

Iron Deficiency Anemia, β -Thalassemia Minor, and Anemia of Chronic Disease

A Morphologic Reappraisal

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Abstract

We observed increased numbers of an infrequently referenced poikilocyte, the prekeratocyte, in iron deficiency anemia (IDA) compared with β -thalassemia minor and anemia of chronic disease (ACD) and, therefore, chose to quantify these cells and other morphologic features in these anemias. Prekeratocytes were observed in 31 (78%) of 40 IDAs vs 11 (37%) of 30 β -thalassemias ($P = .001$) and 5 (13%) of 40 ACDs ($P < .001$) and averaged 0.78 per 1,000 RBCs in IDA vs 0.21 in β -thalassemia ($P < .001$) and 0.075 in ACD ($P < .001$). Pencil cells also were more commonly seen and more numerous in IDAs than in β -thalassemia or ACD. Target cells were present in most IDAs and thalassemia and in similar numbers. Basophilic stippling was seen in only 5 (17%) of the β -thalassemias. Our results lend quantitative support to prekeratocytes and pencil cells as morphologic features favoring the diagnosis of IDA but fail to support the diagnostic usefulness of target cells and basophilic stippling in discriminating IDA and β -thalassemia minor.

The peripheral blood morphologic findings in various types of anemias are the subject of abundant anecdotal description but few supportive quantitative data. This limits the application of morphologic data to differential diagnosis. A commonly encountered differential is that of microcytic anemia, which, for practical purposes, is limited to iron deficiency anemia (IDA), thalassemia, and a minority of cases of anemia of chronic disease (ACD). Morphologic features commonly cited as favoring one diagnosis over the others include the presence of pencil cells in IDA and target cells and basophilic stippling in β -thalassemia minor.¹⁻⁵

In addition to these classic morphologic findings, we have observed in smears of IDA increased numbers of rarely described poikilocytes known as prekeratocytes. These are characterized by distinct, sharp-edged, submembranous vacuoles and intact central pallor³ **Image 1**. They were originally described by Bell⁶ as precursor forms in the sequence of spiculated red cell (burr cell) formation. Prekeratocytes are observed in microangiopathic hemolytic anemias and, as the name implies, may give rise to keratocytes, a poikilocyte morphologically similar to the helmet cell.^{3,7} They have often been inappropriately referred to as “blister cells.” True blister cells, described in sickle cell disease, show compartmentalization of their hemoglobin to one side, the remainder of the cell outlined by a thin, hemoglobin-free membrane³ (Image 1, inset). Although these cells are similar to the “eccentrocytes” or “ghost cells” of severe oxidant hemolysis, they are readily identified by context.

Because of the lack of quantitative data supporting classic morphologic descriptions of the microcytic anemias, we endeavored to test the discriminatory power of prekeratocytes and other morphologic features in the differential diagnosis of IDA, β -thalassemia minor, and ACD.

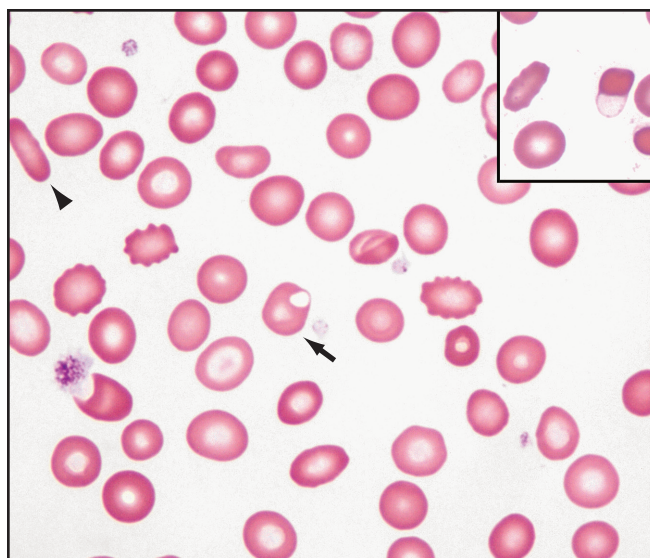


Image 1 Prekeratocyte (arrow) and pencil cell (arrowhead) in iron deficiency anemia (Wright-Giemsa, $\times 1,000$). Inset, Blister cell in sickle cell disease (Wright-Giemsa, $\times 1,000$).

Materials and Methods

We selected 40 IDA, 30 β -thalassemia minor, and 40 ACD cases from the hematology laboratory of Dynacare Laboratories, Froedtert Hospital/Medical College of Wisconsin, Milwaukee. IDA was defined as a microcytic anemia (mean corpuscular volume [MCV], $<80 \mu\text{m}^3$ [80 fL]; hemoglobin concentration, $<12.0 \text{ g/dL}$ [120 g/L] for females and $<13.0 \text{ g/dL}$ [130 g/L] for males) in patients with a concurrent serum ferritin level of $32 \mu\text{g/L}$ (71 pmol/L) or less⁸ or a previously established diagnosis of iron deficiency, using the same diagnostic criteria. β -Thalassemia was defined as microcytosis with 3.5% hemoglobin A₂ or more.⁹ ACD was defined by anemia (hemoglobin $<12.0 \text{ g/dL}$ [120 g/L] for females and $<13.0 \text{ g/dL}$ [130 g/L] for males) in patients with chronic illness with a serum ferritin level of 240 ng/mL (548 pmol/L) or more and a serum iron level of $80 \mu\text{g/dL}$ (14.3

$\mu\text{mol/L}$) or less or a total iron binding capacity of $310 \mu\text{g/dL}$ (55.5 $\mu\text{mol/L}$) or less, or a bone marrow examination showing markedly increased storage iron and fewer than 20% erythroid precursors containing sideroblastic iron.¹

Peripheral venous blood samples were collected in EDTA-anticoagulated tubes, and blood smears were prepared with a Wright-Giemsa stain. CBC count data were obtained from an automated hematology analyzer (Advia 2120, Siemens Medical Solutions Diagnostics, Tarrytown, NY). Prekeratocytes, pencil cells, target cells, and RBCs with basophilic stippling were enumerated in 20 $\times 50$ oil immersion fields, using a Miller disk. Morphologic features were assessed in a blinded manner, without knowledge of the iron studies or hemoglobin electrophoretic data. Prekeratocytes, pencil cells, target cells, and RBCs with basophilic stippling per 1,000 RBCs were calculated. Categorical data were analyzed using a χ^2 or Fisher exact test, and quantitative data were compared using a Mann-Whitney *U* test. This study was approved by the Medical College of Wisconsin Institutional Review Board.

Prekeratocytes were defined as RBCs with 1 or more sharp-edged, submembranous vacuoles and central pallor (Image 1, arrow). Pencil cells were defined as elongated, hypochromic RBCs, in which the long axis was more than 3 times the length of the short axis (Image 1, arrowhead).¹⁰ Target cells were defined as RBCs with a central hemoglobinized area surrounded by an area of pallor. Coarse basophilic stippling was defined as easily identified, uniformly distributed basophilic inclusions.

Results

Complete RBC parameters were available for 34 of 40 IDA, 18 of 30 β -thalassemia, and 37 of 40 ACD cases. Average values and ranges for all cases are shown in **Table 1**.

Prekeratocytes were observed in the 20 $\times 50$ fields examined in 31 (78%) of 40 IDAs vs 11 (37%) of 30 β -thalassemias ($P = .001$) and 5 (13%) of 40 ACDs ($P < .001$). Mean numbers of prekeratocytes per 1,000 RBCs were higher in IDA than

Table 1
RBC Parameters*

	IDA	ACD	β -Thalassemia
RBC count ($\times 10^6/\mu\text{L}$)	2.9 (1.5-5.8)	3.3 (2-4.4)	5.4 (4.3-6.7)
Hemoglobin (g/dL)	7.6 (2.7-11.8)	9.5 (5.8-11.6)	11.5 (9.4-14.5)
Hematocrit (%)	26 (9.2-40)	29.2 (17-36)	35.7 (29.8-44)
MCV (μm^3)	65.9 (52-79)	90.2 (71-114)	66.6 (55-85)
MCH (pg/cell)	18.9 (13-25)	29.5 (22-36)	21.6 (16-26)
MCHC (g/dL)	28.5 (24-32)	32.6 (31-36)	32.2 (29-34)
RDW (%)	20.6 (16.7-31.2)	17.1 (13.4-24)	16.3 (13.8-22.1)

ACD, anemia of chronic disease; IDA, iron deficiency anemia; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RDW, RBC distribution width.

* Data are given as mean (range). For RDW, the numbers of samples for IDA, ACD, and β -thalassemia were 34, 37, and 18, respectively. Values are given in conventional units. Conversions to Système International are as follows: RBC count ($\times 10^{12}/\text{L}$), multiply by 1.0; hemoglobin concentration (g/L), multiply by 10.0; hematocrit proportion of 1.0, multiply by 0.01; MCV (fL), multiply by 1.0; MCH (pg/cell), multiply by 1.0; MCHC (g/L), multiply by 10.0.

in β -thalassemia ($P < .001$) and ACD ($P < .001$) **Table 2** and **Figure 1**. A cutoff of more than 0.35 prekeratocytes per 1,000 RBCs produced a sensitivity of 67.5% and a specificity of 73% for IDA vs β -thalassemia and a sensitivity and specificity of 67.5% and 97.6%, respectively, for IDA vs ACD. Prekeratocytes per 1,000 RBCs were negatively correlated with the mean corpuscular hemoglobin concentration (MCHC) and MCV in IDA **Table 3** and **Figure 2**. Also, in IDA, a trend toward negative correlation between prekeratocytes per 1,000 RBCs and hemoglobin was observed.

Pencil cells were observed in 28 (70%) of 40 IDAs vs 9 (30%) of 30 β -thalassemias ($P = .002$) and 5 (13%) of 40 ACDs ($P < .001$) and averaged more per 1,000 RBCs in IDA

vs β -thalassemia and ACD (Table 2 and Figure 1). A cutoff of more than 0.5 pencil cells per 1,000 RBCs produced a sensitivity of 65% and specificities of 86.7% and 97.5% for IDA vs β -thalassemia and IDA vs ACD, respectively. Pencil cells per 1,000 RBCs were negatively correlated with MCV (Table 3, Figure 2) and target cells per 1,000 RBCs ($R = -0.409$; $P = .009$) and positively correlated with prekeratocytes per 1,000 RBCs ($R = 0.326$; $P = .040$) in IDA.

Target cells were observed in 38 (95%) of 40 IDAs vs 28 (93%) of 30 β -thalassemias ($P = 1.00$) and 26 (65%) of 40 ACDs ($P < .001$). Mean target cells per 1,000 RBCs were 16.9 in β -thalassemia compared with 12.4 in IDA ($P = .330$) and 1.0 in ACD ($P < .001$) (Table 2 and Figure 1). There was a trend toward

Table 2
Quantitative Morphologic Findings*

	IDA (n = 40)	β -Thalassemia (n = 30)	$P^\dagger$	ACD (n = 40)	$P^\dagger$
Prekeratocytes present [‡]	31 (78)	11 (37)	.001	5 (13)	<.001
Mean prekeratocytes/1,000 RBCs	0.78	0.21	<.001	0.08	<.001
Pencil cells present [‡]	28 (70)	9 (30)	.002	5 (13)	<.001
Mean pencil cells/1,000 RBCs	1.2	0.23	<.001	0.03	<.001
Target cells present [‡]	38 (95)	28 (93)	1.00	26 (65)	<.001
Mean target cells/1,000 RBCs	12.4	16.9	.330	1.0	<.001
Basophilic stippling present [‡]	1 (3)	5 (17)	—	2 (5)	—

ACD, anemia of chronic disease; IDA, iron deficiency anemia.
* Data are given as number (percentage) unless otherwise indicated.
† Compared with IDA.
‡ Observed in 20 $\times$ 50 fields.

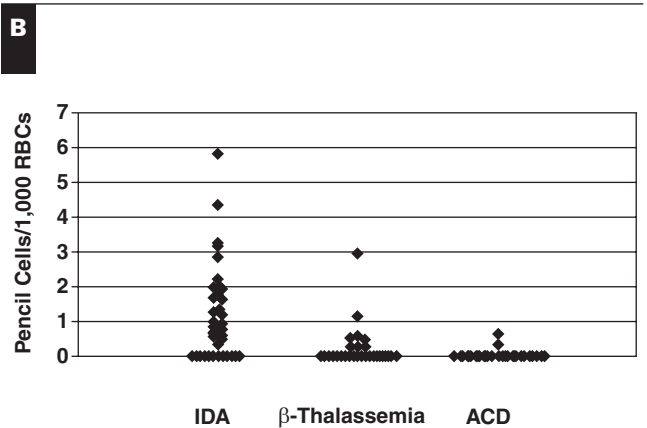
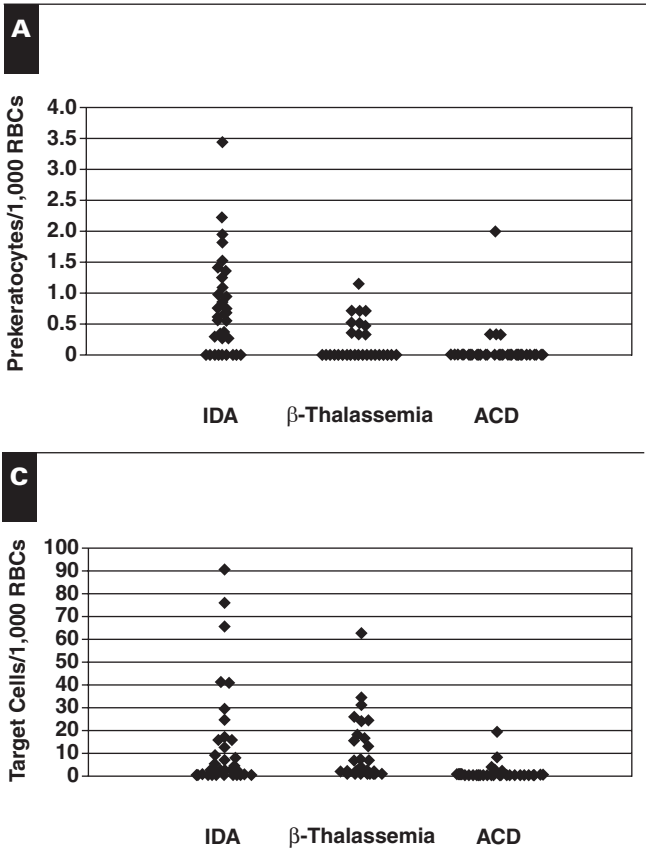
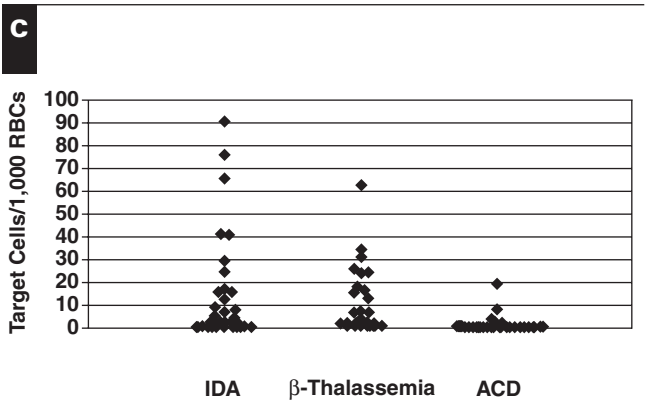


Figure 1 Prekeratocytes (A), pencil cells (B), and target cells (C) per 1,000 RBCs in iron deficiency anemia (IDA) vs β -thalassemia vs anemia of chronic disease (ACD).



a negative correlation between target cells per 1,000 RBCs and MCV ($R = -0.427$; $P = .06$) in β -thalassemia.

Basophilic stippling was seen in 20 $\times 50$ fields in only 5 (17%) of 30 β -thalassemias, 2 (5%) of 40 ACDs, and 1 (3%) of 40 IDAs.

Statistical analysis of the RBC indices identified several relationships. In IDA, the following indices were positively correlated: hemoglobin concentration and MCHC ($R = 0.729$; $P < .001$), hemoglobin concentration and MCV ($R = 0.430$; $P = .006$), and MCHC and MCV ($R = 0.584$; $P < .001$), and a negative correlation was found between RBC distribution width

(RDW) and MCHC ($R = -0.339$; $P = .05$). In addition, the mean MCHC was 28.5 g/dL (285 g/L) in IDA compared with 32.2 g/dL (322 g/L) in thalassemias ($P < .001$) and 32.6 g/dL (326 g/L) in ACD ($P < .001$).

Finally, for comparison with morphologic data, cutoffs using RBC indices were calculated. An MCHC cutoff of 31 g/dL (310 g/L) or less produced a sensitivity of 90% and a specificity of 87% for IDA vs β -thalassemia, while an RDW cutoff of 18 fL or more produced a sensitivity of 82% and a specificity of 89% for IDA vs β -thalassemia. The same MCHC cutoff yielded a sensitivity of 90% and a specificity of 80% for IDA

Table 3
Correlations Between RBC Indices and RBC Morphologic Features in Iron Deficiency Anemia*

	Prekeratocytes		Pencil Cells		Target Cells	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Hemoglobin	-0.295	.064	-0.106	.514	0.218	.176
MCV	-0.476	.002	-0.352	.026	-0.005	.975
MCHC	-0.486	.001	-0.234	.146	-0.064	.696
RDW	0.182	.302	0.014	.939	0.183	.301

MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RDW, RBC distribution width.

* Prekeratocytes, pencil cells, and target cells are given per 1,000 RBCs. *P* values $< .05$ were considered statistically significant.

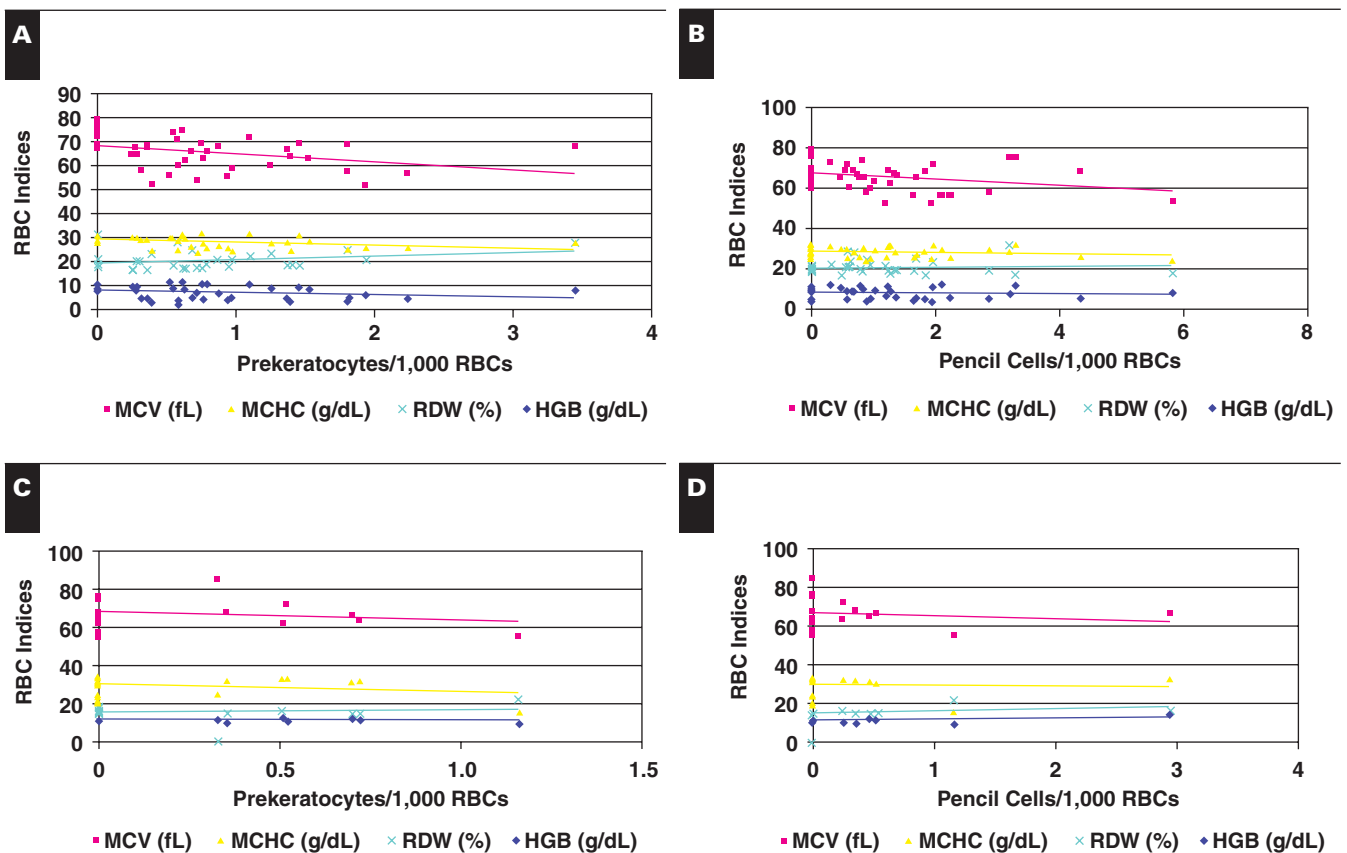


Figure 2 Relationships between RBC indices and prekeratocytes and pencil cells per 1,000 RBCs in iron deficiency anemia (A and B) and β -thalassemia (C and D).

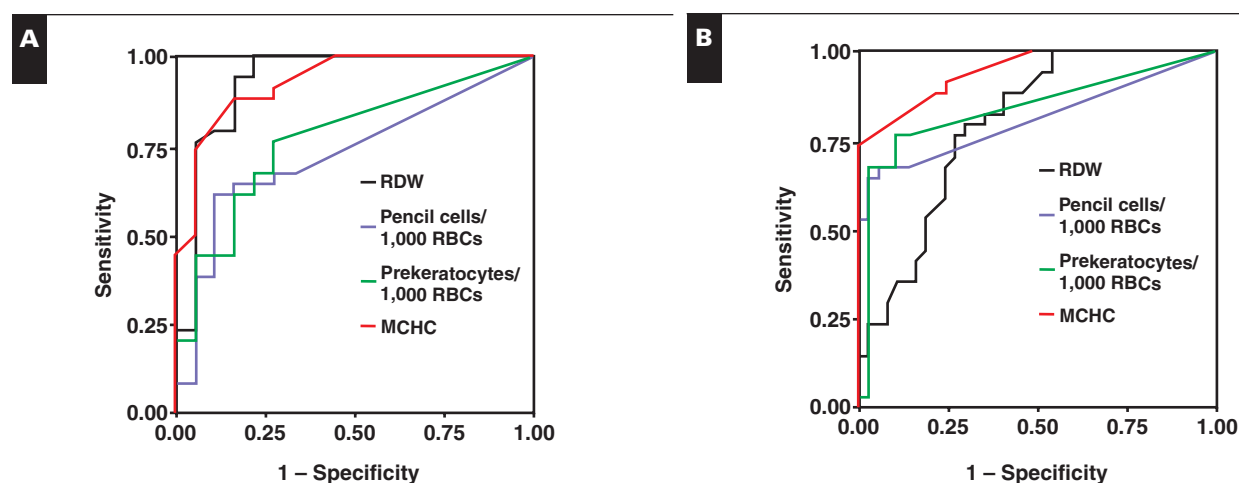


Figure 3 Receiver operating characteristic curves for the discrimination of iron deficiency anemia from thalassemia (**A**) or anemia of chronic disease (**B**), using mean corpuscular hemoglobin concentration (MCHC), RBC distribution width (RDW), prekeratocytes per 1,000 RBCs, and pencil cells per 1,000 RBCs. Areas under the curve were as follows: **A**, RDW, 0.931; MCHC, 0.929; prekeratocytes/1,000 RBCs, 0.770; pencil cells/1,000 RBCs, 0.729; **B**, RDW, 0.795; MCHC, 0.945; prekeratocytes/1,000 RBCs, 0.839; pencil cells/1,000 RBCs, 0.812.

vs ACD, while the RDW cutoff of 18 fL or more yielded a sensitivity and specificity of 82% and 40%, respectively, for IDA vs ACD. Comparisons of the morphologic and RBC indices in discriminating these disorders are illustrated using receiver operating characteristic curves, which reveal areas under the curve of 0.931, 0.929, 0.770, and 0.729 for RDW, MCHC, prekeratocytes per 1,000 RBCs, and pencil cells per 1,000 RBCs, respectively, when comparing IDA with β -thalassemia **Figure 3A** and 0.795, 0.812, 0.839, and 0.945 for RDW, pencil cells per 1,000 RBCs, prekeratocytes per 1,000 RBCs, and MCHC, respectively, when comparing IDA with ACD **Figure 3B**.

Discussion

The relative contribution of blood smear review to the diagnosis of various types of anemia remains controversial.^{11,12} Nevertheless, analysis of blood cell morphologic features remains a widespread practice. Instead of being evidence-based, however, the morphologic rules used in the differential diagnosis of anemias are anecdotally derived and canonically perpetuated in innumerable textbooks of hematology. The present study was originally initiated based on an experiential observation that an unusual poikilocyte known as the prekeratocyte seemed confined to blood smears from patients with IDA, a finding that was previously not described, to our knowledge, in the medical literature. A quantitative morphologic analysis was undertaken to confirm this observation and to evaluate the validity of other commonly described morphologic features of IDA, β -thalassemia minor, and ACD. We restricted our analysis to easily quantifiable morphologic features: pencil cells, target

cells, and basophilic stippling. The first is a commonly cited feature of IDA, and the second and third are classically described features of β -thalassemia minor.

In line with prior descriptions, we found that pencil cells were observed more often and in greater numbers in cases of IDA than in thalassemia or ACD. However, they were not uniformly present in iron deficiency when 20 $\times$ 50 fields were assessed (absent in about one third of cases) and were seen in almost 30% of thalassemias. A cutoff of 0.5 or more pencil cells per 1,000 RBCs (roughly >1 pencil cell per 10 $\times$ 50 fields) produced sensitivity and specificity of 65% and 86.7%, respectively, for IDA vs β -thalassemia.

In contrast, 2 morphologic features, generally considered characteristic of thalassemia minor, were not found to be useful in the distinction of thalassemia from IDA. Target cells were present in most cases of thalassemia and IDA, with similar average numbers in each disorder. Basophilic stippling, which was seen in only 1 case of IDA, was surprisingly only present in 17% of thalassemias, limiting its diagnostic usefulness. Target cells were present in the majority of cases of ACD, but in far fewer numbers than in IDA or thalassemia. Thus, frequent target cells could be useful in ruling out ACD.

Prekeratocytes have rarely been mentioned in the hematology literature and their distribution in various anemic states poorly described. Anecdotally, they have been mentioned as features of microangiopathic hemolytic anemias and renal failure.⁶ In the present study, we confirmed our observation that they were characteristic of IDA, finding them in 78% of cases. While they were also present in 37% of β -thalassemias and 13% of ACDs, they were found in far fewer numbers in these disorders. Consequently, a cutoff of more than 0.35 prekeratocytes per

1,000 RBCs (corresponding roughly to >1 prekeratocyte per 20 ×50 fields) achieved a sensitivity of 67.5% and a specificity of 73% for IDA vs β -thalassemia, while the same morphologic cut-off yielded a sensitivity and a specificity of 67.5% and 97.5%, respectively, for IDA vs ACD.

With respect to the application of these results to practical differential diagnosis, several points must be made. First, the characteristics of our cohorts limit the general applicability of our data. Specifically, the patients with IDA were more anemic on average than patients with thalassemia or ACD, and, thus, the IDA cases are not representative of the cases most likely to cause differential diagnostic problems (ie, mild IDA).^{11,12} This is particularly relevant given our observation that prekeratocytes and pencil cells increase in number in IDA with decreasing MCHC and MCV, respectively, both indices well known to decrease in more severe cases. Similarly, only 13% of ACD cases were microcytic. While this is representative of ACD in general,² it is perhaps not representative of the ACD cases most likely to be confused with IDA or thalassemia.

Second, when comparing the RBC indices and morphologic cutoffs, the cutoffs of 31 g/dL (310 g/L) for MCHC and 18 fL for RDW achieved a higher discriminatory power than either of the cutoffs for prekeratocytes or pencil cells in differentiating IDA from β -thalassemia or ACD. The superiority of MCHC over morphologic parameters in differentiating IDA from the other anemias is illustrated in that the receiver operating characteristic curves found no significant improvement in discriminatory power when combinations of morphologic parameters and RBC indices were attempted (data not shown). Consequently, we do not advocate a major role for poikilocyte enumeration in the diagnosis of microcytic anemia. However, these morphologic findings may help in some circumstances to support or undermine a diagnostic impression based on other clinical and laboratory data, helping to guide targeted laboratory testing for definitive diagnosis.

Finally, it must be noted that the RBC indices data collected in this study are likely to be unique to Advia technology because measurement of these parameters is not uniform across hematology analyzers; thus our results may not be strictly generalizable.

Only 1 quantitative morphologic anemia study comparing the morphologic features of RBCs, RBC indices, and serum iron studies in IDA has been published.¹⁰ Rodgers et al¹⁰ reported inverse relationships between the numbers of 2 poikilocytes—elliptocytes and tailed poikilocytes (dacryocytes)—and several RBC indices, including hemoglobin, hematocrit, and RBC concentration. They reported increased numbers of these poikilocytes as anemia worsened. Additional findings in that study were negative correlations between the percentage of elliptocytes and mean corpuscular hemoglobin and the percentage of target cells and MCV, while a positive correlation was identified between the percentage of

tailed poikilocytes and RDW. Statistically significant relationships were not observed for the numbers of pencil cells, schistocytes, spherocytes, or echinocytes compared with RBC indices and iron studies. Because these authors limited assessment to only IDA cases, the application of these morphologic data to differential diagnosis was not investigated.

We have confirmed anecdotal observations of the association of pencil cells with IDA, as compared with thalassemia and ACD. In addition, we have documented that increased prekeratocytes are also preferentially seen in IDA, a finding that has not been previously reported. Finally, our data fail to support the commonly held belief that target cells and basophilic stippling are helpful morphologic features in the identification of β -thalassemia minor. These results may be of use in some circumstances to further guide the laboratory workup of microcytic anemias.

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