no published guidelines on recommended frequency of screening with biochemical

measures, our clinics have routinely obtained ferritin and TS only every few years (while continuing to obtain a CBC annually) if results have been normal, minimizing the additional cost of screening. Future research is needed to provide guidance on the optimum frequency of screening in the DS population. In clinics with limited resources, using the RDW value alone from the CBC may provide the best combination of accuracy and economy, since our data indicated that high RDW had the best discriminability of the individual tests. Ferritin alone had lower discriminability as an individual measure, but because of its high specificity for ID/IDA it provided additional utility when combined with RDW. TS alone, however, had the highest sensitivity for ID at 93%. While TS may provide additional utility based on its high sensitivity, it should also be considered that in some settings it may need to be manually calculated by mathematically dividing the serum iron value by the TIBC value.

Treatment with a multivitamin with iron or iron supplementation is recommended when criteria are met for ID/IDA or based on clinical judgment when individual measures are abnormal. While there are limited guidelines available for treatment of ID without IDA, our clinics have routinely recommended an age-appropriate multivitamin with 100% of the recommended daily allowance of iron if TS or ferritin are low, with repeat CBC, TS, and ferritin in 3 months. If IDA is present (defined by ID with low hemoglobin), the recommended treatment is ferrous sulfate supplementation, 3–6 mg/kg elemental iron per day with follow-up testing recommended in 4 weeks.<sup>30</sup> An argument has been made to provide prophylactic iron supplementation to the general population,<sup>13</sup> but iron overload in infancy has been associated with worse neurodevelopmental outcomes.<sup>31,32</sup> Further, prophylactic iron supplementation in DS would be concerning due to increased risk of injury by free radicals from excess iron.<sup>33</sup> Unnecessary iron supplementation in individuals with DS could also exacerbate any concerns with chronic constipation, a condition that is common in individuals with DS.<sup>34</sup> Therefore, specificity in identifying those at risk through laboratory measures is important so a multivitamin with iron can be provided to those with ID prior to the development of IDA.

Our study was limited in that our sample did not include a control group to directly compare hematological and biochemical values between children with and without Down syndrome. The data set was also limited in terms of the ability to assess potential covariates of dietary factors, comorbid conditions, and socioeconomic status. The presence of other medical conditions such as thyroid disease, gastrointestinal issues, feeding problems, attention deficit hyperactivity disorder, and obstructive sleep apnea could have had potential impacts on ID/IDA that were not assessed in the current study. There were relatively few minorities and individuals with Hispanic ancestry in the data set, limiting the ability to generalize the findings. The International Down Syndrome Patient Database is a clinic-based cohort and, as such, not necessarily a population-based one, so the prevalence findings might not be generalizable to individuals with DS at large. Finally, our study's definitions of ID/IDA were not based on the gold standard of diagnosis via bone marrow biopsy to examine iron stores. There is no current clinical consensus on the precise definition of iron deficiency through laboratory measures, as multiple factors can impact iron status, usage, and influence on red blood cell mass and hemoglobin concentration.



Future studies may investigate the role of reticulocyte hemoglobin, a relatively recently studied test to diagnose ID prior to the development of IDA in children. Lower hemoglobin in reticulocytes reflects earlier changes in bone marrow iron status than hemoglobin measured from the entire population of red blood cells.<sup>35</sup> Reticulocyte hemoglobin has been found to have better accuracy than hemoglobin for detecting ID in infants and children from the general population,<sup>35,36</sup> but has not yet been tested in a large representative population. Further, the potential accuracy of this measure in children with DS is unknown, as it is possible that reticulocyte size could also be larger in children with DS than in typically developing children.

We conclude that all children with DS who are over 1 year of age and are not taking iron-fortified formula should be offered regular, intermittent screening for ID/IDA. Our data suggest that the combination of a CBC (which provides information on hemoglobin and RDW) and ferritin with CRP provides the best utility for detecting ID/IDA. TS may also be considered when available as an additional measure to the CBC and ferritin, as TS had the highest sensitivity for ID of any individual laboratory measure. Given the high prevalence of macrocytosis in DS and the poor utility of currently recommended screening measures to identify those with ID prior to the development of IDA, the use of these laboratory screening measures should be considered to allow for accurate and specific identification of children at an early age who can benefit from intervention. Future randomized, placebo-controlled trials are needed to establish neurodevelopmental effects of universal screening by laboratory measures and treatment of ID/IDA in individuals both with and without DS, compared with the standard of care of screening only those with known risk factors.<sup>37</sup> Future studies in DS are also needed to assess the effects of common comorbidities and dietary factors on ID/IDA.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## REFERENCES

1. Starc TJ. Erythrocyte macrocytosis in infants and children with Down syndrome. *J Pediatr*. 1992;121:578–581. [PubMed: 1403393]

2. Dixon NE, Crissman BG, Smith PB, Zimmerman SA, Worley G, Kishnani PS. Prevalence of iron deficiency in children with Down syndrome. *J Pediatr.* 2010;157:967–71 e961. [PubMed: 20650467]
3. Roizen NJ, Amarose AP. Hematologic abnormalities in children with Down syndrome. *Am J Med Genet.* 1993;46:510–512. [PubMed: 8322810]
4. Akin K Macrocytosis and leukopenia in Down's syndrome. *JAMA.* 1988;259:842.
5. Lozoff B, Smith JB, Kaciroti N, Clark KM, Guevara S, Jimenez E. Functional significance of early-life iron deficiency: outcomes at 25 years. *J Pediatr.* 2013;163:1260–1266. [PubMed: 23827739]
6. Georgieff MK. Long-term brain and behavioral consequences of early iron deficiency. *Nutr Rev.* 2011;69 suppl 1:S43–48. [PubMed: 22043882]
7. Lozoff B, Beard J, Connor J, Barbara F, Georgieff M, Schallert T. Long-lasting neural and behavioral effects of iron deficiency in infancy. *Nutr Rev.* 2006;64 5 pt 2:S34–43. discussionS72–91 [PubMed: 16770951]
8. Siu AL, US Preventive Services Task Force. Screening for iron deficiency anemia in young children: USPSTF Recommendation Statement. *Pediatrics.* 2015;136:746–752. [PubMed: 26347426]
9. Baker RD, Greer FR, Committee on Nutrition American Academy of Pediatrics. Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0–3 years of age). *Pediatrics.* 2010;126:1040–1050. [PubMed: 20923825]
10. Bull MJ, Committee on Genetics. Health supervision for children with Down syndrome. *Pediatrics.* 2011;128:393–406. [PubMed: 21788214]
11. Lavigne J, Sharr C, Ozonoff A, et al. National down syndrome patient database: insights from the development of a multicenter registry study. *Am J Med Genet A.* 2015;167A:2520–2526. [PubMed: 26249752]
12. Jonker FA, Boele van Hensbroek M, Leenstra T, et al. Conventional and novel peripheral blood iron markers compared against bone marrow in Malawian children. *J Clin Pathol.* 2014;67:717–723. [PubMed: 24915849]
13. White KC. Anemia is a poor predictor of iron deficiency among toddlers in the United States: for heme the bell tolls. *Pediatrics.* 2005;115:315–320. [PubMed: 15687438]
14. Maxim LD, Niebo R, Utell MJ. Screening tests: a review with examples. *Inhal Toxicol.* 2014;26:811–828. [PubMed: 25264934]
15. Oski FA. Iron deficiency in infancy and childhood. *N Engl J Med.* 1993;329:190–193. [PubMed: 8515791]
16. Akkermans MD, Uijterschout L, Vloemans J, et al. Red blood cell distribution width and the platelet count in iron-deficient children aged 0.5–3 years. *Pediatr Hematol Oncol.* 2015;32:624–632. [PubMed: 26558306]
17. Aslinia F, Mazza JJ, Yale SH. Megaloblastic anemia and other causes of macrocytosis. *Clin Med Res.* 2006;4:236–241. [PubMed: 16988104]
18. Colon-Otero G, Menke D, Hook CC. A practical approach to the differential diagnosis and evaluation of the adult patient with macrocytic anemia. *Med Clin North Am.* 1992;76:581–597. [PubMed: 1578958]
19. Davidson RJ, Hamilton PJ. High mean red cell volume: its incidence and significance in routine haematology. *J Clin Pathol.* 1978;31:493–498. [PubMed: 649776]
20. Breedveld FC, Bieger R, van Wermeskerken RK. The clinical significance of macrocytosis. *Acta Med Scand.* 1981;209:319–322. [PubMed: 7234509]
21. Mei Z, Flores-Ayala RC, Grummer-Strawn LM, Brittenham GM. Is erythrocyte protoporphyrin a better single screening test for iron deficiency compared to hemoglobin or mean cell volume in children and women?. *Nutrients.* 2017;9:E557. [PubMed: 28561801]
22. Bogen DL, Duggan AK, Dover GJ, Wilson MH. Screening for iron deficiency anemia by dietary history in a high-risk population. *Pediatrics.* 2000;105:1254–1259. [PubMed: 10835066]
23. Boutry M, Needlman R. Use of diet history in the screening of iron deficiency. *Pediatrics.* 1996;98 6 pt 1:1138–1142. [PubMed: 8951266]
24. Centers for Disease Control and Prevention. Recommendations to prevent and control iron deficiency in the United States. *MMWR Recomm Rep.* 1998;47:1–29.

25. Lozoff B, Jimenez E, Smith JB. Double burden of iron deficiency in infancy and low socioeconomic status: a longitudinal analysis of cognitive test scores to age 19 years. *Arch Pediatr Adolesc Med.* 2006;160:1108–1113. [PubMed: 17088512]
26. McCann JC, Ames BN. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. *Am J Clin Nutr.* 2007;85:931–945. [PubMed: 17413089]
27. Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. *J Nutr.* 2001;131(2S-2):649S–666S. discussion 666S-668S [PubMed: 11160596]
28. Oatley H, Borkhoff CM, Chen S, et al. Screening for iron deficiency in early childhood using serum ferritin in the primary care setting. *Pediatrics.* 2018;142:e20182095.
29. Sullivan KD, Evans D, Pandey A, et al. Trisomy 21 causes changes in the circulating proteome indicative of chronic autoinflammation. *Sci Rep.* 2017;7:14818. [PubMed: 29093484]
30. Powers JM, Buchanan GR, Adix L, Zhang S, Gao A, McCavit TL. Effect of low-dose ferrous sulfate vs iron polysaccharide complex on haemoglobin concentration in young children with nutritional iron-deficiency anemia: a randomized clinical trial. *JAMA.* 2017;317:2297–2304. [PubMed: 28609534]
31. Hare DJ, Arora M, Jenkins NL, Finkelstein DI, Doble PA, Bush AI. Is early-life iron exposure critical in neurodegeneration? *Nat Rev Neurol.* 2015;11:536–544. [PubMed: 26100754]
32. Lozoff B, Castillo M, Clark KM, Smith JB. Iron-fortified vs low-iron infant formula: developmental outcome at 10 years. *Arch Pediatr Adolesc Med.* 2012;166:208–215. [PubMed: 22064877]
33. Barone E, Arena A, Head E, Butterfield DA, Perluigi M. Disturbance of redox homeostasis in Down syndrome: role of iron dysmetabolism. *Free Radic Biol Med.* 2018;114:84–93. [PubMed: 28705658]
34. Sharr C, Lavigne J, Elsharkawi IM, et al. Detecting celiac disease in patients with Down syndrome. *Am J Med Genet A.* 2016;170: 3098–3105. [PubMed: 27605215]
35. Ullrich C, Wu A, Armsby C, et al. Screening healthy infants for iron deficiency using reticulocyte hemoglobin content. *JAMA.* 2005;294: 924–930. [PubMed: 16118382]
36. Bakr AF, Sarette G. Measurement of reticulocyte hemoglobin content to diagnose iron deficiency in Saudi children. *Eur J Pediatr.* 2006;165: 442–445. [PubMed: 16520941]
37. Larson LM, Phiri KS, Pasricha SR. Iron and cognitive development: what is the evidence?. *Ann Nutr Metab.* 2017;71 suppl 3:25–38. [PubMed: 29268256]

Table 1

Iron deficiency (ID) and ID anemia (IDA) by demographic information for 856 participants across 5 sites<sup>a</sup>

	Normal	ID <sup>b</sup>	IDA <sup>c</sup>	P value
Sex	<i>n</i> = 785	<i>n</i> = 46	<i>n</i> = 10	0.199
Female	447 (56.9%)	28 (60.9%)	3 (30%)	
Male	338 (43.1%)	18 (39.1%)	7 (70%)	
Race	<i>n</i> = 766	<i>n</i> = 42	<i>n</i> = 10	0.880
Caucasian	689 (89.95%)	38 (90.48%)	8 (80%)	
African American	46 (6%)	3 (7.14%)	1 (10%)	
Asian	20 (2.61%)	1 (2.38%)	1 (10%)	
Native American	2 (0.26%)	0	0	
Other	9 (1.17%)	0	0	
Ethnicity	<i>n</i> = 696	<i>n</i> = 42	<i>n</i> = 8	0.707
Hispanic	22 (3.1%)	2 (4.7%)	0	
Non-Hispanic	674 (93.9%)	40 (93%)	8 (100%)	
Age	<i>n</i> = 800	<i>n</i> = 46	<i>n</i> = 10	0.002
12–36 months	157 (19.63%)	18 (39.1%)	3 (30%)	
3–5 years	117 (14.63%)	10 (21.74%)	0 (0%)	
5–11 years	294 (36.75%)	8 (17.39%)	2 (20%)	
11–19 years	185 (23.13%)	10 (21.74%)	3 (30%)	
>19 years	47 (5.88%)	0 (0%)	2 (20%)	
Mean (year), SD	8.78 ± 6.53	6.1 ± 5.3	12.94 ± 12/62	
Median (year)	8	4	11	

*RDW* red cell distribution width, *TS* transferrin saturation.

<sup>a</sup>The total number of subjects varies by demographic variable due to missing data from some participants.

<sup>b</sup>ID = two abnormal biochemical markers of iron (low ferritin and low TS), or one abnormal biochemical marker plus elevated RDW.

<sup>c</sup>IDA = ID plus a low hemoglobin concentration.

**Table 2**

Prevalence of iron deficiency (ID)/ID anemia (IDA) by site

Site	Normal <i>n</i> = 800	ID <sup>a</sup> <i>n</i> = 46	IDA <sup>b</sup> <i>n</i> = 10
Bambino Gesù <i>n</i> = 532	502 (94.4%)	27 (5.1%)	3 (0.6%)
DUMC <i>n</i> = 115	111 (96.5%)	3 (2.6%)	1 (0.9%)
MGH <i>n</i> = 82	78 (95.1%)	2 (2.4%)	2 (2.4%)
NCH <i>n</i> = 64	47 (73.4%)	13 (20.3%)	4 (6.3%)
UPMC <i>n</i> = 63	62 (98.4%)	1 (1.6%)	0 (0%)

*DUMC* Duke University Medical Center, *MGH* Massachusetts General Hospital, *NCH* Nationwide Children's Hospital, *UPMC* University of Pittsburgh Medical Center.

<sup>a</sup>ID = two abnormal biochemical markers of iron (low ferritin and low transferrin saturation [TS]), or one abnormal biochemical marker plus elevated red cell distribution width (RDW).

<sup>b</sup>IDA = ID plus a low hemoglobin concentration.

Table 3

Hematological and biochemical values for iron deficiency (ID)/ID anemia (IDA)

	No ID/IDA	ID <sup>a</sup>	IDA <sup>b</sup>
Hgb (g/dL)			
12–36 months	13.1 ± 1.1 <i>n</i> = 157 (88.2%)	12.1 ± 0.9 <i>n</i> = 18 (10.1%)	8.2 ± 3.4 <i>n</i> = 3 (1.7%)
>36 months	14.2 ± 1.2 <i>n</i> = 643 (94.8%)	13.5 ± 1.2 <i>n</i> = 28 (4.1%)	10.3 ± 1.6 <i>n</i> = 7 (1.0%)
All ages	14.0 ± 1.3 <i>n</i> = 800 (93.5%)	12.9 ± 1.3 <i>n</i> = 46 (5.4%)	9.7 ± 2.3 <i>n</i> = 10 (1.2%)
MCV (fL)			
12–36 months	87.4 ± 4.7 <i>n</i> = 142 (89.3%)	77.1 ± 9.8 <i>n</i> = 15 (9.4%)	66.4 ± 15.2 <i>n</i> = 2 (1.2%)
>36 months	91.6 ± 5.7 <i>n</i> = 629 (96.2%)	86.3 ± 5.3 <i>n</i> = 18 (2.8%)	77.0 ± 6.5 <i>n</i> = 7 (1.1%)
All ages	90.8 ± 5.8 <i>n</i> = 771 (94.8%)	82.1 ± 8.8 <i>n</i> = 33 (4.1%)	74.7 ± 9.1 <i>n</i> = 9 (1.1%)
RDW (%)			
12–36 months	14.6 ± 1.4 <i>n</i> = 142 (89.3%)	17.9 ± 2.6 <i>n</i> = 15 (9.4%)	20.6 ± 4.7 <i>n</i> = 2 (1.2%)
>36 months	14.5 ± 9.7 <i>n</i> = 629 (96.2%)	16.5 ± 1.1 <i>n</i> = 18 (2.8%)	19.5 ± 1.8 <i>n</i> = 7 (1.1%)
All ages	14.5 ± 8.8 <i>n</i> = 771 (94.8%)	17.2 ± 2.0 <i>n</i> = 33 (4.1%)	19.8 ± 2.3 <i>n</i> = 9 (1.1%)
Ferritin (ng/mL)			
12–36 months	51.8 ± 102.0 <i>n</i> = 134 (87.0%)	23.8 ± 24.9 <i>n</i> = 17 (11.0%)	12.3 ± 9.0 <i>n</i> = 3 (1.9%)
>36 months	60.2 ± 49.8 <i>n</i> = 561 (94.4%)	19.2 ± 22.0 <i>n</i> = 26 (4.4%)	5.3 ± 1.9 <i>n</i> = 7 (1.2%)
All ages	58.6 ± 63.3 <i>n</i> = 695 (92.9%)	21.0 ± 23.0 <i>n</i> = 43 (5.7%)	7.4 ± 5.7 <i>n</i> = 10 (1.3%)
CRP (mg/dL)			
12–36 months	0.6 ± 1.0 <i>n</i> = 105 (88.2%)	1.8 ± 4.6 <i>n</i> = 13 (10.9%)	2.6 <i>n</i> = 1 (0.8%)
>36 months	0.7 ± 3.0 <i>n</i> = 524 (96.1%)	0.2 ± 0.4 <i>n</i> = 17 (3.1%)	0.6 ± 1.1 <i>n</i> = 4 (0.7%)

	No ID/IDA	ID <sup>a</sup>	ID <sup>a</sup>
All ages	0.7 ± 2.8 <i>n</i> = 629 (94.7%)	0.9 ± 3.1 <i>n</i> = 30 (4.5%)	± 1.3 <i>n</i> = 5 (0.7%)
Ferritin (ng/mL) in setting of normal CRP (<1.2 mg/dL)			
12–36 months	36.9 ± 25.0 <i>n</i> = 94 (89.5%)	22.2 ± 30.9 <i>n</i> = 10 (9.5%)	13.0 <i>n</i> = 1 (1.0%)
>36 months	57.3 ± 45.8 <i>n</i> = 486 (96.2%)	8.6 ± 7.7 <i>n</i> = 16 (3.2%)	5.0 ± 0.0 <i>n</i> = 3 (0.6%)
All ages	54.0 ± 43.7 <i>n</i> = 580 (95.1%)	13.8 ± 20.6 <i>n</i> = 26 (4.3%)	7.0 ± 4.0 <i>n</i> = 4 (0.7%)
TS (%)			
12–36 months	21.5 ± 9.2 <i>n</i> = 108 (84.4%)	9.5 ± 3.6 <i>n</i> = 17 (13.3%)	7.5 ± 6.4 <i>n</i> = 3 (2.3%)
>36 months	25.9 ± 11.5 <i>n</i> = 522 (94.6%)	10.5 ± 5.4 <i>n</i> = 28 (5.1%)	7.3 ± 3.9 <i>n</i> = 2 (0.4%)
All ages	25.2 ± 11.3 <i>n</i> = 630 (92.2%)	10.1 ± 4.8 <i>n</i> = 45 (6.6%)	7.4 ± 4.5 <i>n</i> = 8 (1.2%)

*CRP*-reactive protein, *Hgb* hemoglobin, *MCV* mean corpuscular volume, *RDW* red cell distribution width, *TS* transferrin saturation.

<sup>a</sup>ID = two abnormal biochemical markers of iron (low ferritin and low TS), or one abnormal biochemical marker plus elevated RDW.

<sup>b</sup>ID = ID plus a low hemoglobin concentration.

Table 4

Diagnostic utility of individual measures for detecting iron deficiency (ID)<sup>a</sup>/ID anemia (IDA)<sup>b</sup>, % (95% confidence interval)

		Sensitivity	Specificity	PPV	NPV	ROC
Low Hgb	ID	0 (0–8)	98 (97–99)	0 (0)	95 (94–95)	0.49
	IDA	100 (69–100)	99 (98–100)	56 (39–71)	100 (100)	1.00
Low MCV	ID	9 (2–21)	99 (97–99)	25 (10–50)	95 (95)	0.54
	IDA	30 (7–65)	98 (97–99)	19 (7–41)	99 (99)	0.64
High RDW	ID	91 (79–98)	91 (89–93)	38 (32–44)	99 (99–100)	0.91
	IDA	100 (69–100)	88 (86–90)	9 (8–11)	100 (100)	0.94
Low ferritin	ID	28 (16–44)	99 (97–99)	50 (33–67)	96 (95–97)	0.64
	IDA	80 (44–97)	98 (97–99)	31 (20–44)	100 (99–100)	0.89
Low ferritin (normal CRP)	ID	50 (31–69)	99 (98–100)	79 (57–91)	97 (96–98)	0.75
	IDA	75 (19–99)	97 (96–99)	16 (8–28)	100 (99–100)	0.86
Low TS	ID	93 (82–99)	86 (83–88)	27 (24–31)	100 (99–100)	0.90
	IDA	80 (44–97)	82 (80–85)	5 (4–7)	100 (99–100)	0.81
Low TS (normal CRP)	ID	90 (73–98)	87 (84–89)	26 (22–31)	99 (98–100)	0.88
	IDA	100 (40–100)	84 (80–86)	4 (3–5)	100 (100)	0.92

CRP-c-reactive protein, *Hgb* hemoglobin, *MCV* mean corpuscular volume, *NPV* negative predictive value, *PPV* positive predictive value, *RDW* red cell distribution width  
*ROC* receiver operating characteristic, *TS* transferrin saturation.

<sup>a</sup>ID = two abnormal biochemical markers of iron (low ferritin and low TS), or one abnormal biochemical marker plus elevated RDW.

<sup>b</sup>IDA = ID plus a low hemoglobin concentration.



Table 5

Diagnostic utility of combined measures for detecting iron deficiency (ID)<sup>a</sup>/ID anemia (IDA)<sup>b</sup> (at least one positive value), % (95% confidence interval)<sup>c</sup>

		Sensitivity	Specificity	PPV	NPV	ROC
High RDW or low ferritin	ID	100 (92–100)	91 (89–93)	38 (33–44)	100 (100)	0.95
	IDA	100 (69–100)	87 (85–89)	8 (7–10)	100 (100)	0.94
High RDW or low TS	ID	100 (92–100)	78 (75–81)	21 (19–23)	100 (100)	0.89
	IDA	100 (69–100)	75 (72–78)	5 (4–5)	100 (100)	0.88
Low ferritin (with normal CRP) or low TS <sup>d</sup>	ID	96 (88–100)	86 (84–89)	33 (29–37)	100 (99–100)	0.91
	IDA	80 (44–97)	82 (79–84)	5 (4–7)	100 (99–100)	0.81

*NPV* negative predictive value, *PPV* positive predictive value, *RDW* red cell distribution width, *ROC* receiver operating characteristic, *TS* transferrin saturation.

<sup>a</sup>ID = two abnormal biochemical markers of iron (low ferritin and low TS), or one abnormal biochemical marker plus elevated RDW.

<sup>b</sup>IDA = ID plus a low hemoglobin concentration.

<sup>c</sup>Note that these analyses do not include the requirement for both values to be abnormal, as these would be reflective of our a priori definitions of ID/IDA and would have perfect discriminability by definition.

<sup>d</sup>This combination of measures reflects criteria used by some clinics for detection and treatment of ID.