Evaluation of the Reticulocyte Production Index in the Pediatric Population

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ABSTRACT

Objectives: *Since hematologic values vary with age in children, we evaluated the agreement between the "traditional" reticulocyte production index (RPI) and an RPI by age (RPI/A)–adjusted normal values.*

Methods: *A retrospective, observational, and analytical study was performed on CBCs of children with anemia younger than 18 years. The agreement and clinical repercussions of the RPI values were analyzed with an RPI/A developed with theoretical values for different ages.*

Results: *A total of 5,503 tests were analyzed and no systematic error between the two indices was found; however, there were significant proportional differences at higher values that resulted in lower RPI/A in children younger than 15 days and higher RPI/A in children aged 15 days and older. No agreement was observed at any age. The proportion of arregenerative anemia diagnosed using RPI/A was higher in children younger than 15 days and lower in those 15 days and older.*

Conclusions: *RPI is not an adequate tool for evaluating the erythropoietic capacity of bone marrow in the pediatric population. The disagreement between the results can be explained by the difference in normal hematologic values between children and adults.*

Key Points

- The reticulocyte production index (RPI) has been shown to be a good indicator of the bone marrow erythropoietic response to anemia in adults, but it has not been validated in children.
- There is no agreement between RPI and RPI by age values at any age in children.
- RPI is not an adequate tool for evaluating the erythropoietic capacity of bone marrow in the pediatric population.

Reticulocytes are defined as "any nonnucleated red cell which contains two or more particles of blue-stained material, visible without fine microscope adjustment, after exposure to supravital stains, such as brilliant cresyl blue or new methylene blue."[1,2] This reticular structure, or granules, is formed by precipitation of the rough endoplasmic reticulum and the remaining polyribosomes.[1-4] Reticulocytes originate from nucleated RBCs, and in their last stage of maturation, they lose their nucleus. These cells remain in the bone marrow for 3 to 3.5 days before being released into circulation, where they remain in that state for approximately 24 hours before becoming mature erythrocytes.[5-8] During this period, reticulocytes finish synthesizing the hemoglobin and other proteins that characterize mature erythrocytes; change their shape from larger, mobile, and irregular cells to discoid cells that are biconcave, smaller, and more uniform; degrade residual organelles; and remove specific proteins from the plasma membrane.[4,8-12] Maturation is complete when the basophilic filamentous substance that characterizes the reticulocyte disappears.[4]

When erythropoiesis is stimulated, the production of reticulocytes can increase between 15 and 20 times at the expense of greater production and reduced maturation time in the bone marrow, which results in

less-mature erythroid elements being sent to the peripheral blood.[4,5,9,13-17] Upon being released prematurely, reticulocytes must complete the maturation processes in the bloodstream, thus increasing their residence time in the circulation.[4,9,18] Therefore, the reticulocyte population can become considerably heterogeneous due to differences in the maturation stages of circulating cells.[8,15]

The analysis of reticulocytes is an important tool for evaluating erythropoietic activity because the number of reticulocytes reflects the equilibrium of cells released from the bone marrow, their stage of maturity, and their rate of transformation into mature erythrocytes; thus, reticulocyte analysis is one of the most useful and cost-effective laboratory tests for studying anemia and controlling the response to treatment.[4,8,9,19,20]

Although reticulocyte counts can be performed by automated analyzers, they are one of the few hematologic tests that are still performed manually.[2,9] For identification, the technique described by Brecher[21] is used, in which peripheral blood anticoagulated with EDTA is mixed with a supravital stain (new methylene blue or 1% brilliant cresyl blue) and observed with a light microscope. The results can be expressed as a percentage and/or a count.[2,9,22] These values do not accurately reflect the erythropoietic capacity of the bone marrow, so two indices were created to try to relate the percentage of reticulocytes with the extent of anemia: the corrected reticulocyte count (CRC) and the reticulocyte production index (RPI):[16, 18,23]

CRC = reticulocyte (%) × patient hematocrit / normal hematocrit

RPI = (reticulocyte (%) × patient hematocrit / normal hematocrit) / maturation time

The latter was developed by Hillman[16] in 1969, and its purpose is to evaluate the response capacity of bone marrow in the presence of anemia, correct the reticulocyte count according to the hematocrit value, and determine the delayed maturation time in peripheral blood with various levels of anemia **Table 1**.[24] Therefore, an RPI of more than 3 indicates an appropriate bone marrow response (regenerative anemia), while an RPI of less than 2 indicates an inadequate compensatory response to correct the anemia (arregenerative anemia).[16]

The RPI has been shown to be a good indicator of the responsiveness of bone marrow to anemia and can guide the diagnostic study of the etiology and pathophysiology of anemia.[9,15,25-27] In pediatrics, the RPI has been widely used without considering that it was developed from a study conducted in adults. Considering that normal hematologic values vary with age in children,[24] there is a need to determine whether there is adequate agreement between the "traditional" RPI and an RPI based on normal values adjusted for different pediatric ages (RPI/A).

Table 1

Hematocrit Values for Calculation of the Reticulocyte Production Index According to Age

Age	Average Hematocrit by Age, %[24]	Maturation Time in Peripheral Blood, d			
		2.5	2	1.5	1
Original study[16]	45	≤19.9	20.0-29.9	30.0-39.9	≥40.0
1-3 days	56	≤30.9	31.0-40.9	41.0-50.9	≥51.0
4-13 days	51	≤25.9	26.0-35.9	36.0-45.9	≥46.0
14-29 days	43	≤17.9	18.0-27.9	28.0-37.9	≥38.0
1-2 months	35	≤9.9	10.0-19.9	20.0-29.9	≥30.0
2-6 months	35	≤9.9	10.0-9.9	20.0-29.9	≥30.0
6 months to 2 years	36	≤10.9	11.0-20.9	21.0-30.9	≥31.0
2-6 years	37	≤11.9	12.0-21.9	22.0-31.9	≥32.0
6-12 years	40	≤14.9	15.0-24.9	25.0-34.4	≥35.0
12-18 years (females)	41	≤15.9	16.0-25.9	26.0-35.9	≥36.0
12-18 years (males)	43	≤17.9	18.0-27.9	28.0-37.9	≥38.0

Materials and Methods

To evaluate the RPI in pediatric patients, a retrospective observational and analytical study was designed. The sample of patients was obtained from the database of our laboratory (SysLAB; SysLab Engineering) between January 1, 2016, and December 31, 2018. Only validated CBC results, with all erythrocyte indices and a manual count of concomitant reticulocytes for patients between 0 days and 17 years, 11 months and 29 days of life were extracted. From this sample, tests were selected only if they indicated anemia, defined as a hematocrit or hemoglobin concentration less than 2 standard deviations from the norm for age and sex.[24] To guarantee the anonymity of the samples, personal information was excluded from the extracted results, and the samples were coded by a third party that did not participate in this research.

The hematologic counts were performed with a Sysmex XT-4000i automated hematologic counter from Sysmex Corporation. The manual counting of reticulocytes was performed according to the specifications of the National Committee for Clinical Laboratory Standards: equivalent volumes of EDTA-collected blood and brilliant cresyl blue staining were mixed in a Khan tube, the mixture was incubated for 10 minutes in a thermoregulated bath at 37°C, and then a smear was performed on glass slides; the count was

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Table 2
Results of Indices for Different Age Groups

Age	No.	RETCOR, Mean ± SD	RETCORR/A, Mean ± SD	RPI, Mean ± SD (95% CI)	RPI/A, Mean ± SD (95% CI)	*P* Value
1-3 days	19	7.12 ± 9.82	5.72 ± 7.89	4.30 ± 5.80 (1.51-7.10)	2.64 ± 3.58 (0.91-4.36)	.2947
4-13 days	53	1.68 ± 1.28	1.49 ± 1.13	1.02 ± 0.74 (0.81-1.22)	0.76 ± 0.56 (1.01-1.57)	.0448[a]
14-29 days	76	2.50 ± 2.47	2.62 ± 2.59	1.29 ± 1.22 (1.01-1.57)	1.46 ± 1.47 (1.13-1.80)	.4265
1-2 months	191	2.15 ± 1.51	2.77 ± 1.95	1.07 ± 0.75 (0.96-1.18)	1.83 ± 1.29 (1.65-2.02)	<.0001[a]
2-6 months	304	2.64 ± 2.06	3.32 ± 2.48	1.32 ± 1.04 (1.20-1.44)	2.27 ± 1.80 (2.07-2.47)	<.0001[a]
6 months—2 years	911	2.25 ± 2.79	2.77 ± 3.35	1.22 ± 1.42 (1.13-1.31)	2.02 ± 2..31 (1.87-2.17)	<.0001[a]
2-6 years	1,174	2.16 ± 3.59	2.49 ± 3.38	1.20 ± 1.95 (1.08-1.31)	1.50 ± 2.84 (1.33-1.66)	.0028[a]
6-12 years	1,703	2.18 ± 3.63	2.42 ± 3.85	1.21 ± 1.95 (1.12-1.30)	1.53 ± 2.44 (1.42-1.65)	<.0001[a]
12-18 years (females)	451	1.78 ± 1.64	2.27 ± 4.64	1.05 ± 0.96 (0.96-1.14)	1.25 ± 1.18 (1.14-1.36)	.0060[a]
12-18 years (males)	621	2.79 ± 4.10	2.73 ± 3.28	1.59 ± 2.24 (1.42-1.7)	1.74 ± 2.40 (1.55-1.92)	.2835

CI, confidence interval; RETCOR, corrected reticulocyte count; RETCOR/A, corrected reticulocyte count by age; RPI, reticulocyte production index; RPI/A, reticulocyte production index by age.
[a]Significant difference.

performed with a light microscope at ×100 for a total of 1,000 erythrocytes per smear.[2]

The RPI was calculated according to the formula by Hillman[16] using the values reported in their study (Table 1). To calculate the RPI/A, the results were classified according to the age groups published by Brugnara et al,[24] using the average hematocrit for each age group as a reference (Table 1). Then, a theoretical determination of the maturation times was performed for each group using the slope of the curve described by Hillman[16] for the relationship between the decrease in hematocrit and the delayed maturation time of the reticulocytes in the peripheral blood, using the average hematocrit for each age as the point of intercept (Table 1).[24]

For descriptive statistics, the mean was used as a measure of centralization, and the standard deviation was used as a measure of dispersion. As it has been widely suggested that the usual correlation and regression methods are insufficient to evaluate the agreement between quantitative measures,[28] the Passing-Bablok nonparametric regression model was used, and the slopes and intercepts were calculated with a confidence interval of 95%; the model was validated using the cumulative sum (CUSUM) linearity test. To analyze the agreement of the results from a clinical perspective, paired results were compared using the Bland-Altman analysis[28]; no statistical comparison was made with the RPI/A because no quality control specifications indicate a desirable or minimum bias for the RPI, so the interpretation of the results was limited only to the comparability between the results of the RPI and RPI/A; a linear regression model for the mean differences between RPI and RPI/A was elaborated to determine the existence of systematic error between both indices, with a level of statistical significance of $P < .05$.[2] To evaluate the clinical impact of the possible differences observed between the traditional RPI and the RPI/A, the patients were classified as having "regenerative anemia," with an RPI of more than 3, or "arregenerative anemia," with an RPI of less than 2. The proportion of patients in each category was compared using the χ^2 test, with a level of statistical significance of $P < .05$.

For the statistical analysis, MS-Excel 2013 (Microsoft) and Analyze-it (Analyze-it Software) were used.

The study was conducted according to the standards of good practice in clinical research established by our institution. As this is a study in which patients were not accessed for clinical information, the request for informed consent was not considered necessary.

Results

Information was collected from a total of 10,724 validated examinations of patients between 0 days and 17 years, 11 months and 29 days of life from January 1, 2016, to December 31, 2018. Of these, 5,503 examinations met the criterion of anemia for the patient's age.[24] The number of

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Table 3
Passing-Bablok Analysis to Compare the Results of the RPI and the RPI/A

Age	Parameter	Estimated	95% Limits of Agreement	CUSUM
1-3 days	Intercept	–7.129 E-17	–0.022 to 1.645 E-16	0.619
	Slope	0.6027	0.603 to 0.643[a]	
4-13 days	Intercept	0.02059	–0.089 to 0.0206	0.686
	Slope	0.6618	0.662 to 0.882[a]	
14-29 days	Intercept	–2.533 E-17	–2.533 E-17 to 1.527 E-16	0.999
	Slope	1.047	1.047 to 1.047[a]	
1-2 months	Intercept	5.811 E-17	–1.130 E-16 to 5.811 E-17	0.999
	Slope	1.714	1.714 to 1.714[a]	
2-6 months	Intercept	–1.232 E-16	–1.232 E-16 to –1.232 E-16[a]	0.999
	Slope	1.714	1.714 to 1.714[a]	
6 months to 2 years	Intercept	–4.228 E-18	–1.202 E-16 to –4.228 E-18[a]	0.999
	Slope	1.667	1.667 to 1.667[a]	
2-6 years	Intercept	–7.893 E-17	–6.019 E-16 to –7.893 E-17[a]	0.999
	Slope	1.622	1.622 to 1.622[a]	
6-12 years	Intercept	–2.190 E-17	–2.190 E-17 to 5.551 E-17	0.999
	Slope	1.125	1.125 to 1.125[a]	
12-18 years (females)	Intercept	–1.372 E-17	–1.372 E-17 to –1.372 E-17[a]	0.999
	Slope	1.098	1.098 to 1.098[a]	
12-18 years (males)	Intercept	–3.204 E-17	–3.204 E-17 to –3.204 E-17[a]	0.999
	Slope	1.047	1.047 to 1.047[a]	

CUSUM, cumulative sum; RPI, reticulocyte production index; RPI/A, reticulocyte production index by age.
[a]Level of statistical significance: $P < .05$.

samples for each age group and the descriptive statistics for the RPI and RPI/A can be seen in Table 2. While the average values of the RPI/A are significantly higher between 1 month and 12 years of life, this only serves a descriptive purpose since this statistical test compares the means of the indices and therefore their dispersion but not the agreement between the two indices.

To compare the indices, the nonparametric Passing-Bablok regression model was used with a confidence interval of 95% to highlight the significant differences between them Table 3. The results of each group demonstrated a linear relationship with the CUSUM model, which validates the inferences obtained from this regression. In the groups younger than 2 months of age and between 6 and 12 years of age, the 95% agreement limits for the intercept of the obtained equations included 0, which means that there was no significant systematic error between the two indices; in the remaining groups, although the intercept did not include 0, its values tended toward 0, which represents an imperceptible systematic error. In contrast, the 95% limits of agreement for the slope did not include 1 in any of the equations obtained, and the confidence intervals were almost equal to the slope, which reflects significant proportional differences between both indices and shows lower RPI/A values for children younger than 15 days and higher values from 15 days of age onward.

To analyze the agreement from a clinical perspective, the difference in results was compared using the Bland-Altman analysis. In each age group, it was determined that the confidence limits for each observed mean difference did not include 0, which suggests that these indices are not concordant at any age Figure 1. The regression line calculated for the mean differences in each group demonstrated a nonconstant proportional systematic bias, with the differences becoming larger as the magnitude of the measured variable increased Table 4.

To evaluate whether the differences observed between the RPI and RPI/A values had a clinical impact on the diagnosis of anemia, the results were classified as "regenerative anemia" with an RPI of more than 3 and "arregenerative anemia" with an RPI of less than 2. The trend demonstrated in previous models was also observed in this model, which resulted in a greater number of arregenerative anemias with RPI/A in those younger than 15 days of life and a greater number of regenerative anemias in those older than 15 days of life; furthermore, there was a significant difference between males from 1 month to 12 years and females from 1 month to 18 years of age Table 5.

Discussion

Since 1937, when Krumbhaar[29] described for the first time that the number of circulating reticulocytes varied according to "the intensity of the demand and the capacity of the bone marrow to respond," an attempt has been made to understand the reticulocyte maturation process to determine its usefulness in the study of anemia.[4] In this scenario, Hillman[16] in 1969 published a study that

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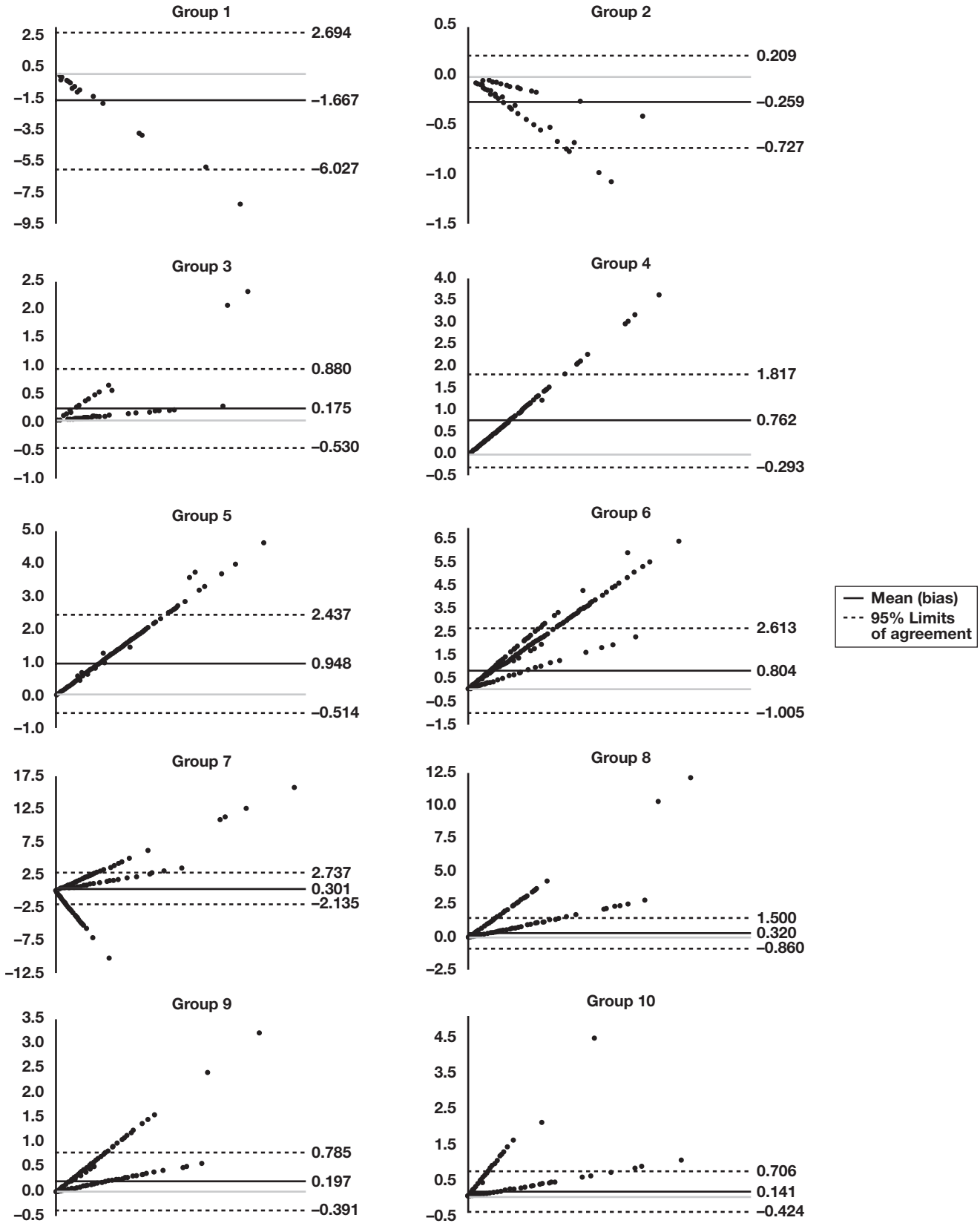

Figure 1 Bland-Altman analysis to compare the results of the reticulocyte production index (RPI) and the RPI by age (RPI/A).

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Table 4
Bland-Altman Linear Regression Calculation to Compare the Results of the RPI and the RPI/A

Age	Parameter	Mean ± SD (95% CI)	Standard Error	*P* Value
1-3 days	Mean difference	–1.67 ± 2.22[a] (–2.74 to –0.59)	0.51	—
	Slope	–0.473 (–0.493 to –0.453)	0.010	<.0001[a]
4-13 days	Mean difference	–0.26 ± 0.24[a] (–0.33 to –0.19)	0.03	—
	Slope	–0.292 (–0.356 to –0.229)	0.032	<.0001[a]
14-29 days	Mean difference	0.18 ± 0.36[a] (0.09 to 0.26)	0.04	—
	Slope	0.185 (0.140 to 0.230)	0.023	<.0001[a]
1-2 months	Mean difference	0.76 ± 0.01[a] (0.69 to 0.84)	0.04	—
	Slope	0.526 (0.520 to 0.528)	0.001	<.0001[a]
2-6 months	Mean difference	0.95 ± 0.76[a] (0.86 to 1.03)	0.04	—
	Slope	0.534 (0.530 to 0.539)	0.002	<.0001[a]
6 months to 2 years	Mean difference	0.80 ± 0.92[a] (0.74 to 0.86)	0.03	—
	Slope	0.478 (0.470 to 0.487)	0.004	<.0001[a]
2-6 years	Mean difference	0.30 ± 1.24[a] (0.23 to 0.37)	0.04	—
	Slope	0.384 (0.363 to 0.405)	0.011	<.0001[a]
6-12 years	Mean difference	0.32 ± 0.60[a] (0.29 to 0.35)	0.01	—
	Slope	0.222 (0.215 to 0.230)	0.004	<.0001[a]
12-18 years (females)	Mean difference	0.20 ± 0.30[a] (0.17 to 0.23)	0.01	—
	Slope	0.204 (0.186 to 0.222)	0.009	<.0001[a]
12-18 years (males)	Mean difference	0.14 ± 0.29[a] (0.12 to 0.16)	0.01	—
	Slope	0.069 (0.061 to 0.077)	0.004	<.0001[a]

CI, confidence interval; RPI, reticulocyte production index; RPI/A, reticulocyte production index by age.
[a]Level of statistical significance of $P < .05$.

Table 5
Proportion of Regenerative and Regenerative Anemia According to the RPI and the RPI/A

		RPI, %			RPI/A, %			
Age	No.	<2	2-3	>3	<2	2-3	>3	*P*
1-3 days	19	52.6	15.8	31.6	68.4	5.3	26.3	.4766
4-13 days	53	86.8	9.4	3.8	96.2	3.8	0.0	.4095
14-29 days	76	86.8	6.6	6.6	84.2	5.3	10.5	.6590
1-2 months	191	91.6	5.8	2.6	68.1	20.4	11.5	<.0001[a]
2-6 months	304	80.6	11.5	7.9	60.2	17.1	22.7	<.0001[a]
6 months to 2 years	911	83.0	6.8	10.2	71.7	10.2	18.1	<.0001[a]
2-6 years	1,174	82.9	8.9	8.2	77.4	7.2	15.4	<.0001[a]
6-12 years	1,703	84.7	8.0	7.3	78.7	9.1	12.2	<.0001[a]
12-18 years (females)	451	84.9	10.9	4.2	79.4	12.9	7.8	.0420[a]
12-18 years (males)	621	75.4	12.7	11.9	71.5	15.0	13.5	.3006

RPI, reticulocyte production index; RPI/A, reticulocyte production index by age.
[a]Significant difference.

evaluated the production of reticulocytes in adults with healthy bone marrow against the different degrees of anemia and found that a constant volume was maintained. With the decrease in hematocrit, they demonstrated an inverse linear relationship between the iron transit time in the bone marrow and the increase in the magnitude of anemia; this relationship was associated with the appearance of large polychromatic reticulocytes in the circulation. This suggests the premature release of reticulocytes from the bone marrow when they are less mature and therefore have not reached their full functionality; their maturation period is displaced toward the periphery, which increases the time they spend in circulation.[16,18] This increased number of circulating reticulocytes comprises a heterogeneous population of cells at different maturation stages; therefore, considering only their number would not provide adequate information about the actual compensatory capacity of normal bone marrow.[8,15,16] On the basis of these findings, the researchers determined the maturation time of circulating reticulocytes for various levels of anemia, proposing that reticulocyte counts should be corrected for a normal hematocrit and for maturation time according to the extent of anemia, thus creating the reticulocyte production index.[16,18]

The objective of this study was to evaluate the application of the RPI in children. For this, a theoretical model

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of the RPI/A was created based on the projection of the model described by Hillman,[16] using normal hematologic values for children of different ages. The results obtained showed that although there was no significant systematic error between the two indices, there was no agreement, and significant proportional differences occurred between the two indices at all ages. From a clinical point of view, these differences had an impact on the diagnosis of the type of anemia, producing up to 24% disagreement in the classification of arregenerative anemia and up to 15% disagreement regarding regenerative anemia, especially in groups with lower normal hematocrit levels (between 1 and 6 months of age).

The difference between the two indices lies in the normal hematocrit values for the different pediatric ages. The original study in which the RPI was proposed was performed with adult men and used a hematocrit of 45% as the average value for the first correction. The average hematocrit values published for children are higher than this value before 15 days and lower at older ages.[24] This variation in the denominator of the first correction (the corrected reticulocyte count) caused the difference between the two indices, and there was an inverse directionality to the difference between the hematocrits (Table 1). While this mathematical relationship is predictable, an evaluation of this index in the pediatric population has not been published.

The lack of information about the way erythropoiesis behaves in its basal state at different ages, or about its capacity to respond to the presence of anemia and the modification of the maturation time of reticulocytes, makes it difficult to extrapolate the results obtained from a study conducted in adults; therefore, there is no theoretical basis for applying the RPI/A in clinical practice or for developing a predictive model, demonstrating that the RPI is not a reliable tool for the pediatric population.

The strength of this study is that it has a large sample size, which decreases the possibility of random error in the results. Its main bias is derived from the study design, in which the results are extracted from a theoretical model, elaborated on extrapolated reference values from a study conducted in adults. Furthermore, since this study only sought to compare the results between both indices, it was not considered to include clinical information that might have been relevant to understand the etiology of the anemia and if the results really corresponded to the response capacity of bone marrow, which would have conferred a clinical context to these theoretical results.

The possibility of replicating the study by Hillman[16] in children is very unlikely given that performing it required daily phlebotomies to maintain a stable hematocrit for 3 to 5 weeks, the administration of colloids to maintain a constant blood volume, the administration of radioisotopes to evaluate the transit of iron in the bone marrow and the life of the erythrocytes, the collection of blood samples twice a day for several days, and the application of a very subjective system for classifying the degree of maturation of the reticulocytes.[16,30]

An alternative for adjusting the reticulocyte count to the extent of anemia in children is the automated analysis of reticulocytes and their maturation parameters, which can reliably predict the effectiveness of erythropoiesis earlier than conventional parameters can.[4,31,32] Among these analyses, the immature reticulocyte fraction has demonstrated clinical utility for monitoring the treatment of neonatal anemia; managing the treatment of nutritional anemias in kidney transplant patients; detecting occult bleeding, hemolysis, and aplastic crisis in hemolytic anemia; and diagnosing and monitoring aplastic anemias.[2,4,31,33]

Conclusion

For pediatric patients, the RPI is not an adequate tool for evaluating the response of the bone marrow in the presence of anemia due to differences in hematologic values between children and adults and the absence of information on the maturation time of reticulocytes in children.

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References

1. World Health Organization and International Council for Standardization in Haematology. *ICSH Guidelines for Reticulocyte Counting by Microscopy on Supravitally Stained Preparations*. Geneva, Switzerland: World Health Organization; 1992.
2. National Committee for Clinical Laboratory Standards. *Methods for Reticulocyte Counting (Flow Cytometry and Supravital Dyes)*. Approved Guideline. H44-A. Wayne, PA: NCCLS; 1997.
3. Krumbhaar EB, Chanutin A. Studies on experimental plethora in dogs and rabbits. *J Exp Med.* 1922;35:847-871.
4. Piva E, Brugnara C, Spolaore F, et al. Clinical utility of reticulocyte parameters. *Clin Lab Med.* 2015;35:133-163.
5. Baldini M, Pannacciulli I. The maturation rate of reticulocytes. *Blood.* 1960;15:614-629.
6. Stryckmans PA, Cronkite EP, Giacomelli G, et al. The maturation and fate of reticulocytes after in vitro labeling with tritiated amino acids. *Blood.* 1968;31:33-43.

DOI: 10.1093/ajcp/aqaa020

7. Skadberg O, Brun A, Sandberg S. Human reticulocytes isolated from peripheral blood: maturation time and hemoglobin synthesis. *Lab Hematol.* 2003;9:198-206.
8. Koury MJ, Koury ST, Kopsombut P, et al. In vitro maturation of nascent reticulocytes to erythrocytes. *Blood.* 2005;105:2168-2174.
9. McKenzie SB. Introduction to anemia. In: McKenzie SB, ed. *Clinical Laboratory Hematology*. Upper Saddle River, NJ: Pearson/Prentice Hall; 2004:161-188.
10. Ney PA. Normal and disordered reticulocyte maturation. *Curr Opin Hematol.* 2011;18:152-157.
11. Wilson MC, Trakarnsanga K, Heesom KJ, et al. Comparison of the proteome of adult and cord erythroid cells, and changes in the proteome following reticulocyte maturation. *Mol Cell Proteomics.* 2016;15:1938-1946.
12. Ovchynnikova E, Aglialoro F, von Lindern M, et al. The shape shifting story of reticulocyte maturation. *Front Physiol.* 2018;9:829.
13. Hillman RS, Finch CA. Erythropoiesis. *N Engl J Med.* 1971;285:99-101.
14. Coulombel L, Tchernia G, Mohandas N. Human reticulocyte maturation and its relevance to erythropoietic stress. *J Lab Clin Med.* 1979;94:467-474.
15. Finch CA. Erythropoiesis, erythropoietin, and iron. *Blood.* 1982;60:1241-1246.
16. Hillman RS. Characteristics of marrow production and reticulocyte maturation in normal man in response to anemia. *J Clin Invest.* 1969;48:443-453.
17. Hamstra RD, Block MH. Erythropoiesis in response to blood loss in man. *J Appl Physiol.* 1969;27:503-507.
18. Hillman RS, Finch CA. The misused reticulocyte. *Br J Haematol.* 1969;17:313-315.
19. Cline MJ, Berlin NI. The reticulocyte count as an indicator of the rate of erythropoiesis. *Am J Clin Pathol.* 1963;39:121-128.
20. Secic D, Omerbasic A, Drljo M, Dizdarevic A. New simplified formula for RPI reticulocyte production index. *HealthMED.* 2011;5:1815-1820.
21. Brecher G. New methylene blue as a reticulocyte stain. *Am J Clin Pathol.* 1949;19:895.
22. Riley RS, Ben-Ezra JM, Goel R, et al. Reticulocytes and reticulocyte enumeration. *J Clin Lab Anal.* 2001;15:267-294.
23. Hillman RS, Finch CA. The detection of anemia. In: Hillman RS, Finch CA, eds. *Red Cell Manual.* 7th ed. Philadelphia, PA: F. A. Davis; 1996:39-69.
24. Brugnara C, Oski FA, Nathan DG. Diagnostic approach to the anemic patient. In: Orkin SH, Nathan DG, Ginsburg D, et al, eds. *Nathan and Oski's Hematology of Infancy and Childhood.* 7th ed. Philadelphia, PA: Saunders Elsevier; 2009:455-466.
25. Koepke JF, Koepke JA. Reticulocytes. *Clin Lab Haemat.* 1986;8:169-179.
26. Gilmer PR Jr, Koepke JA. The reticulocyte: an approach to definition. *Am J Clin Pathol.* 1976;66:262-267.
27. Bessman JD. Reticulocytes. In: Walker HK, Hall WD, Hurst JW, eds. *Clinical Methods: The History, Physical, and Laboratory Examinations.* 3rd ed. Boston, MA: Butterworth-Heinemann; 1990:735-738.
28. Twomey PJ, Kroll MH. How to use linear regression and correlation in quantitative method comparison studies. *Int J Clin Pract.* 2008;62:529-538.
29. Krumbhaar EB. Modern concepts of anemia from the clinical standpoint. *Bull N Y Acad Med.* 1937;13:501-511.
30. Heilmeyer L, Westharer R. Reifungsstadien an uberlebenden reticulocyte in vitro und ihre bedentung fur die schatzung der taglichen hamoglobin produktion in vivo. *Ztsch Klein Med.* 1932;121:361-369.
31. Böck A, Herkner KR. Reticulocyte maturity pattern analysis as a predictive marker of erythropoiesis in paediatrics, part I: evaluation of age-dependent reference values. *Clin Lab Haematol.* 1994;16:247-251.
32. Buttarello M. Laboratory diagnosis of anemia: are the old and new red cell parameters useful in classification and treatment, how? *Int J Lab Hematol.* 2016;38(suppl 1):123-132.
33. Chesney A, Good D, Reis M. Clinical utility of flow cytometry in the study of erythropoiesis and nonclonal red cell disorders. *Methods Cell Biol.* 2011;103:311-332.

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