Using Reticulocyte Hemoglobin Equivalent to Screen for Iron Deficiency May Be Problematic

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Introduction
Iron deficiency (ID) is the most common nutritional deficiency globally, with a prevalence of 9.2% among children age 12 to 35 months in the United States. Iron deficiency anemia (IDA) is found in 2.1% of children in this age group.1 The developmental delays associated with ID motivate pediatricians to diagnose the iron deficient child as early and accurately as possible,2 ideally before the development of IDA. Unfortunately, traditional markers of iron stores, such as transferrin saturation or serum ferritin, are not sensitive in this age group, and both can be affected by systemic inflammation.3,4 In 2010, the American Academy of Pediatrics (AAP) published a clinical report recommending that children be screened for ID with either ferritin and C-reactive protein (CRP) or reticulocyte hemoglobin content (CHr).1 The report did not favor one testing strategy over another. Prior work has shown that CHr is an early and sensitive marker of ID,5,6 and as a result, our clinic transitioned to testing for ID exclusively with this test.

Currently, 2 tests exist for assessing the amount of hemoglobin in reticulocytes. CHr is an older, more studied value resulted by Advia machines; reticulocyte hemoglobin equivalent (Ret-He) is a newer, less-documented value resulted by Sysmex machines. Several cutoff levels for the CHr test have been proposed,5,6 but pediatric data are lacking for the Ret-He test, and the manufacturer recommends normative testing for each machine and population (personal communication, Dr Meyers).

Pediatric primary care clinicians at our urban safety-net hospital began using Ret-He to screen for ID in early 2013 when the test first became available at our institution. Subsequently, several clinicians noticed a high number of positive screens compared with their prior experience and given the estimated disease prevalence.1 The authors began a quality improvement initiative to standardize our clinic’s screening practice. A literature review established that normal values reported with the Ret-He test had been established for adults only, leading to concerns about the appropriateness of this test in our patients.

Methods
A retrospective data review of all Ret-He values drawn for patients aged 7 to 25 months at the time of test was performed. We searched de-identified data from January 2013, when the Ret-He test first became available at our institution, until January 2014. We examined only those results drawn on the same day as a routine health care maintenance visit. If a patient had multiple lab values meeting these criteria, only the first value was retained. Premature children were excluded, given their higher risk of ID and likely iron exposure. We identified the proportion of patients who would have screened positive for ID using various thresholds of the lower limit of normal for the Ret-He test and the proportion with IDA using a hemoglobin less than 11 g/dL.1 We calculated similar proportions using pediatric lower limit thresholds for the CHr test that are sex-specific.7 While these values are not validated for the Ret-He test, we compare them to show their potential utility in interpreting Ret-He values. We also classified subjects as having microcytosis using a mean cell volume of less than 77fL.8 If other iron and complete blood count indices were resulted on the same day, they were examined. Descriptive statistics and graphics were calculated using SAS 9.3.9

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Results

We found 365 unique patient samples meeting our criteria. The average patient age was 14.4 months, 49% of the sample was male, and the mean Ret-He value was 28.6 pg (standard deviation 3.24; range 16.5-34.6). Only 11 children had simultaneously drawn ferritin, and only 25 had simultaneously drawn iron and total iron-binding capacity (TIBC).

A histogram plot of Ret-He values was made with a normal distribution curve overlaid in blue (Figure 1). Also on the histogram are 3 vertical lines, which indicate 3 previously suggested reference range lower limits. As expected, the proportion of patients who would have screened positive for ID decreased as the lower threshold of normal Ret-He decreased (Table 1). We found a microcytic profile in 42% of the sample, and an increasing proportion of patients with low Ret-He who also had microcytosis as thresholds for Ret-He were lowered.

Discussion

As a result of instituting the Ret-He test as a stand-alone screen for ID, our clinic saw a significant number of
children identified as iron deficient. Using our laboratory’s lower limit of normal (30.1 pg), 61.1% of our patients were diagnosed with ID, significantly higher than the expected 9% to 10% prevalence. Ret-He normative values are not available for children, but norms do exist for the CHr test, which also measures the hemoglobin in reticulocytes in identical units (pg). A lower limit of normal of 30.1 pg does not represent a pediatric distribution, and our lab has since clarified the language in their reporting of values.

While this study illustrates some of the important concerns clinics must address when instituting a new test, such as verification of appropriate normative values and early and careful monitoring of trends in results, it is limited by its retrospective nature, and we cannot draw any conclusions about the potential validity of the Ret-He test in other settings. Unfortunately, we were also limited in our ability to evaluate the accuracy of the test by the fact that our clinicians made a near complete switch to the new methodology and stopped using other testing strategies to assess for the presence of a true physiologic abnormality. The number of simultaneously drawn iron, TIBC, and ferritin tests was insufficient for us to draw definitive conclusions about a patient’s iron status. Finally, without knowing the number of iron prescriptions given as a result of the many false-positive Ret-He values obtained, we cannot ascertain the financial impact of the implementation of this test in our clinic.

The 2010 AAP report that endorses the use of CHr to test for ID refers to “CHr” only. Currently, both CHr and Ret-He may be seen in various practice settings. Both tests are widely used and there are demonstrations of equivalency between their results. However, the reference range of one test should not be applied to another, and each test should have its own range established from the population being tested. There are published, age-specific pediatric guidelines for the CHr test; however, in our review, there are no established reference intervals in a pediatric population for the Ret-He test on Sysmex machines. The manufacturer has confirmed the absence of pediatric standards for this measure via personal communication (oral, with Dr Meyers). Interestingly, when applying the CHr norms to our Ret-He values, we arrived at the closest approximation of the expected prevalence of ID and IDA in our sample population.

Finally, another important limitation of the tests of the hemoglobin in reticulocytes is that they cannot distinguish between ID and thalassemia trait. Therefore, its use in a population with a high expected prevalence of thalassemia trait, such as our urban clinic, may not be appropriate. Tests that can differentiate the 2 conditions would be highly valued when screening a population largely made up of minority groups known to have a high prevalence of thalassemia trait who are also predisposed to ID.

Because of concerns for a high false-positive rate, our clinicians have stopped exclusively using Ret-He to detect ID and IDA, and many have reverted to more traditional methods such as ferritin with CRP. While we believe there is excellent potential for the Ret-He or CHr tests to be put into practice in pediatric clinics, more studies are needed to establish normal ranges for the Ret-He test in a pediatric population, and we plan to pursue a study of our patients to establish normative values for our clinic. Similar studies in other populations should pay particular attention to the racial makeup of the test population and the prevalence of thalassemia. Although it is recommended that normative studies be performed at each institution prior to test use, published reference ranges would at least serve as a better standard than using adult norms.

Author Contributions
Drs. Meyers, Sobota, and Hatoun contributed to the study concept, the study design, and the revision of the manuscript. Dr. Hatoun was responsible for data acquisition and analysis, as well as for drafting the manuscript.

Declaration of Conflicting Interests
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