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ORIGINAL ARTICLE

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A bone marrow report of absent stainable iron is not diagnostic of iron deficiency

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Abstract The absence of stainable iron in a bone marrow aspirate is widely considered to be diagnostic of iron deficiency anemia (IDA). We re-evaluated this concept by studying a cohort of 108 consecutive bone marrow specimens from an unselected series of patients who were seen at a hematology clinic and in whom iron stores were reported as being absent. A review of the pathologic reports revealed 19 inadequate specimens and 15 with decreased, but not absent, iron stores. Thus, only 74 of the 108 cases had been accurately reported. In 37 of these cases, adequate clinical and laboratory data were available and allowed further analysis. In 18 patients, careful review of patient history, physical examination, results of endoscopic procedures, and follow-up information failed to suggest the presence of IDA (group A). The review process in the remaining 19 patients suggested the possibility of IDA (group B). Significant differences between groups A and B were observed in serum ferritin (P=0.001) and red blood cell mean corpuscular volume (P=0.004). In contrast, the two groups did not differ significantly in hemoglobin concentration, serum iron, total iron-binding capacity, transferrin saturation, or erythrocyte sedimentation rate. These observations suggest that a pathology report of absent bone marrow hemosiderin may be inaccurate in more than 30% of cases and, even when accurate, may not necessarily signify the presence of IDA. Measurement of the serum ferritin level is needed to confirm a clinical diagnosis.

Keywords Iron deficiency anemia · Bone marrow · Ferritin · Hemosiderin · Iron

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Introduction

Potassium ferrocyanide (Prussian blue) stain is applied to tissue samples in order to visualize microscopically nonheme iron in cells [24]. Accordingly, the examination of Prussian-blue-stained bone marrow aspirate for the presence or absence of histiocytic iron granules has been considered the "gold standard" in evaluating iron-depleted states [1, 8]. However, very few studies have addressed the accuracy of the test, and the value of other tests has mostly been compared against this "standard." Therefore, the derived sensitivity and specificity of the alternative tests lie in their predicting the presence or absence of stainable bone marrow iron rather than in their detecting true iron deficiency [11, 19]. Furthermore, absent stainable iron in a bone marrow aspirate may not be an accurate indicator of marrow iron stores, since significantly different amounts may be demonstrated in the corresponding needle-biopsy specimen [6, 14].

Other methods of assessing body iron stores include the measurement of serum ferritin, serum iron, total iron binding capacity (TIBC), and percent transferrin saturation [9, 18]. Less frequently, iron deficiency may be assessed by measuring soluble transferrin receptor [17, 19, 20] and erythrocyte protoporphyrin concentration [13, 15, 16, 21]. In addition, the possibility of iron deficiency is suggested by a low red blood cell mean corpuscular volume [12], an increased red cell distribution width [23], and a blood smear showing anisocytosis, poikilocytosis, elliptocytosis, and hypochromasia. However, the diagnostic sensitivity and specificity of each of the above noninvasive tests, with the exception of serum ferritin, are less than optimal [10, 22]. Nevertheless, the combined use of multiple parameters, including serum ferritin, is often adequate in the clinical diagnosis of iron deficiency [4]. However, bone marrow examinations are often performed in a hematology clinic for the investigation of both unexplained anemia and other primary bone marrow disease. In the current study, we examined the accuracy and clinical relevance of a bone marrow report of absent hemosiderin in 108 consecutive cases from our hematology clinic.

Materials and methods

After approval by the institutional review board, we identified a consecutive series of bone marrow studies reporting absent stainable iron. The test specimens were obtained from bone marrow procedures performed at our institution for various indications. Iron stains (Prussian blue) were performed on one slide. For each batch of iron studies examined, a quality control of the reagents was performed on a smear previously read as showing increased iron stores. The slides were fixed with methyl alcohol for 1 min, decanted, and allowed to air-dry. Afterward, they were placed into a Coplin jar containing one part 2% hydrochloric acid to two parts potassium ferrocyanide, followed by incubation in an incubator at 37°C for 20 min. After being washed with distilled water, the slides were placed into a Coplin jar and counterstained with Nuclear Fast Red for 15 min. The slide was then washed with distilled water, fan-dried, and examined by a pathologist. All the test slides in the current study were re-reviewed by one of the authors (J.D.H.).

Patients in whom the absence of bone marrow hemosiderin was confirmed were classified into two groups on the basis of an extensive historical and laboratory review: group A, those who were considered not to have true iron deficiency on the basis of history, physical examination, results of stool blood tests and endoscopic procedures, and follow-up information, and group B, those who were clinically suspected of having iron deficiency. The two groups were compared with regard to their serum ferritin concentrations, serum iron, TIBC, percent transferrin saturation, MCV, and hemoglobin level.

Results

Of the total of 108 consecutive bone marrow specimens that had a report of absent bone marrow hemosiderin and that were re-reviewed, 19 (18%) did not allow an accurate assessment of iron stores and hence were deemed inadequate. An additional 15 specimens (14%) revealed decreased or increased, but not absent, stainable iron and were also excluded from further analysis. Therefore, only 74 specimens (69%) were thought to be accurately reported. Of these, only 37 had adequate laboratory and historical information that allowed the classification of the patients into the aforementioned group A (clinically not consistent with iron deficiency) and group B (clinically consistent with iron deficiency). Group A consisted of 17 patients (median age: 65 years; 12 of them female) and group B of 18 patients (median age: 60 years; 8 of them female). The underlying diseases in both groups of patients are outlined in Table 1. The clinical suspicion of bleeding in the 15 patients from group B was based on a history of gastrointestinal or menstrual bleeding, the identification of a bleeding lesion by upper or lower endoscopy, or a positive stool blood test with documented response to iron-replacement therapy. Of the 18 patients in group B, eight also had co-morbid conditions that may have contributed to their anemia, including rheumatoid arthritis, myelodysplastic syndrome, multiple myeloma, liver disease, and hypersplenism.

Blood measurements of serum ferritin, serum iron, TIBC, percent transferrin saturation, hemoglobin, MCV, and erythrocyte sedimentation rate are outlined in Table 2. Only serum ferritin and MCV values were significantly different between groups A and B (Table 2). In general, only 38% of the patients had serum ferritin values that were less than 20 µg/l, confirming the presence of true IDA. The proportion of patients with similarly

Table 1 Underlying diseases of group A (clinically not suspected of having iron deficiency) and group B (clinically suspected of having iron deficiency) patients with the absence of stainable bone marrow iron

Group A (n=17)	n	Group B (n=18)	n
Lymphoma	3	Bleeding	15
Acute myeloid leukemia	1	Polycythemia vera	1
Acute lymphocytic leukemia	1	Paroxysmal nocturnal hemoglobinuria	1
Multiple myeloma	3	Celiac sprue	1
Systemic mastocytosis	1		
Myeloproliferative disorder	4		
Hemolysis	2		
Inflammatory disorder	2		
Pernicious anemia	1		

Table 2 Blood studies for investigating iron deficiency in 35 patients with the absence of stainable bone marrow iron (values are median and range): comparison of subgroups with (group B) or without (group A) the clinical suspicion of iron deficiency (*iron* serum iron level, *TIBC* total iron-binding capacity, *Hgb* hemoglobin, *MCV* mean red cell corpuscular volume, *ESR* erythrocyte sedimentation rate, *NS* not significant). Normal ranges: ferritin 20–300 µg/l, iron 6–26 µmol/l, TIBC 43–69 µmol/l, transferrin saturation 14%–50%, Hgb 120–175 g/l, MCV 81.2–98.3 fl, ESR 0–29 mm/1 h. These normal values include lowest and highest values in both sexes

	All patients n=35	Group A n=17	Group B n=18	P
Ferritin (µg/l)	29 (2–544)	60 (5–544)	13 (2–96)	0.001
Iron (µmol/l)	5.5 (1.9–11.2)	5.5 (1.9–9.8)	5 (2–11.2)	NS
TIBC (µmol/l)	58 (38–84)	60 (42–71)	55 (38–84)	NS
Transferrin saturation (%)	10 (3–29)	11 (3–15)	7 (3–29)	NS
Hgb (g/l)	106 (68–170)	106 (70–170)	105 (68–153)	NS
MCV (fl)	82 (65–100)	85 (75–100)	78 (65–96)	0.004
ESR (mm in 1 h)	20 (0–139)	12 (0–139)	25 (0–116)	NS

Table 3 Laboratory parameters (with values expressed in mean and range) of 35 patients with "absent" stainable bone marrow iron classified by their serum ferritin concentrations (*Hgb* hemoglobin, *MCV* mean red cell corpuscular volume, *RDW* red cell distribution width, *ESR* erythrocyte sedimentation rate, *Iron* serum iron level, *TIBC* total iron binding capacity, *IDA* iron deficiency anemia)

	Serum ferritin concentration (μg/l)			
	n=13 0–20	*n*=10 >20–50	*n*=6 >50–100	*n*=6 >100
Hgb (g/l)	107 (70–153)	114 (83–150)	122 (79–170)	99 (74–117)
MCV (fl)	78.3 (64.6–96.4)	80.7 (69.5–87.2)	81.9 (73.4–87.6)	93.7 (82.8–103.1)
RDW	17.8 (14.1–21.5)	16.3 (12.0–24.4)	16.7 (12.7–27.8)	16.3 (12.8–20.5)
ESR (mm in 1 hr)	24 (0–56)	31 (2–116)	8.8 (0–20)	74.5 (2–139)
Iron (μmol/l)	4 (1.9–8.9)	6.6 (2–11.2)	6 (5.5–6.5)	5.8 (5.3–6.0)
TIBC (μmol/l)	64 (49–84)	50 (38–64)	61 (60–62)	40 (33–44)
Transferrin saturation (%)	5 (3–16)	12.2 (5–29)	10 (9–11)	14.7 (12–18)
% IDA[a]	11/13 (84.6%)	5/10 (50%)	1/6 (16.7%)	0/6 (0%)

[a] Clinically suspected

low ferritin values, 11% for group A and 65% for group B, validated our clinical impression. Accordingly, the patients were divided into four groups on the basis of serum ferritin levels: 0–20, >20–50, >50–100, and >100 μg/l (Table 3). Not surprisingly, the incidence of clinically suspected cases of IDA was 84.6% in the first group and 0% in the fourth group. Table 3 further illustrates the inadequacy of other laboratory measurements in the diagnosis of IDA. The increase in erythrocyte sedimentation rate, which correlated with the increase in serum ferritin, is consistent with the assertion that the anemia in the group of patients with the higher values of ferritin was related to chronic disease rather than to IDA.

Discussion

Based on our clinical practice in a hematology clinic, we suspected the inaccuracy of bone marrow hemosiderin staining in the assessment for IDA in an unselected group of patients with suspected hematologic disease. In addition to potential inaccuracies attributable to methodology, we discovered that visual inspection for bone marrow hemosiderin by experienced hematopathologists was not always reproducible. In the current study, only 69% of the reports were believed to be accurate on re-review. An interobserver difference of 31% is unacceptably high for a supposed "gold standard," and further consequences in patient convenience and cost may not be trivial. For example, even when another explanation for anemia was apparent, the bone marrow report in 10 patients instigated additional diagnostic workup, including upper and lower endoscopic examinations, which were largely unrevealing.

Of more importance, less than half the patients with "absent" stainable bone marrow iron had clinical evidence that supported the possibility of IDA. The relative accuracy of our clinical impression with regard to the presence or absence of IDA was validated by the demonstrated significant difference between the two groups in serum ferritin and MCV. Furthermore, subclassification of the study population according to serum ferritin concentration revealed seven patients (20%) with values above 100 μg/l, which confirmed the clinical evidence that IDA was doubtful. At the same time, the finding that 85% of the patients with low serum ferritin were believed clinically to have IDA suggests the potential superiority of measuring serum ferritin over assessing bone marrow hemosiderin in detecting IDA in certain circumstances. We also confirmed the inadequacy of other iron studies in this regard.

The laboratory diagnosis of IDA has not been perfected, because a true "gold standard" is lacking. The problem is further complicated by the multifactorial nature of anemia in the elderly and the chronically sick. Many physicians continue to regard a bone marrow iron study as the "gold standard" for the diagnosis of IDA, despite previous observations to the contrary [2, 3, 5, 7]. The current study clearly demonstrates that absent bone marrow hemosiderin is not tantamount to a patient having IDA, especially in the presence of a primary bone marrow disorder such as chronic myeloid disorders. The true incidence of false positivity of "absent" bone marrow iron may not be estimated from this type of study for a variety of reasons, including patient and referral center selection bias. Nonetheless, our observations underscore the value of serum ferritin measurement in complementing and/or confirming the clinical impression based on a pathology report of bone marrow iron studies.

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