Diagnosis of Iron Deficiency Anemia in the Elderly by Transferrin Receptor–Ferritin Index

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Background: The diagnosis of iron deficiency anemia (IDA) in the elderly is difficult because of the prevalence of chronic diseases, which can cause anemia with high ferritin levels, even in the presence of iron deficiency. Therefore, we studied the sensitivity and specificity of a serum transferrin receptor assay, which is not affected by chronic diseases, in the diagnosis of IDA in elderly patients.

Methods: We performed a prospective controlled study of 49 consecutive male and female patients older than 80 years who were admitted to an acute geriatric department. Bone marrow aspirate confirmed IDA in all 49 patients. Fourteen additional patients, also older than 80 years, with anemia but without evidence of iron deficiency on results of bone marrow examination, served as a control group. All patients underwent evaluation by means of a detailed medical history and results of complete physical examination, routine blood tests, and specific tests for diagnosis and evaluation of anemia. Examination of bone marrow aspirate was performed for all patients. Levels of transferrin receptor in serum were determined by means of a specific enzyme-linked immunosorbent assay. The transferrin receptor–ferritin index (TR-F index) was defined as the ratio of serum transferrin receptor level to log ferritin level.

Results: Only 8 patients could be diagnosed as having IDA by means of routine blood test results (serum iron, ferritin, and transferrin saturation levels). In contrast, the TR-F index disclosed IDA in 43 of the 49 patients, thus increasing the sensitivity from 16% to 88%.

Conclusions: The diagnosis of IDA in the elderly by means of routine blood tests has a very low sensitivity. The TR-F index is much more sensitive, and when results are positive, the TR-F index can eliminate the need for bone marrow examination.

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A NEMIA IS a common clinical problem at all ages, especially among the elderly. Its prevalence among adults older than 70 years is about 2%, and this rises to 28% in men 85 years and older. Iron deficiency anemia (IDA) accounts for about half of these cases. In industrialized nations, IDA is rarely due to dietary deficiency. Gastrointestinal tract abnormality can be identified in more than half of elderly patients with IDA, including gastric tumors, colonic polyps, and carcinoma of the colon. Thus, the presence of IDA in the elderly demands investigation of the upper and lower gastrointestinal tract. However, x-ray or gastroscopy/colonoscopy investigation in the elderly involves great inconvenience and hazardous complications. Therefore, an accurate assay for IDA in elderly patients with high specificity and sensitivity is mandatory.

The diagnosis of IDA in the elderly is often difficult owing to the presence of multiple abnormalities. The routine diagnostic tests have a low sensitivity in this group of patients. Serum iron and transferrin saturation levels have a limited value in the diagnosis of IDA in elderly patients. An increased level of free erythrocyte protoporphyrin is a late and nonspecific finding of IDA. Serum ferritin level is considered the best single test for the diagnosis of iron deficiency because its concentration is proportional to total-body iron stores. However, in the elderly, serum ferritin level is not a reliable test, because levels increase with age and because the lower reference range for elderly subjects is not well defined. Moreover, levels of serum ferritin increase in chronic disorders and in malignancy, which are common in the elderly. Thus, in more than half of elderly patients with IDA, serum ferritin level is high or within
PATIENTS AND METHODS

SUBJECTS

We studied 106 consecutive patients older than 80 years who were admitted to the Department of Acute Geriatric Medicine, Kaplan Medical Center, Rehovot, Israel, between January 1, 1995, and December 31, 1997, with a diagnosis of anemia (hemoglobin level, <13.0 g/dL in men and <12.0 g/dL in women). Exclusion criteria included acute gastrointestinal tract bleeding, inactivity or refusal to sign an informed consent, significant vitamin B12 or folic acid deficiency, current iron therapy, known malignancy, and renal or hepatic failure. Sixty-three patients were eligible for the study. All patients underwent a complete medical history and a thorough physical examination on admission, with routine laboratory tests including a complete blood cell count, erythrocyte sedimentation rate, kidney and liver function tests, and serum levels of iron, transferrin, ferritin, vitamin B12, folic acid, C-reactive protein, and TR. Examination of bone marrow aspirate was performed in all patients. All blood samples were drawn before any blood transfusions or iron supplements were given to the patients. All patients agreed to participate in the study by signing an informed consent that was approved by the hospital ethics committee.

DIAGNOSIS OF IDA BY MEANS OF BONE MARROW EXAMINATION

Bone marrow was aspirated from the sternum or iliac crest. The smears were stained using the combined May-Grünwald and Giemsa methods (Orion Diagnostica, Helsinki, Finland), and the iron stores were stained by the Prussian blue method. The presence of less than 10% of normoblasts stained blue was considered evidence for diagnosis of iron deficiency.

DIAGNOSIS OF IDA BY MEANS OF ROUTINE LABORATORY TESTS

Blood cell counts were measured with an automated analyzer (Technicon H1; Technicon Instruments Corp, Tarrytown, NY). Serum iron level (reference range, 59-158 µg/dL [10.6-28.3 µmol/L]) for men and 37-145 µg/dL [6.6-26.0 µmol/L] for women) was measured using an iron FZ assay (Hoffmann-La Roche, Basel, Switzerland) based on a guanidine hydrochloride/Ferrozine reaction. Transferrin level (reference range, 200-400 mg/dL [2.0-4.0 g/L]) was measured with an immunoturbidimetric assay (Boehringer Mannheim, Mannheim, Germany). Transferrin saturation level was calculated with the following equation:

$$\frac{\text{Iron} \times 23}{\text{Transferrin} \times 23} \times 100$$

where iron level is measured in micromoles per liter and transferrin level in grams per liter (reference value, 20%). The ferritin level (reference range, 24-300 ng/mL [53.9-674.1 pmol/L] for men and 15-307 ng/mL [33.7-689.8 pmol/L] for women) was measured using a chemiluminescence assay (Access Immunossay System; Beckman Instruments Inc, Chaska, Minn). Iron deficiency was diagnosed by means of routine laboratory test results when serum iron, transferrin saturation, and ferritin levels were all abnormal.²²

DIAGNOSIS OF IDA BY MEANS OF TR

Serum TR assays were performed using a commercially available kit based on polyclonal antibodies in a sandwich enzyme-linked immunosorbent assay format (Clinigen; R&D Systems, Minneapolis, Minn). According to the assay kit from the manufacturers, the central 95th percentile of the reference distribution of TR concentration is 0.85 to 3.05 mg/L (n=1000). To make the test more specific, we calculated the ratio of TR to log ferritin level (TR-F index). An index value of greater than 1.5 was considered diagnostic of iron deficiency.²⁴,²⁵ All TR values herein are presented as TR-F index. To ascertain analytic quality, all TR assays in the present study were performed in duplicate.

CONTROL GROUP

The control group consisted of 14 patients older than 80 years. These patients also underwent investigation for IDA, but the results of their bone marrow aspirate examinations demonstrated that more than 10% of normoblasts contained iron, thus excluding the diagnosis of IDA.

STATISTICAL ANALYSIS

Sensitivity was defined as $[TP/(TP+FN)] \times 100$, and specificity as $[TN/(TN+FP)] \times 100$, where TP is true positive; TN, true negative; FP, false positive. Positive predictive value was defined as $[TP/(TP+FP)] \times 100$; negative predictive value, $[TN/(TN+FN)] \times 100$. Unless otherwise indicated, data are given as mean±SD.

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the reference range, probably due to the concomitant presence of other illnesses.¹³

The definitive test for the diagnosis of IDA is the presence of less than 10% of normoblasts stained by Prussian blue in a bone marrow aspiration sample.¹⁰ However, this procedure is invasive, painful, and expensive, and therefore is not performed regularly. Thus, an alternative sensitive and noninvasive test for the diagnosis of IDA in the elderly is needed.

Transferrin receptor (TR) is a transmembrane glycoprotein that is expressed on most cells, especially those that require high iron levels such as immature erythroid cells.¹⁷ It has a major role in the internalization of iron into the cells.¹⁸ Transferrin receptor is susceptible to proteolysis, thus producing soluble serum TR forms.¹⁹ The serum levels of TR reflect the amount of membranous TR, which inversely correlates to iron storage levels.²⁰ Kohgo et al²¹ developed a radioimmunoassay for the measurement of serum TR. They were the first to report that high serum TR levels correlate with iron deficiency. Flow- ers et al²² developed an enzyme-linked immunosorbent assay for the detection of TR in patients’ serum samples. Using this assay, Skikne et al²³ also demonstrated that high serum TR levels are specific markers for IDA. Serum TR levels increase in IDA, but not in inflammatory states.²⁴-²⁷ The value of serum TR measurement in the diagnosis of IDA in the elderly is not precisely defined, since most previous studies focused on younger popula-
normoblasts, thus excluding the diagnosis of IDA. Only 8 patients (group 1) were identified by means of the routine laboratory tests (combination of serum iron, transferrin saturation, and ferritin assay): 35 additional patients were identified by means of the TR-F index (group 2); and 6 patients (group 3) were not identified by means of routine laboratory tests or TR-F index.

MCV, mean corpuscular volume; CRP, C-reactive protein; and ESR, erythrocyte sedimentation rate.

As can be seen in Table 1, the mean hematocrit levels of these 8 patients (9.4±0.9 g/dL) was similar to that of the other groups. However, the mean corpuscular volume and mean serum levels of iron, transferrin saturation, ferritin, and C-reactive protein were significantly lower compared with those of the other groups of patients. The TR-F index clearly identified IDA in these 8 patients, with a mean value of 4.2±1.3.

By using the TR-F index, we were able to identify another 35 (71%) of the 49 patients who had bone marrow–proved IDA. None of these 35 patients could have been identified by means of the routine laboratory tests for IDA. In 6 other patients with bone marrow–proved IDA (12%), the diagnosis could not be established by means of either method. These 6 patients had high levels of ferritin and C-reactive protein (Table 1).

The control group of 14 patients had anemia with mean hemoglobin levels of 10.3±0.7 g/dL, which were similar to the hemoglobin levels of the 49 patients with bone marrow–proved IDA (Table 1). In 13 (93%) of these patients, the TR-F index was lower than 1.5, excluding iron deficiency as the cause of anemia. The only patient in the control group who had slightly raised levels of TR-F index (1.68) demonstrated low levels of serum iron (19.0 µg/dL [34 pmol/L]) and transferrin saturation (16%) with high levels of ferritin (847 ng/mL [1903.2 pmol/L]). Although we cannot rule out the possibility that this patient had an early stage of iron deficiency, combined with anemia due to chronic disease (ACD), the presence of a normal content of iron stores in the bone marrow does not support this assumption.

| Table 1. Routine Laboratory Tests and TR-F Index in 49 Study Patients and 14 Control Patients* |
|----------------------------------|-------------------|-------------------|-------------------|-------------------|
| **Variables†**                  | **Routine Laboratory Tests** (Group 1) | **>1.5 (Group 2)** | **≤1.5 (Group 3)** | **Control Patients§** |
| No. (%) of patients             | 8/49 (16)         | 35/49 (71)        | 6/49 (12)         | 14                |
| Hb, g/dL (men, >13.0 g/dL; women, >12.0 g/dL) | 9.4 ± 0.9         | 10.3 ± 0.9        | 9.4 ± 0.6         | 10.3 ± 0.7        |
| MCV, fl (80-99 fl)             | 77.0 ± 5.2        | 89.0 ± 6.7        | 90.4 ± 3.9        | 88.6 ± 6.2        |
| Reticulocytes, % (0.5%-1.5%)   | 1.5 ± 0.7         | 1.9 ± 4.8         | 0.67 ± 0.4        | 1.12 ± 2.0        |
| Serum iron, µg/dL (36.9-145.3 µg/dL) | 20.7 ± 7.8       | 45.8 ± 27.4       | 58.1 ± 55.3       | 115.1 ± 113.4 #   |
| Transferrin saturation, % (20%-45%) | 5.3 ± 2.8   | 19.1 ± 11.0       | 29.3 ± 28.5       | 42.1 ± 9.9 #      |
| Ferritin, ng/mL†† (men, 24-300 ng/mL; women, 15-307 ng/mL) | 13.7 ± 5.2    | 144.3 ± 151.3     | 385.1 ± 336.4 ** | 363.9 ± 222.2 ‡‡ |
| CRP, mg/L (0.01-8.00 mg/L)     | 19.3 ± 14.9       | 48.1 ± 45.4       | 58.3 ± 38.4       | 54.5 ± 33.0       |
| ESR, mm/h (men, 0-20 mm/h; women, 0-30 mm/h) | 51.6 ± 17.8 | 65.6 ± 40.3       | 62.0 ± 27.8       | 64.6 ± 31.2       |
| TR-F index (≤1.5)             | 4.2 ± 1.3         | 2.1 ± 0.5         | 1.1 ± 0.3 #       | 1.1 ± 0.2 #       |

*Unless otherwise indicated, data are given as mean ± SD. TR-F index indicates transferrin receptor–ferritin index; IDA, iron deficiency anemia; Hb, hemoglobin; MCV, mean corpuscular volume; CRP, C-reactive protein; and ESR, erythrocyte sedimentation rate.
†Unless otherwise indicated, data in parentheses indicate reference ranges or reference values.
‡Indicates 49 patients with IDA as defined by bone marrow evaluation results (<10% blue-stained normoblasts). Only 8 patients (group 1) were identified by means of routine laboratory test results (combination of serum iron, transferrin saturation, and ferritin assays); 35 additional patients were identified by means of the TR-F index (group 2); and 6 patients (group 3) were not identified by means of routine laboratory tests or TR-F index.
§Control group represents 14 patients with anemia in whom results of bone marrow aspirate examination demonstrated more than 10% blue-stained normoblasts, thus excluding the diagnosis of IDA.
¶To convert to micromoles per liter, multiply by 0.179.
#P < .001 compared with all other groups.
‡‡To convert to picomoles per liter, multiply by 2.247.
††To convert to micromoles per liter, multiply by 2.247.
†††P < .001 compared with group 1; P < .005 compared with group 2.
The present study clearly demonstrated the important role of the TR-F index in the diagnosis of IDA in elderly patients with anemia. The TR-F index has high specificity (93%) and sensitivity (88%) for the diagnosis of IDA in the elderly compared with the low sensitivity (16%) of the routine laboratory tests now used for the evaluation of IDA.

Iron deficiency anemia is a serious medical problem in the elderly. The high rate of gastrointestinal tract malignancy in these patients makes the study of the gastrointestinal tract obligatory. However, these studies are inconvenient and carry a high risk for complications, especially in elderly patients. Thus, one should not recommend those studies without a clear indication, ie, a definite diagnosis of IDA. Serum iron, transferrin saturation, and ferritin levels often conceal the diagnosis of IDA in elderly patients, mainly because of the coexistence of ACD in many of those patients. The present study confirms these previous observations, demonstrating a very low sensitivity of the routine laboratory tests for the diagnosis of IDA in the elderly (Table 1 and Table 2). The use of routine laboratory tests in our studies did not disclose the diagnosis of IDA in 41 (84%) of 49 patients. This low sensitivity for IDA diagnosis is not acceptable. Therefore, bone marrow examination is used for more accurate diagnosis of IDA.

The TR serum levels have been shown to be sensitive markers for IDA. We used the TR–log ferritin ratio, the TR-F index, because it was shown to improve the diagnostic efficiency for IDA compared with serum TR level alone or the TR-ferritin ratio. The TR-F index is an accurate marker for IDA, because it represents the total-body iron stores and the availability of iron for erythropoiesis. To our knowledge, our report is the first study of patients older than 80 years in whom the results of bone marrow examinations and routine blood tests and TR-F index have been compared. In agreement with previous observations in younger groups of patients, we found the TR-F index to be a specific and sensitive test for the diagnosis of IDA in the elderly (Table 2). Our data also support the previous observation of Gimferrer et al., who demonstrated that the TR assay exceeds the diagnostic value of routine laboratory tests for IDA during the early stages of iron depletion.

The routine laboratory tests pointed to the diagnosis of IDA in 8 patients with low mean corpuscular volume and very low levels of serum iron, transferrin saturation, and ferritin. In 35 other patients with higher serum iron levels, the diagnosis could be established only by means of the TR-F index (Table 1). Moreover, in most elderly patients, IDA develops concomitantly with ACD. The most important clinical issue is not to differentiate between those 2 morbidities, but rather to have a good diagnostic tool to identify IDA in the presence of ACD. The routine laboratory tests for IDA are of no value in this situation (Tables 1 and 2). In contrast, the TR-F index findings led to the diagnosis of IDA in 19 patients with high inflammatory variables (C-reactive protein level, >20 mg/dL; erythrocyte sedimentation rate, >50 mm/h), whereas results of routine laboratory tests led to the diagnosis of IDA in only 1 of those 19 patients. Although measurement of TR-F index is about 5 times more expensive than routine laboratory tests, it is less expensive than bone marrow examination, and its diagnostic efficiency is much better than that of the routine laboratory tests for IDA. Thus, it is a cost-effective assay for the diagnosis of IDA.

This study supports the high specificity and sensitivity of the TR-F index in the diagnosis of IDA in elderly patients. It is a simple, noninvasive test. A positive finding on the TR-F index (>1.5) can accurately establish a diagnosis of IDA and may eliminate the need for bone marrow examination. On the other hand, a normal finding on the TR-F index in elderly patients with inflammatory variables does not exclude IDA; thus a bone marrow examination should be considered in those patients.

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