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Anemia in Alcoholics

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Anemia is a frequent complication of alcoholism. It has been found in 13 to 62% of chronic alcoholics hospitalized for acute or chronic illnesses (8, 14, 20, 42). Megaloblastic and sideroblastic changes in the bone marrow are said to account for the anemia in the majority of cases (8, 20). There are many other potential causes of a low hematocrit, however, in alcoholics (6).

Most previous studies have focused on the pathophysiology and mechanisms of anemia in this patient group. There are no published guidelines for the diagnostic evaluation and management of this common problem. Direct and indirect effects of alcohol ingestion may affect standard tests used in the diagnosis of anemia, such as the mean corpuscular volume (23), serum and erythrocyte folate (9, 42), serum iron, total iron-binding capacity and ferritin (10, 24, 36). We undertook a prospective study of a consecutive series of anemic alcoholic patients. Although we confirmed many of the findings of previous workers, we differed in our assessment of the underlying causes of anemia. We also found the sensitivity, specificity, and predictive values of certain laboratory tests important in the approach to anemia in nonalcoholic patients to be so limited as to cast question on their usefulness. On the basis of our findings, we have developed recommendations for diagnosis and therapy.

Methods

During the periods January through June 1982 and May through October 1984, patients for study were identified from the daily admission lists of the general medical wards of Harlem Hospital Center. Admission lists include the names of all patients hospitalized in the previous 24 hours and their major diagnoses, including drug or alcohol dependence. All patients with a history of heavy alcohol abuse who were anemic (hematocrit <35%, women; hematocrit <40%, men) were consecutively interviewed as early as possible following admission. Detailed dietary and drinking information was obtained from all patients able to provide a history. Patients were considered alcoholic when they admitted to ≥80 g ethanol consumption per day and/or manifested a serious complication of alcohol abuse, e.g., withdrawal seizures or delirium tremens. All who agreed to venepuncture and bone marrow aspiration were included in the study. "Time of evaluation" was the interval between registration in the hospital emergency room and bone marrow aspiration. Diet was graded in the following manner based on the week before admission: 0 = no food for one week; 1 = occasional meals; 2 = one meal/day; 3 = two meals/day; 4 = three meals/day.

Standard methods were used to determine the complete automated blood count, including mean corpuscular volume (MCV), liver function tests, and iron and total iron binding capacity (TIBC) (12). Serum ferritin was measured by radioimmunoassay using an antibody precipitation technique (Becton-Dickinson Immunodiagnostics, Orangeburg, New York). The absolute reticulocyte count was calculated by multiplying the percentage reticulocyte count by the red blood cell count (RBC). Plasma folate and cobalamin levels were measured by radioassays using a milk binder and purified intrinsic factor, respectively ("Quantaphase," Bio-Rad Laboratories, Oakland, California). The normal range for the serum cobalamin using this assay in our laboratory is 230–950 pg/ml. Erythrocyte folate concentrations were determined by microbiological assay (16). Plasma folate was measured in every patient, and RBC folate in a subgroup of 55 consecutive patients. The normal ranges for plasma and RBC folate levels (2.1–13.0 ng/ml and 150–810 ng/ml RBC, respectively) were based on measurements performed on blood samples drawn from 69 nonalcoholic, nonanemic healthy medical personnel and were calculated as the mean ±2 SD after log transformation.

Both Wright's and Prussian blue stains of the peripheral blood were performed. Neutrophil hypersegmentation was considered to be present when 5% or more granulocytes with 5 lobes or any granulocytes with 6 or more lobes per 100 neutrophils were noted. An abnormal number of siderocytes (1) in the peripheral smear was defined as 5 or more per thousand erythrocytes on the Prussian blue stain. The macroovalocyte percentage was determined by counting the number of macroovalocytes seen among 500 erythrocytes in well-spread areas of the blood smear; microcyte percentage was determined in the same manner. Blood smears were considered "dimorphic" when ≥2% hypochromic microcytes were seen in the presence of a second population of normocytic and/or macrocytic erythrocytes. The degree of megaloblastic change in the myeloid and erythroid series in bone marrow smears was graded separately in each series from 0 to 4. Sideroblastic change was diagnosed in patients with 10% or more ringed sideroblasts in Prussian blue stains of marrow smears. Aggregation of marrow macrophage iron characteristic of the anemia of chronic disease was said to be present

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when numerous coarse aggregates greater than 5 microns in diameter were seen (29). "Acute blood loss" was diagnosed in patients with hematemesis, melena, and/or visible rectal bleeding. Smears of peripheral blood and bone marrow were read by one of the authors without knowledge of the patient's clinical and laboratory data.

Ferrous sulfate therapy was begun in iron-deficient patients and oral folic acid and parenteral cyanocobalamin in those with megaloblastic marrow changes within 24 to 36 hours of evaluation. Serial complete blood counts and reticulocyte determinations were performed at least 3×/week following admission.

For comparison with this prospective, randomly selected series, the records of 3 groups of patients were reviewed: 1) all patients seen by the hematology consultation services with a diagnosis of megaloblastic erythropoiesis associated with chronic alcoholism admitted to Harlem Hospital Center and Columbia-Presbyterian Medical Center from 1968 through 1982, 2) 32 consecutive alcoholic patients with absent iron stores on bone marrow iron stains seen by the hematology consultation service at Harlem Hospital Center between 1980 and 1983 in whom values for serum ferritin, iron, and TIBC were available, and 3) 52 non-alcoholic patients, seen at the two hospitals in 1981, whose hematocrit values were less than 30% and who have unequivocal iron deficiency anemia (low serum ferritin and/or absent marrow iron stores with a subsequent response to iron therapy).

Standard statistical methods were used, including Student's t test, coefficient of correlation, and the chi-square (χ^2) test with Yates' correction for continuity (33). Since the relationships between a large number of variables were assessed statistically, only P values of less than 0.01 were considered significant, in order to reduce the likelihood of erroneous conclusions. The data of the patient with pernicious anemia were excluded from the analysis of time of evaluation and plasma and RBC folate; a patient who appeared to have inadvertently received vitamin therapy before venepuncture was excluded from the analysis of folate and cobalamin concentrations.

The sensitivity of a test result was defined as the percentage of patients with a given diagnosis in whom the finding was present; specificity was the percentage of patients lacking the diagnosis in whom the finding was absent. Positive predictive value was defined as the percentage of patients with the disorder among subjects in whom the diagnostic finding was present, i.e., the probability that the condition was present if the test was positive. Negative predictive value was the percentage of patients lacking a given disorder among subjects in whom the finding was absent, i.e., the probability that the condition was not present if the test was negative (13).

Results

Overall characteristics

Among approximately 250 anemic alcoholic patients who were interviewed at admission, 121 (83 men, 38 women; 115 black, 6 Hispanic) agreed to the study. The mean age was 51 (range, 29–79) years and mean hematocrit was 27% (range, 10–39). The primary reasons for admission are listed in Table 1. Sixty-four patients (52.9%) were evaluated in the first 24 hours of hospitalization, 48 (39.7%) in the first 25 to 48 hours and 9 (7.4%) within 49 to 63 hours. (The patients who refused to participate in the study did not differ from those who were included in age, sex, or severity of anemia but had a lower incidence of marked macrocytosis [MCV >110 fl]).

TABLE 1. Primary diagnosis at admission in 121 anemic alcoholics

Diagnosis	Number	Percent
Withdrawal seizures and/or delirium tremens	32	26.4
Infection*	23	19.0
Liver disease†	20	16.5
Gastrointestinal bleeding	16	13.2
Anemia	10	8.3
Pancreatitis	6	5.0
Miscellaneous‡	14	11.6

* 18 had pneumonia.
† Encephalopathy 13, alcoholic hepatitis 5, tense ascites 2
‡ Intracranial hemorrhage 3, renal failure 2, syncope 2, peripheral neuropathy 2, rhabdomyolysis 2, hypercalcemia 1, alcoholic ketoacidosis 1, Wernicke's encephalopathy 1

Patients were able to indicate their specific preferred beverage in 102 instances. Hard liquor was the predominant drink in 62 cases (60.8%), wine in 35 (34.3%), and beer in 5 (4.9%). Among drinkers of hard liquor, vodka was the preferred beverage in 29; whiskey in 12; rum in 11; and gin in 10. Wine drinkers imbibed inexpensive, fortified wines. Ingestion of more than 1 beverage was reported by 19 of 102 patients; in 9 additional instances, patients reported drinking any alcoholic beverage that could be obtained. It was possible to estimate the daily consumption of ethanol from the information provided by 100 patients; in only 2 it was less than 100 g. The mean (± 1 SD) daily consumption was 5.6 ± 4.8 g ethanol/kg body weight.

The dietary rating was "0" in 45 patients (41.7%); "1" in 30 patients (27.8%); "2" in 12 patients (11.1%); and "3–4" in 21 patients (19.4%). Seventy-seven patients (63.6%) had signs of hepatic disease on physical examination, including: hepatomegaly and/or tenderness in 46 instances (59.7%); liver enlargement and/or tenderness plus jaundice, 11 (14.3%); ascites and/or encephalopathy with 1 or more of the previous signs, 20 (26.0%). Twenty-five patients (20.7%) had documented infection.

Causes of anemia

As shown in Table 2, in only 54 patients (44.6%) was there evidence of a single "cause" of anemia; 45 had 2 apparent causes (37.2%); and 22 had 3 or more causes (18.2%). Aggregated macrophage iron was present in a total of 98 patients (81.0%); megaloblastic change in the bone marrow in 41 (33.9%); and absent iron stores in 16 (13.2%). The only patient with megaloblastic change as the sole cause

TABLE 2. Diagnostic findings in 121 alcoholic patients with anemia

Finding	Number of Patients
Megaloblastic marrow changes	
alone*	1
plus ringed sideroblasts† (RS)	3
plus aggregated macrophage iron	17
plus absent iron stores	3
plus aggregated macrophage iron and acute blood loss	4
plus aggregated macrophage iron and RS	10
plus aggregated macrophage iron, RS, and blood loss	3
Ringed sideroblasts	
alone	0
plus aggregated macrophage iron	7
plus aggregated macrophage iron and acute blood loss	5
Absent iron stores	
alone	5
plus acute blood loss	8
Marrow macrophage iron aggregation	
alone	45
plus acute blood loss	7
Acute blood loss‡	
alone	3

* Alone = no other etiology for anemia apparent
† ≥ 10 percent of marrow normoblasts
‡ Clinical diagnosis (see text); 13 of 30 with acute blood loss were hypotensive.

was found to have pernicious anemia (hematocrit 14%, MCV 111 fl, low plasma cobalamin, antibody to intrinsic factor, and normal plasma folate). Ringed sideroblasts were present in the bone marrow of 41 patients and constituted 10% or more of nucleated erythroid cells in 28 (23.1%); there was no case in which these changes were the only apparent reason for the anemia. Although acute blood loss was a presenting finding in 30 patients (24.8%), marrow examination revealed additional possible causes of anemia in 27 (Table 2). One patient with leukocytosis and thrombocytosis had ringed sideroblasts and aggregated marrow macrophage iron, in addition to Philadelphia chromosome-positive chronic myelogenous leukemia; the abnormal sideroblasts were no longer present in the bone marrow after 2 weeks of hospitalization. No patient had evidence of overt hemolytic anemia (i.e., stable or falling hematocrit associated with reticulocytosis and decreased plasma haptoglobin).

Megaloblastic change in the bone marrow

Patients with and without megaloblastic change in the bone marrow are compared in Table 3. Among subjects with megaloblastic morphology, anemia, macrocytosis, macroovalocytosis, reticulocytopenia, elevation of serum lactic dehydrogenase (LDH), and depression of plasma and RBC folate levels were significantly more pronounced and neutrophil hypersegmentation and sideroblastic marrow change significantly more common than in those without megaloblastic change. There was considerable overlap, however, in each of these findings between the groups with and without megaloblastic morphology.

Myeloid and erythroid megaloblastic changes in the bone marrow: Of 41 patients with megaloblastic marrow findings, megaloblastic change was present in the myeloid series in 38, accompanied by megaloblastic morphology in the erythroid series in 17 (41.5%) and occurring alone in 21 (51.2%). Three subjects had erythroid changes only. Isolated myeloid change was slightly but not significantly more common in marrows obtained more than 24 hours after hospitalization than in those obtained earlier. Mean MCV values and numbers of reticulocytes did not differ between patients with both myeloid and erythroid changes versus those with myeloid changes alone. Two subjects with myeloid changes alone had absent marrow iron stores.

In 2 of the 3 subjects with megaloblastic change in the erythroid series only, the hematocrit exceeded 32% and the MCV was normal. Macroovalocytes were present in 2 and hypersegmented neutrophils in only 1. In 1 patient the plasma folate was normal and in 2 the RBC folate was normal.

Patients with marked (3 to 4+) megaloblastic change in the erythroid and/or myeloid series tended to have more severe anemia, lower plasma folate levels, and higher LDH values than those with less marked megaloblastic morphology although the difference was significant only for the LDH levels (data not shown).

MCV: Elevation of the MCV (>100 fl) was frequent in patients with and without megaloblastic change (Fig. 1). In addition, the MCV was within the normal range (80–100 fl) in 15 patients (36.6%) with megaloblastic marrows, only 2 of whom had absent iron stores. In subjects with megaloblastic hematopoiesis, the MCV and RBC were inversely correlated ($r = -0.47$, $p < 0.005$), as has previously been reported in normal subjects (27). The degree of MCV elevation did not correlate with the hematocrit, severity of megaloblastic change, the presence of ringed sideroblasts, absolute number of reticulocytes, LDH, plasma or RBC folate concentrations, or evidence of liver disease by physical examination or liver function tests.

Macroovalocytes: Macroovalocytosis was present on the blood smears of 90% of the patients with megaloblastic marrow morphology. The degree of macroovalocytosis inversely correlated with hematocrit ($r = -0.52$, $p < 0.001$) and RBC ($r = -0.45$, $p < 0.005$) among subjects with megaloblastic hematopoiesis. Percent macroovalocytosis was corre-

TABLE 3. Comparison of anemic alcoholics with and without megaloblastic change in the bone marrow

Measurement	Megaloblastic Change* Mean ± 1 SD		Finding	Megaloblastic Change†	
	Present	Absent		Present (%)	Absent (%)
Hematocrit (%)	24 ± 8	28 ± 7‡	MCV >100 fl	27/41§	26/80‡
RBC ($\times 10^6/\mu l$)	2.31 ± .86	2.96 ± .75‖		(65.9)	(32.5)
MCV (fl)	104 ± 12	95 ± 10‖	MCV >110 fl	11/41	2/80‖
Macroovalocytosis (%)	4.3 ± 3.9	0.6 ± 1.1‖		(26.8)	(2.5)
Reticulocytosis ($\times 10^3/\mu l$)	42 ± 47	70 ± 53‡	Macroovalocytes	37/41	26/80‖
WBC ($\times 10^3/\mu l$)	7.7 ± 5.2	9.0 ± 5.6	present	(90.2)	(32.5)
Platelets ($\times 10^3/\mu l$)	158 ± 105	196 ± 160	Macroovalocytes	23/41	3/80‖
LDH (U/L)	666 ± 1031	293 ± 120‡	>3%	(56.1)	(3.8)
Plasma folate (ng/ml)	2.8 ± 2.2	4.8 ± 4.5‡	Hypersegmentation	32/41	4/80‖
RBC folate (ng/ml RBC)	109 ± 67	251 ± 159‖	present	(78.0)	(5.0)
Plasma cobalamin (pg/ml)	623 ± 461	1059 ± 993‡	LDH >225 U/L	30/37	44/60
Serum ferritin (ng/ml)	843 ± 942	502 ± 615		(81.1)	(73.3)
Serum iron (μg/dl)	111 ± 63	80 ± 54	Plasma folate	17/39	19/80
Transferrin saturation (%)	55 ± 32	43 ± 29	<2.1 ng/ml	(43.6)	(23.8)
			RBC folate	11/16	12/39
			<150 ng/ml	(68.8)	(30.8)
			Serum ferritin	25/41	39/80
			>300 ng/ml	(61.0)	(48.8)
			Serum iron	5/24	2/62
			>175 ug/dl	(20.8)	(3.2)
			Ringed sideroblasts	16/41	12/80‡
			>10%	(39.0)	(15.0)

* Differences between mean values were assessed by Student's t-test.
† Differences between percentages were assessed by calculation of χ^2 using Yates' correction for continuity.
‡ $p < 0.01$
§ Number of patients with finding divided by total number in whom test was done.
‖ $p < 0.001$

lated with MCV (r = 0.39) and inversely correlated with RBC folate (r = −0.61) and plasma folate (r = −0.23) but none of these correlations was significant. In all 4 patients with megaloblastic change in whom macroovalocytes were not observed, the plasma folate was normal, both myeloid and erythroid change were less than 3+, and neutrophil hypersegmentation was absent; the hematocrit was greater than 30% and MCV was normal in 3.

Macroovalocytes were also present on the blood smears of 26 (32.5%) of the 80 patients in whom megaloblastic hematopoiesis was absent. The mean number of macroovalocytes and the incidence of macroovalocytosis exceeding 3% were significantly lower in the 80 patients lacking megaloblastic marrows, however, than in subjects in whom megaloblastic change was present (Table 3). Among subjects lacking megaloblastic change, the 3 patients with greater than 3% macroovalocytosis included 1 with sideroblastic change and normal plasma folate and low erythrocyte folate concentrations. In the other 2 patients the MCV and plasma and erythrocyte folate levels were normal and the marrow showed no abnormality other than macrophage iron aggregation.

Among the 26 patients lacking megaloblastic change in whom macroovalocytes were present, 3 (11.5%) also had neutrophil hypersegmentation. Seven of the 26, all of whom lacked neutrophil hypersegmentation, had sideroblastic changes and 3 were iron-deficient. Aggregation of macrophage iron was present in 22 instances (84.6%) and was the sole marrow morphologic abnormality in 15 (57.7%). Among patients without megaloblastic hematopoiesis, those with macroovalocytes were more anemic (mean RBC 2.64 ± 0.73 × $10^6/\mu l$) than those without macroovalocytes (mean RBC 3.12 ± 0.71 × $10^6/\mu l$, t = 2.82, $p < 0.01$) but had similar MCV values (96 ± 10 fl vs. 95 ± 10 fl).

Hypersegmented neutrophils: Neutrophil hypersegmentation was 15 times more common in patients with megaloblastic change than in those without (Table 3) and was present in the smears of 78% of patients with megaloblastic marrows. In subjects with megaloblastic change, those with hypersegmentation were more anemic than those with normal granulocyte morphology (mean RBC 2.07 ± 0.71 vs. 3.18 ± 0.77 × $10^6/\mu l$, t = 4.08, $p < 0.001$). Among patients with megaloblastic change, the mean plasma folate of patients with neutrophil hypersegmentation (2.4 ± 1.8 ng/ml) was lower than that of subjects lacking hypersegmented neutrophils (4.1 ± 2.8 ng/ml, t = 2.18, $0.05 > p > 0.01$). Two of the 3 patients who lacked myeloid megaloblastic change in the bone marrow also lacked hypersegmented neutrophils. The leukocyte differential count, absolute number of granulocytes, and

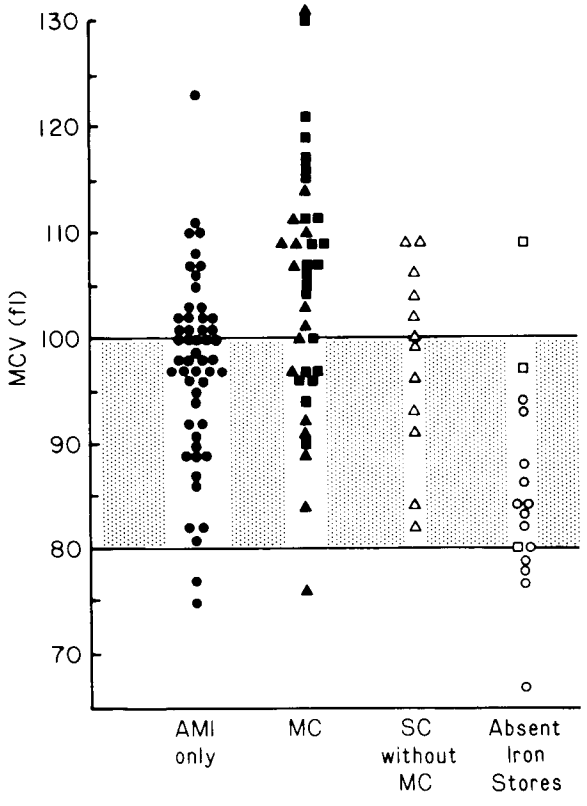

FIG. 1. Mean corpuscular volume (MCV) in 118 anemic alcoholics in relation to different morphologic findings in bone marrow. ● = aggregated macrophage iron (AMI) without megaloblastic or sideroblastic change; ■ = megaloblastic change (MC) without sideroblastic change (SC); ▲ = both megaloblastic and sideroblastic change; □ = both megaloblastic hematopoiesis and absent iron stores; △ = sideroblastic without megaloblastic change; ○ = absent iron. The 3 patients with combined megaloblastic morphology and absent iron stores (□) appear only in the "absent iron" column. Three patients with anemia due only to acute blood loss are not included in the figure (MCV's 95, 102, and 103 fl).

incidence of infection of patients with and without neutrophil hypersegmentation did not differ.

There were 4 patients with hypersegmented neutrophils on the blood smear but no evidence of megaloblastic change in the bone marrow, 3 of whom had macroovalocytosis. In all 4 macrophage iron was abundant and aggregated. None was infected or had renal failure. The plasma folate was normal in 2 of the 4 patients and the RBC folate was normal in 2 of the 3 in whom it was measured. Follow-up data were available in 3 patients. Neutrophil hypersegmentation cleared in 1 by the 10th hospital day but persisted unchanged in 2 others 18 and 31 days after admission, in the latter case despite treatment with folic acid.

LDH: Elevations of serum LDH were seen in the majority of patients regardless of whether megaloblastic hematopoiesis was present (Table 3). Marked elevation of serum LDH (>1000 U/L) was seen exclusively in association with megaloblastic change, but this finding was present in only 4 patients. LDH and hematocrit were inversely correlated in patients with megaloblastic change ($r = -0.45$, $p < 0.005$) but not in patients lacking megaloblastic morphology. The 30 subjects with megaloblastic marrows in whom LDH values were elevated did not differ from the 7 with normal LDH levels in MCV, severity of anemia, plasma folate concentration, the presence of neutrophil hypersegmentation or macroovalocytosis, or the severity of myeloid megaloblastic change. However, erythroid megaloblastic morphology was absent from the bone marrow of 6 of the patients with normal LDH values. In the remaining subject 4+ changes in both the erythroid and myeloid series were associated with an LDH value of 218 U/L. The LDH was 660 U/L in a single patient with erythroid hypoplasia and megaloblastic change in the myeloid series.

Plasma folate: Although the mean plasma folate concentration was lower in the patients with megaloblastic marrows (Table 3), the majority of patients with megaloblastic change had normal plasma folate levels (Fig. 2A). Among subjects with megaloblastic change, those with low folate concentrations did not differ significantly from those with normal levels in severity of anemia, degree of megaloblastic change or MCV elevation, or the presence of macroovalocytosis or neutrophil hypersegmentation. The mean plasma folate of patients with megaloblastic morphology who had eaten food in the hospital prior to study did not differ significantly from that in patients who had not been fed (2.6 ± 2.1 vs. 2.5 ± 2.2 ng/ml, respectively). The patients studied within 24 hours of admission also did not differ significantly from those studied at more than 24 hours (3.0 ± 2.2 vs. 2.5 ± 2.3 ng/ml, respectively). Plasma folate did not correlate with clinical or biochemical evidence of liver disease.

RBC folate: The mean RBC folate concentration was significantly lower in patients with megaloblastic hematopoiesis than in those without (Table 3). There was considerable overlap, however, between the 2 groups (Fig. 2B), and 12 of 23 patients with low erythrocyte folate levels (< 150 ng/ml RBC) lacked megaloblastic morphology. Of these 12 patients macroovalocytes were present in 6 and neutrophil hypersegmentation in 1. All of the patients with megaloblastic change had RBC folate values below 250 ng/ml RBC; this was also true, however, of 19 of 39 subjects (48.7%) who lacked megaloblastic marrows and of 14 of 68 normal controls (20.6%) (Fig. 2B).

In 5 (31.3%) of the 16 patients with megaloblastic

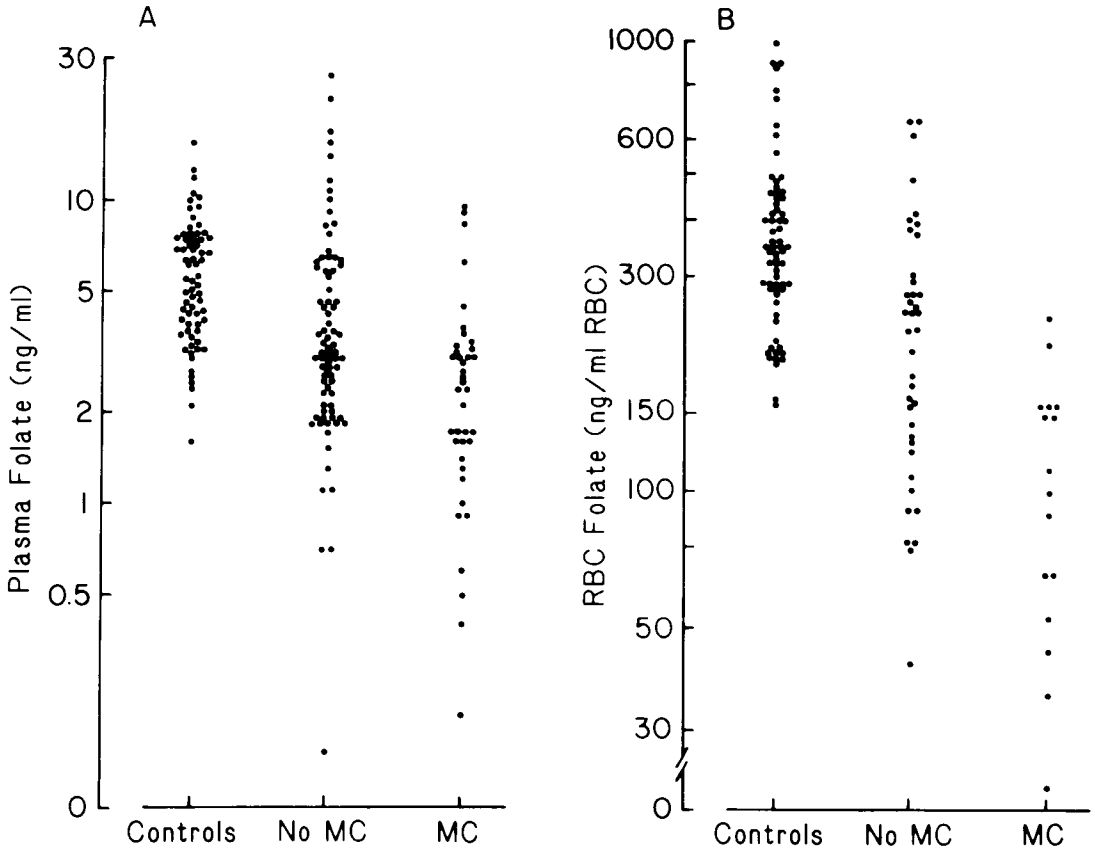

FIG. 2. **A.** Plasma folate concentrations in 69 healthy, non-alcoholic medical personnel (normal range, 2.1–13.0 ng/ml), 80 anemic alcoholics lacking megaloblastic change (MC), and 39 anemic alcoholics with megaloblastic marrows. **B.** Erythrocyte folate levels in 68 healthy controls (normal range, 150–810 ng/ml RBC), 39 anemic alcoholics lacking megaloblastic morphology, and 16 anemic alcoholics with megaloblastic change.

hematopoiesis in whom RBC folate concentrations were measured, the value was in the lower part of the normal range (Fig. 2B). Anemia was more severe among subjects with megaloblastic change and low RBC folate concentrations (mean RBC 2.14 ± 0.69 × $10^6/\mu l$) than those with normal erythrocyte folate values (3.11 ± 0.66 × $10^6/\mu l$, $t = 2.63$, $0.02 > p > 0.01$). Otherwise there were no apparent differences between the 2 subgroups. Both the plasma and RBC folate concentrations were normal in 4 (25%) of the 16 patients with megaloblastic hematopoiesis in whom both tests were done.

Plasma cobalamin: Cobalamin levels were elevated (>950 pg/ml) in 37 (31.1%) of 119 patients, all of whom had clinical and/or biochemical evidence of liver disease. Plasma cobalamin was normal in 78 (65.5%) and slightly subnormal (170–185 pg/ml) in 4, 2 of whom had megaloblastic morphology.

Iron studies: The serum ferritin was within the normal range (20–300 ng/ml) in 15 (36.6%) of 41 subjects with megaloblastic hematopoiesis and elevated in 25 (61.0%). In the 3 patients with megaloblastic change and absent iron stores, serum ferritin values were 15, 25, and 46 ng/ml. The serum iron was low (<65 μg/dl) in 8 of 24 patients with megaloblastic change, including the 3 with absent iron stores. In addition, in each of the 3 iron-deficient patients with megaloblastic change the TIBC was elevated (>400 μg/dl) and transferrin saturation was decreased (<16%). The serum iron was elevated (>175 μg/dl) in 5 (20.8%) of 24 subjects with megaloblastic hematopoiesis, 2 of whom had coexisting sideroblastic marrow change.

Sensitivity, specificity and predictive value of various tests in relation to megaloblastic marrow change: As seen in Table 4, the sensitivity, specificity, and positive and negative predictive values of elevations in MCV or LDH or decreases in plasma and RBC folate levels were generally low in identifying patients with megaloblastic marrows. The combination of low plasma and low RBC folate values in the same patient was moderately specific but highly insensitive for megaloblastic hemato-

TABLE 4. Sensitivity, specificity and predictive values* of several diagnostic findings in identification of anemic alcoholic patients with megaloblastic marrow change

Finding	%			
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
MCV >100 fl	65.9	67.5	50.9	79.4
MCV >110 fl	26.8	97.5	84.6	72.2
LDH >225 U/L	81.1	26.7	40.5	69.6
Low plasma folate (PF)†	43.6	76.3	47.2	73.5
Low RBC folate (RBCF)‡	68.8	69.2	47.8	84.4
Both PF, RBCF low	37.5	87.2	54.5	77.3
Either PF, RBCF low	75.0	61.5	44.4	85.7
Neutrophil hypersegmentation (NH)	78.0	95.0	88.9	89.4
Macroovalocytes on smear (MO)	90.2	67.5	58.7	93.1
MO > 3%	56.1	96.3	88.5	81.1
Both NH, MO present	78.0	96.3	91.4	89.5
Either NH, MO present	90.2	66.3	57.8	93.0

* See Methods for definitions
† Plasma folate <2.1 ng/ml
‡ RBC folate <150 ng/ml RBC

poiesis. The presence of neutrophil hypersegmentation was more specific for megaloblastic change than it was sensitive; the reverse was true for macroovalocytosis. If more than 3% of erythrocytes were macroovalocytes, the specificity of the finding was high but sensitivity low. The positive and negative predictive values of neutrophil hypersegmentation or the combination of hypersegmentation and macroovalocytosis were of the order of 90%. Macroovalocytosis alone had a low positive predictive value but a high negative predictive one.

Sideroblastic change

Twenty-eight patients had ≥10% ringed sideroblasts in the bone marrow (hereafter referred to as those with sideroblastic change). They were significantly older than those without this finding and were also more likely to have macroovalocytes on the blood smear and associated megaloblastic change in the bone marrow (Table 5). The difference in the incidence of macroovalocytosis between subjects with and without sideroblastic change was no longer significant, however, after exclusion of patients with associated megaloblastic morphology (data not shown). The smear was more often dimorphic and plasma and RBC folate values tended to be lower in subjects with sideroblastic change than in those without, but these differences were not significant (Table 5).

Siderocytes: Increased numbers of siderocytes on the peripheral blood smear were observed in 36% of the patients with ≥10% ringed sideroblasts, a finding virtually exclusive to this group (Table 5). In 2 patients ringed sideroblasts were seen on the Prussian blue stain of the peripheral smear in addition to siderocytes; nucleated red blood cells were not seen on the smears of other patients with sideroblastic change. None had Howell–Jolly bodies or a history of splenectomy. There was no relationship between the presence of increased siderocytes and the severity of anemia, megaloblastic change, folate levels, macrocytosis, evidence of liver disease, serum ferritin, iron, transferrin saturation, or reticulocyte count. As an indicator of sideroblastic change, the finding of increased siderocytes on the blood smear was 36.0% sensitive; 98.9% specific, and had a positive predictive value of 90.0% and negative predictive value of 85.0%.

Other features of patients with sideroblastic change: The MCV was high in 14 patients with sideroblastic change (9 of whom had co-existing megaloblastic hematopoiesis), normal in 15 and low in 1 (Fig. 1). In 7 patients with both megaloblastic and sideroblastic change the MCV was low or normal. Aggregated marrow macrophage iron was present in 25 of 28 patients (89.3%) with ≥10 percent ringed sideroblasts. The serum ferritin was elevated in most subjects with sideroblastic change (Table 5), although it was normal in 8 (28.6%). Serum iron was measured in 16 patients with sideroblastic change, and was low in 4, normal in 8, and increased in 4. Mean transferrin saturation was significantly higher in patients with ≥10 percent ringed sideroblasts (Table 5). There was no association between the presence of sideroblastic change and time of evaluation, sex, diet, infection, quantity of ethanol consumed or clinical or biochemical evidence of liver disease.

Absent iron stores

Sixteen patients had absent marrow macrophage iron. Ten were women (62.5%) in contrast to 28 (26.7%) of 105 in whom iron was present in the marrow. The mean MCV of the iron deficient patients was 85 ± 10 fl (range, 67–109, Fig. 1) versus 100 ± 10 fl (range, 75–131) in non-iron deficient subjects ($t = 5.60$, $p < 0.001$). Acute blood loss was present in 8 of 16 subjects with iron deficiency. Hematocrit and RBC values did not differ between patients with and without iron deficiency.

MCV: As seen in Figure 1, the MCV was low in only 4 of the iron deficient subjects, normal in 11, and elevated in 1. The MCV was normal or elevated in 7 of 11 patients with a hematocrit <30%, only 1

TABLE 5. Comparison of patients with and without sideroblastic change in the bone marrow

Measurement	Sideroblastic Change* Mean values (± 1 SD)		Finding	Sideroblastic Change†	
	Present	Absent		Present (%)	Absent (%)
Hematocrit (%)	25 ± 7	27 ± 8	Age > 50 yr	23/26‡ (88.5)	41/93§ (44.1)
MCV (fl)	100 ± 12‖	98 ± 12	Increased sidero-cytes	9/25 (36.0)	1/92§ (1.1)
Plasma folate (ng/ml)	3.4 ± 2.9	4.3 ± 4.2	Macroovalocytes present	23/28 (82.1)	40/93§ (43.0)
RBC folate (ng/ml RBC)	141 ± 71	230 ± 162	Dimorphic blood smear	16/27 (59.3)	37/92 (40.2)
Serum ferritin (ng/ml)	827 ± 839	665 ± 748¶	Transferrin saturation > 60%	10/16 (62.5)	21/54¶ (38.9)
Serum iron (μg/dl)	113 ± 61	89 ± 54¶	Ferritin >300 ng/ml	20/28 (71.4)	44/77¶ (57.1)
Transferrin saturation (%)	67.1 ± 22.6	47.5 ± 30.5**¶	Megaloblastic change present	16/28 (57.1)	12/93** (12.9)
			Mortality	9/26 (34.6)	13/93 (14.0)

* Differences between mean values were assessed by Student's t-test.
† Differences between percentages were assessed by calculation of χ^2 with Yates' correction for continuity.
‡ Two patients aged > 50 yr were both hospitalized twice with sideroblastic change.
§ $p < 0.001$
‖ Range = 76–131 fl
¶ Iron-deficient patients excluded.
** $p < 0.01$

of whom had associated megaloblastic change. There was no correlation between the MCV and hematocrit (r = 0.14), and only a non-significant inverse correlation with the RBC (r = −0.21). In addition, the MCV was not significantly correlated with reticulocyte count, presence of acute blood loss, liver function tests, or evidence of liver disease on physical examination.

Iron studies: The serum ferritin was less than 100 ng/ml in all 16 patients with absent iron stores (mean, 25 ± 15 ng/ml: range, 4–46 ng/ml) and was below 20 ng/ml in 8. In only 5 of 105 non-iron deficient patients was the serum ferritin less than 100 ng/ml, of whom 1 had acute blood loss and 4 had aggregated macrophage iron.

Only 4 of the 16 iron deficient patients had a combination of a low serum iron and high TIBC (>400 μg/dl); the TIBC was low (<250 μ/dl) in 4 of the patients, all of whom had hepatic disease.

In 4 of the 8 patients in whom the ferritin exceeded 20 ng/ml, the TIBC was elevated (associated with a serum iron that was low in 2 and normal in 2). In the remaining 4 patients, the TIBC was normal in 2 and subnormal in 2. In 2 of these 4, the serum iron exceeded 150 μg/dl despite the absence of megaloblastic change; both had liver disease and acute blood loss. The transferrin saturation was below 16% in only 3 subjects with serum ferritin values exceeding 20 ng/ml; in the 5 other cases, the range of transferrin saturation was 16.1 to 61.3%.

In the 14 iron-deficient subjects with abnormal liver function, the serum ferritin was greater than 20 ng/ml in 8 and transferrin saturation exceeded 16 percent in 7.

Sensitivity, specificity, and predictive value of various tests in relation to absent iron stores: Low ferritin and high TIBC values were 100% specific and low MCV values 97.1% specific as indicators of the absence of marrow iron stores; each of these findings alone was highly insensitive in identifying iron-deficient patients, however (Table 6). If either the ferritin was low or the TIBC high, however, the sensitivity improved and the negative predictive value exceeded 95%. A serum ferritin value <100 ng/ml was 100% sensitive and 95% specific for absent marrow iron; ferritin values >100 ng/ml were 100% accurate in predicting the presence of marrow iron. The serum iron and transferrin saturation were of limited value in the diagnosis of iron deficiency.

Comparison with other iron-deficient groups: The sensitivity of the various tests in detecting absent iron stores was assessed in a group of 32 anemic alcoholic patients with absent iron stores who were seen in referral by the Harlem Hospital Center hematology consultation service. Most of these patients had alcoholic liver disease. In only 5 of the 32, equivocal results of serum ferritin and iron/TIBC values were the indication for marrow aspiration; in the remaining patients, the results of

TABLE 6. Sensitivity, specificity, and predictive values* of several diagnostic findings in identification of anemic alcoholics with absent iron stores

Finding	%			
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
MCV <80 fl	25.0	97.1	57.1	89.5
Ferritin <20 ng/ml	50.0	100.0	100.0	86.8
Ferritin <100 ng/ml	100.0	95.2	76.2	100.0
Low iron (<65 μg/dl)	62.5	62.9	27.8	88.0
High TIBC (>400 μg/dl)	37.5	100.0	100.0	87.8
Either low ferritin or high TIBC	75.0	100.0	100.0	96.3
Both low iron, high TIBC	25.0	100.0	100.0	85.4
Transferrin saturation <16%	56.3	87.1	50.0	89.7

* See Methods for definitions.

these tests were not available until after the marrow was obtained. The sensitivity of the various tests in the diagnosis of iron deficiency in these 32 patients was as follows: MCV <80 fl, 61.3%; serum ferritin <20 ng/ml, 62.5%; serum ferritin <100 ng/ml, 100%; serum iron <65 μg/dl, 90.6%; TIBC >400 μg/dl, 56.3%; both low iron and high TIBC, 43.8%; either low ferritin or high TIBC, 81.3%, and transferrin saturation <16%, 81.3%.

In the referral patients in whom the hematocrit was less than 30%, the MCV was 80 fl or greater in 10 of 28 (35.7%), in contrast to 7 (63.6%) of 11 patients in the unselected alcoholic series. In 52 nonalcoholic patients with a hematocrit <30% and proven iron deficiency anemia, the MCV was normal or elevated in 9 (17.3%).

Aggregated macrophage iron in bone marrow smears

Infection was present in 24 of 98 patients (24.5%) with aggregated macrophage iron in contrast to only 1 (4.3%) of 23 in the group without aggregated iron, a nonsignificant difference. The frequency of evidence of liver disease on physical examination did not differ between those with (64.3%) and those without aggregated iron (60.9%); liver function tests were also comparable. However, in almost all of the patients with aggregated iron, an underlying inflammatory condition was apparent (e.g., infection, alcoholic liver disease, acute pancreatitis).

MCV: The MCV was low in 3 patients (3.1%) with aggregated iron, normal in 54 (55.1%) and elevated in 41 (41.8%). Of the 41 patients with an elevated MCV, 20 had megaloblastic hematopoiesis in the bone marrow and an additional 5 had sideroblastic change (with or without megaloblastic morphology). Among the 12 patients with MCV values >110 fl, 10 had associated megaloblastic and/or sideroblastic change; the remaining 2 subjects had evidence of severe hepatic disease (ascites and coagulopathy). The MCV and RBC count were inversely correlated in the 98 subjects with aggregated iron ($r = -0.41$, $p < 0.001$) in contrast to MCV and hematocrit ($r = -0.06$). After exclusion of the 46 patients with megaloblastic and/or sideroblastic marrow change, the correlation between MCV and RBC persisted among the 52 remaining patients with aggregated iron ($r = -0.38$, $p < 0.01$).

Iron studies: Among 98 patients with aggregated iron the serum ferritin was between 50 and 100 ng/ml in 4 (4.1%), 100 to 300 ng/ml in 32 (32.7%), and more than 300 ng/ml in 62 (63.3%), including 40 greater than 500 ng/ml.

In 36 of 66 patients (54.5%) with marrow iron aggregation the serum iron was normal (65–175 μg/dl). In 24 (36.4%) it was low and in 6 (9.1%), all of whom had megaloblastic and/or sideroblastic changes, it was elevated. The TIBC was normal (250–400 μg/dl) in 11 of 67 cases (16.4%) with aggregated iron, and low in the remainder. Transferrin saturation was less than 16% in 9 of 66 cases (13.6%), including 3 of the 4 subjects with serum ferritin values between 20 and 100 ng/ml.

As seen in Table 7, sensitivity, specificity, and predictive value were relatively low for all serum tests of iron status as indicators of the presence of aggregated macrophage iron in bone marrow, with the exception of the sensitivity and positive predictive value of serum ferritin concentrations >100 ng/ml.

Effect of coexisting megaloblastic and/or sideroblastic changes in bone marrow: Megaloblastic hematopoiesis without sideroblastic change was present in the bone marrow of 21 subjects with iron aggregation, 10% or more ringed sideroblasts were found in 12, and combined megaloblastic and sideroblastic change in 13. In patients with coexisting megaloblastic and/or sideroblastic erythropoiesis, anemia was significantly more severe (mean hematocrit 24 ± 8 vs. 30 ± 6%, $p < 0.001$) than in those with aggregated iron alone. Red cell size was also larger (MCV 103 ± 12 vs 97 ± 9 fl) and percent

TABLE 7. Sensitivity, specificity, and predictive values* of iron studies in identification of anemic alcoholic patients with aggregated marrow iron

Finding	%			
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Ferritin >100 ng/ml	95.9	73.9	94.0	81.0
Low iron (>65 μg/dl)	36.4	40.0	66.7	16.0
Low TIBC (<250 μg/dl)	83.6	71.4	90.3	57.7
Iron, TIBC both low	31.8	80.0	84.0	26.2
Transferrin saturation >16%	86.4	45.0	83.8	50.0

* See Methods for definitions.

transferrin saturation was higher (65 ± 27 vs. 42 ± 29%) in subjects with these coexisting changes, but these differences were not significant.

Admission reticulocyte counts

In the overall group, the 30 subjects with acute blood loss had a higher mean absolute reticulocyte count (74 ± 51 × $10^3/\mu l$) than those without blood loss (57 ± 53 × $10^3/\mu l$) but this difference was not significant. The admission reticulocyte count exceeded the upper limit of normal (120 × $10^3/\mu l$) in only 5 (16.7%) of the patients with acute blood loss and in 13 (10.9%) of 119 patients in the overall group; in 2 it was greater than 200 × $10^3/\mu l$. As noted earlier, reticulocytopenia was more severe in patients with megaloblastic marrows and less marked in those with iron deficiency.

Changes in reticulocyte count

Table 8 shows changes in the reticulocyte count of a subgroup of 41 patients who were not transfused and were observed for 7 to 15 days in the hospital. Although increments in the numbers of circulating reticulocytes commonly occurred, they were often modest. The reticulocyte count failed to rise to greater than 200 × $10^3/\mu l$ in the majority of patients in each listed sub-category. Although the mean peak subsequent reticulocyte count was higher and the percentage with a substantial elevation was greater in patients with megaloblastic and/or sideroblastic change compared to those with aggregated iron in the absence of megaloblastic and sideroblastic erythropoiesis, these differences were not significant.

In Figure 3A, the peak percentage reticulocyte response in patients with megaloblastic marrows is plotted against initial erythrocyte count. The average "expected" response is based on the serial observations of Isaacs, Bethell and their colleagues (35) in patients with megaloblastic anemia due to cobalamin deficiency to whom parenteral liver extract was administered. The peak reticulocyte response in our 17 patients with megaloblastic change fell to the left of the "expected" line, i.e., was lower than "expected," in 14 of the 17 patients (82.4%).

Changes in hematocrit and RBC count

Table 9 shows changes in the hematocrit of 28 patients who were not transfused and were hospitalized for at least 14 days. Despite mean follow-up

TABLE 8. Changes in reticulocyte count in 41 anemic alcoholic patients observed for 7 to 15 days

Diagnostic findings	Number of patients*	Mean (± 1 SD) (range)						
		Days of observation	Initial hematocrit (%)	% Reticulocytes		Absolute reticulocytes (× $10^3/\mu l$)		Number with peak reticulocytes >200 × $10^3/\mu l$ (%)
				Initial	Peak	Initial	Peak	
(A) AMI, excluding MC and SC†	14	10 ± 3 (7–15)	29 ± 5 (18–36)	2.7 ± 2.6 (0.6–9.3)	4.4 ± 3.7 (0.5–11.1)	70 ± 54 (19–181)	118 ± 98 (23–350)	2 (14.3)
(B) MC and/or SC‡	22§	10 ± 3 (7–15)	23 ± 7 (11–33)	2.6 ± 2.6 (0.4–10.0)	8.7 ± 5.9 (0.8–22.0)	60 ± 72 (6–284)	186 ± 107 (13–440)	8 (36.4)
(C) Absent iron stores	5	8 ± 1 (7–10)	25 ± 7 (17–37)	3.1 ± 2.6 (0.8–6.5)	4.7 ± 2.5 (1.0–6.8)	81 ± 58 (34–166)	119 ± 76 (31–240)	1 (20.0)

* Transfused patients excluded; 6 group B patients had acute blood loss, 3 in group C, none in Group A.

† AMI = aggregated macrophage iron; MC = megaloblastic marrow change; SC = sideroblastic change.

‡ AMI 21 cases, MC and SC coexistent 8, MC without SC 9, SC without MC 5.

§ Days of observation, hematocrit, and reticulocyte values in group B did not differ significantly among patients with and without blood loss.

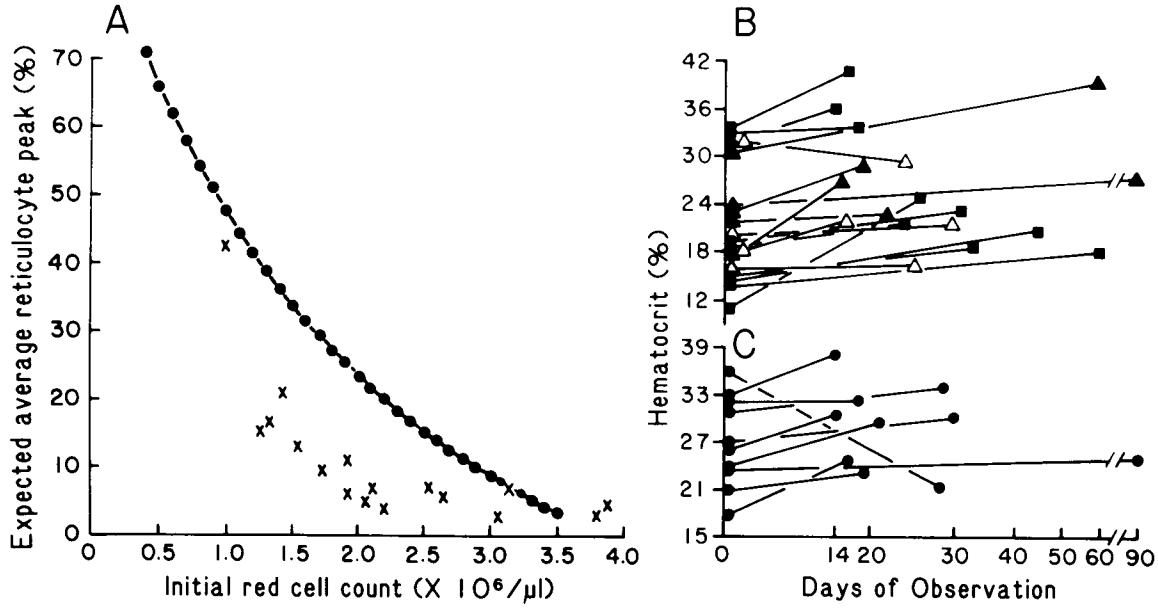

FIG. 3. A. Peak percentage reticulocyte response to folic acid plotted against initial erythrocyte count in 17 alcoholic patients with megaloblastic change observed for 7 to 15 days (x). "Expected" response curve (●) is based on observations made in patients with megaloblastic anemia due to cobalamin deficiency treated with parenteral liver extract by Sturgis and Isaacs (35), from which the figure has been adapted, as in Chanarin (3). B and C. Hematocrit values at admission and after 14 or more days of observation in 28 anemic alcoholic patients. B. Megaloblastic change in the absence of sideroblastic change (■), megaloblastic hematopoiesis with ≥ 10% ringed sideroblasts (▲), and sideroblastic without megaloblastic change (△). C. Aggregated macrophage iron in the absence of megaloblastic and sideroblastic change (●).

TABLE 9. Changes in hematocrit in 28 anemic alcoholic patients over a period of 14 or more days*

Marrow findings	Number of patients	Mean (± 1 SD) Interval (days) (range)	Hematocrit (%) Initial	Hematocrit (%) Follow-up	Hematocrit Change Number (% of group) ≥3 percent	Hematocrit Change Number (% of group) ≥5 percent
(A) AMI, excluding MC and SC†	10	28 ± 23 (14–90)	27 ± 6	29 ± 5	5 (50.0)	4 (40.0)
(B) MC and/or SC‡	18	31 ± 21 (14–90)	22 ± 7	26 ± 7	13 (72.2)	6 (33.3)

* Transfused patients excluded; acute blood loss was present in one group A patient and 3 in group B.
† AMI = aggregated macrophage iron; MC = megaloblastic marrow change; SC = sideroblastic change.
‡ AMI 15 cases, MC without SC 9, both MC and SC 5, SC without MC, 4, absent iron 2.

periods of 28 and 31 days in the 2 groups, increments in hematocrit were often minimal (Table 9 and Fig. 3B). Most patients with megaloblastic hematopoiesis and/or sideroblastic change or both demonstrated only modest increments in hematocrit despite treatment with folic acid and other vitamins (Fig. 3B).

After two weeks of hospitalization, follow-up RBC counts were available in 13 subjects with megaloblastic change, 10 of whom also had aggregated marrow iron. The erythrocyte count in these 13 patients rose from an initial mean value of 2.17 ± 0.83 × $10^6/\mu l$ (range, 1.14–3.29 × $10^6/\mu l$) to 2.58 ± 0.66 × $10^6/\mu l$ (range, 1.79–3.79 × $10^6/\mu l$), a mean increment of 0.41 × $10^6/\mu l$. In contrast, using the formula devised by Ungley (39) on the basis of studies in patients with cobalamin deficiency who were treated with cyanocobalamin, the expected increment at 15 days for patients with this degree of anemia would have been 1.86 × $10^6/\mu l$ (range, 0.98–2.30 × $10^6/\mu l$).

Because of transfusion, death and loss to follow-up, an analysis of long-term changes in MCV was not possible.

Leukocyte and platelet count

Leukopenia (white blood cell count [WBC] <3000/μl) was present in 7 patients (5.8%) at admission and thrombocytopenia (platelet count <150 × $10^3/\mu l$) in 67 (55.4%). Thus thrombocytopenia was much more commonly associated with

anemia than leukopenia in this patient group. All leukopenic patients were infected. Two with leukopenia had megaloblastic change in the bone marrow, and in two others splenomegaly and ascites were present. The mean WBC of patients with infection ($10.7 \pm 7.9 \times 10^3/\mu l$) was slightly but not significantly higher than that of subjects without evidence of infection ($8.0 \pm 4.6 \times 10^3/\mu l$). The WBC correlated with the platelet count in the presence of infection ($r = 0.69$, $p < 0.01$) but not in its absence. Neither WBC nor platelet count correlated with hematocrit, RBC, presence of acute blood loss, quantity of ethanol consumed, diet, liver function tests, evidence of hepatic disease on physical examination, or mortality. A weak correlation was noted between the plasma folate and the platelet count ($r = 0.27$, $p < 0.01$).

Mortality

Twenty-three patients (19.0%) died during their hospitalization; infection was the primary reason for death in 17 instances; hepatic failure, in 3; intracranial hemorrhage, in 1; no definite cause was apparent in 2 patients. Ten of 17 infectious deaths occurred among the 25 subjects in whom infection was found at presentation; 7 patients developed a fatal infection after admission.

There was no correlation between mortality and bone marrow findings, age, or the presence of severe hepatic dysfunction (i.e., encephalopathy, ascites, or coagulopathy) in the overall group of 121 subjects. Patients who died had markedly lower serum TIBC values (mean, 111 ± 45 $\mu g/dl$) than those who survived (248 ± 97 $\mu g/dl$, $t = 5.15$, $p < 0.001$). (A strong correlation between serum transferrin levels and mortality has previously been reported by many investigators in children with malnutrition [28, 31]). Significant, but less striking, correlations were noted between mortality and elevated serum ferritin and low serum albumin, but there was only a weak correlation with severity of anemia (data not shown).

Discussion

Several series of consecutively studied alcoholic patients in whom bone marrow examination and laboratory tests were obtained shortly after hospitalization have been reported (8, 14, 20, 42). Our patient group appears to be comparable in many ways to those previously described from American hospitals (8, 20), including the high incidence of insufficient dietary intake, hepatic dysfunction, megaloblastic and sideroblastic change in the bone marrow, macrocytosis, low serum and erythrocyte folate levels, substantial mortality rate, and a relatively low incidence of iron deficiency. Our focus, however, differed somewhat from those of previous studies in that we attempted to evaluate the diagnostic usefulness of various laboratory determinations and, as far as possible, obtain follow-up studies to indicate the adequacy and rate of recovery after treatment.

The usefulness of various tests in predicting the presence of megaloblastic marrow changes varied considerably. The MCV, unless markedly elevated (>110 fl) was of little value in detecting or excluding megaloblastic hematopoiesis. The finding that many patients with MCV's between 100 and 110 fl had normoblastic marrow morphology is consistent with the observations of numerous workers that chronic alcoholism is associated with modest increases in red cell size in the absence of megaloblastic change (23). Less expected was the finding that more than a third of the patients with megaloblastic morphology had normal MCV's, usually in the absence of associated iron deficiency (Fig. 1). In these patients, the MCV may not have been elevated because of coexistent anemia of chronic disease (34); alternatively, the megaloblastic changes may have been a superimposed finding etiologically unrelated to the anemia. Since most of our patients were black, and alpha-thalassemia involving 2 gene deletions can be anticipated to lower the MCV in approximately 2% of American blacks (5), coexistent thalassemia may account for a fraction of our patients with megaloblastic hematopoiesis and a normal MCV. However, this finding was also frequent in some prior series of predominantly white alcoholics (8, 19).

Coexisting anemia of chronic disease may have "masked" megaloblastic change in the erythroid series, accounting for some patients who showed myeloid megaloblastic change only (34). The latter finding may also be seen during recovery from megaloblastic anemias (3) but we were unable to relate it clearly in our patients to duration of time in hospital before marrow aspiration or to the presence of reticulocytosis.

Macroovalocytosis on blood smears proved to have moderately high sensitivity (90%) for megaloblastic marrows but was of low specificity and positive predictive value (Table 4). The presence of macroovalocytes seemed to be related in part to the severity of anemia, rather than its cause. Substantial numbers of macroovalcoytes (>3%) was a more specific finding, but at the price of a major reduction in sensitivity. Neutrophil hypersegmentation was found to have a high specificity (95%) for megaloblastic hematopoiesis although only moderately high sensitivity (78%). No combination of peripheral smear and/or MCV findings proved to be both highly sensitive and specific. The serum

LDH, often a useful ancillary test in predicting megaloblastic change in nonalcoholics, was of very low specificity in this group of patients with a high incidence of liver disease. In addition, the test was normal in one of 5 patients with megaloblastic morphology in the bone marrow.

Plasma folate concentrations, although generally lower in patients with megaloblastic change, were found to have such low sensitivity and specificity as to be of little diagnostic value (Table 4). Some workers have indicated an "indeterminate" or "suggestive" range of serum or plasma folate in which there is overlap between normals and patients with megaloblastic anemia (7, 19). As can be seen from Figure 2, if the diagnostic "cut-off" point is raised above 2.0 ng/ml in order to accommodate more patients with megaloblastic change, an even larger number of patients without megaloblastic morphology is included, thereby further compromising the specificity of the test. Similar problems were encountered with erythrocyte folate concentration (Fig. 2 and Table 4) or with combinations of the 2 tests of folate status. The frequent finding of low folate levels in alcoholic patients without megaloblastic change is not unexpected, since alcohol administration acutely depresses serum folate (9). Brief periods of dietary deficiency cause a fall in serum levels before the development of megaloblastic change (15) and early depletion of folate stores may account for decreased RBC folate concentrations in the absence of morphologic changes (42).

The observation that the majority of patients with megaloblastic hematopoiesis had normal plasma folate values was unanticipated, however. For comparison, we reviewed the records of 241 chronic alcoholic patients with megaloblastic hematopoiesis seen in hematologic consultation at 2 Columbia hospitals over a 15-year period. After exclusion of 46 patients with cobalamin deficiency (most of whom had pernicious anemia), 25% of the remaining 195 patients were found to have normal values for plasma or serum folate and 19% to have normal erythrocyte folate concentrations when the latter test was available. In addition, other investigators have reported alcoholic patients with megaloblastic anemia who had normal serum folate and cobalamin levels (2, 7, 8, 14, 17, 18, 30, 42). In the consecutive series of Eichner and coworkers, 11 of 26 patients (42.5%) with megaloblastic change were noted to have serum folate concentrations that were not "unequivocally deficient" (7). Eight of 20 patients with megaloblastic marrows studied by Wu et al had both normal serum and red cell folate levels (42). The majority of patients with megaloblastic change had normal serum folate values in the series of Heidemann and colleagues (14) and had normal red cell folate levels in the study of Chalmers et al (2). Six patients with megaloblastic morphology studied by Wu and coworkers had normal hepatic as well as red cell folate concentrations (41).

The reason for the not infrequent association of megaloblastic hematopoiesis and normal tests of folate status in alcoholics is uncertain. Eichner et al (7) attributed anomalous serum levels to the feeding of patients prior to testing, but this did not account for this finding in our series. Nor would resumed vitamin intake be expected to rapidly alter erythrocyte folate values. Hepatic release of folate caused by alcohol-associated liver disease (23) might explain higher than expected plasma levels but there was no correlation between severity of hepatic dysfunction and plasma folate in our patients. Wu and coworkers (42) and Heidemann et al (14) speculated that alcohol causes megaloblastic erythropoiesis by 2 separate mechanisms: the induction of folate deficiency and a direct toxic effect of ethanol on erythroid precursors unrelated to depletion of the vitamin. The latter effect remains to be demonstrated experimentally *in vivo*, with the possible exception of a study briefly reported by Sullivan and Liu (37). Our clinical observations, however, are consistent with this dual hypothesis.

Another question raised by our findings is the importance of megaloblastic change as an etiologic factor in anemic alcoholics. Although megaloblastic change was present in the marrows of 34% of our patients, a figure comparable to that of several previous series (8, 14, 20, 42) in many patients it was not the sole apparent "cause" of anemia. Indeed, multiple etiologic factors contributing to anemia appeared to be the rule rather than the exception (Table 2). Megaloblastic anemia in nonalcoholic patients is most commonly associated with the presence of iron in macrophages in finely particulate form (21, 29, 40). In contrast, in most of our patients with or without megaloblastic change, macrophage iron was coarsely aggregated, the pattern typically associated with the anemia seen in chronic inflammatory disorders (29). The importance of coexisting anemia of chronic disease (probably most frequently due to alcoholic liver disease) is further suggested by the dampened reticulocyte response (Table 8 and Fig. 3A) and failure to show substantial increases in hematocrit in many patients with megaloblastic hematopoiesis (Table 9 and Fig. 3B). Thus, although megaloblastic morphologic changes were present in the marrow of a large percentage of patients at the time of admission, in only a minority of subjects could these changes (or associated severe folate deficiency) be incriminated as the predominant cause of anemia. In 2 previous series, in which the anemia of chronic

disease was only diagnosed in 4 to 18 percent of patients (8, 20), long-term follow-up of the extent of hematologic recovery after vitamin treatment was not reported, patterns of macrophage iron accumulation were not described, and no patient was found to have a combination of the anemia of chronic disease and megaloblastic/sideroblastic changes (i.e., an attempt may have been made to assign patients to one or the other etiologic category). However, Eichner and Hillman noted lack of reticulocyte responses in some patients with severe liver disease or infection (8).

These considerations applied even more strongly to the presence of ringed sideroblasts in marrow erythroid precursors. In no patient was sideroblastic change the sole apparent cause of anemia (Table 2). In nearly every instance, macrophage iron was aggregated, in contrast to the finely particulate form characteristic of sideroblastic anemias in nonalcoholic patients (29). A strong association of sideroblastic change in alcoholics with megaloblastic hematopoiesis and folate deficiency has been noted by some previous investigators (8, 20, 30). Indeed, Pierce and coworkers have argued that folate deficiency is a virtual prerequisite for the development of sideroblastic change in alcoholics (30). Our finding that 12 of 28 patients with sideroblastic change lacked megaloblastic marrow morphology and that serum and erythrocyte folate levels were only marginally decreased in patients with ringed sideroblasts (Table 5) argues against this concept. The only laboratory finding that was strongly suggestive of sideroblastic change was the presence of siderocytes on the peripheral blood smear, which was only seen, however, in a third of the patients. The appearance of "dimorphic" erythrocyte morphology was noted in the majority, but the finding was neither sensitive nor specific (Table 5). An increase in serum iron, a characteristic feature of sideroblastic anemias that are unrelated to alcoholism (22), was present only in a minority of our patients with sideroblastic marrow change. The cause of sideroblastic change in alcoholics, which is rapidly reversible (8, 20, 25), is uncertain, although toxic effects of ethanol on heme synthesis may be important; most investigators have not been successful in demonstrating an etiologic role for pyridoxine deficiency (11, 25). We believe that the finding of sideroblastic change in an alcoholic patient has little diagnostic or therapeutic significance, unless it is present in bone marrow smears obtained more than 2 weeks after admission, in which case nonalcohol-related causes of sideroblastic anemia should be assumed to be operative.

The demonstration of iron deficiency by standard laboratory measures appeared to be less readily accomplished in alcoholic patients. The expected fall in the MCV with increasing anemia was often not apparent, possibly due to the concomitant tendency of alcohol ingestion to increase red cell size. Although none of the standard blood tests of iron status is always diagnostic under the best of circumstances, the high incidence of inflammatory disorders such as infection and alcoholic hepatitis in our patients would tend to elevate the serum ferritin and depress the TIBC (10). In fact, the ferritin was normal and the TIBC nondiagnostic or low in a substantial fraction of patients with iron deficiency anemia in both the consecutive and referral series. An important finding, however, was that the serum ferritin remained below 100 ng/ml in all of the iron deficient patients in both groups despite a high frequency of concomitant infection and liver disease; this 100% sensitivity was associated with a relatively high specificity as well (Table 6). The performance of the serum ferritin thus was superior to that of the serum iron, TIBC, or percent transferrin saturation. Only an elevated TIBC had higher specificity, but at the expense of an unacceptable lack of sensitivity. These observations were consistent with previous reports on patients with inflammatory bowel disease (38), rheumatoid arthritis (32), chronic renal failure (4), and a variety of inflammatory and hepatic disorders (26).

Recommendations

Based on our findings as well as those of others that we have cited above, we have attempted to develop a diagnostic and therapeutic approach to anemia in alcoholics. We recommend the following tests on all anemic alcoholics: MCV, careful inspection of the peripheral blood smear, reticulocyte count, and serum ferritin (Fig. 4). A serum cobalamin should be obtained in a subgroup of patients with any of 3 findings: neutrophil hypersegmentation, more than 3% macroovalocytes, or MCV exceeding 110 fl. Folic acid therapy should then be initiated in this subgroup; if the serum cobalamin level proves to be low, appropriate work-up and treatment would be indicated unless it returns to normal after therapy with folic acid. Bone marrow examination would not be performed routinely in any patient for the purpose of demonstrating megaloblastic or sideroblastic change. In patients with a serum ferritin below 20 ng/ml iron therapy would be started. In those with equivocal ferritin levels (between 20 and 100 ng/ml), the serum iron/TIBC would be determined. Iron therapy would be given if the TIBC were elevated, and a bone marrow aspirate obtained for evaluation of iron stores in the remainder. In those patients with demonstrated iron deficiency, appropriate work-up (with an em-

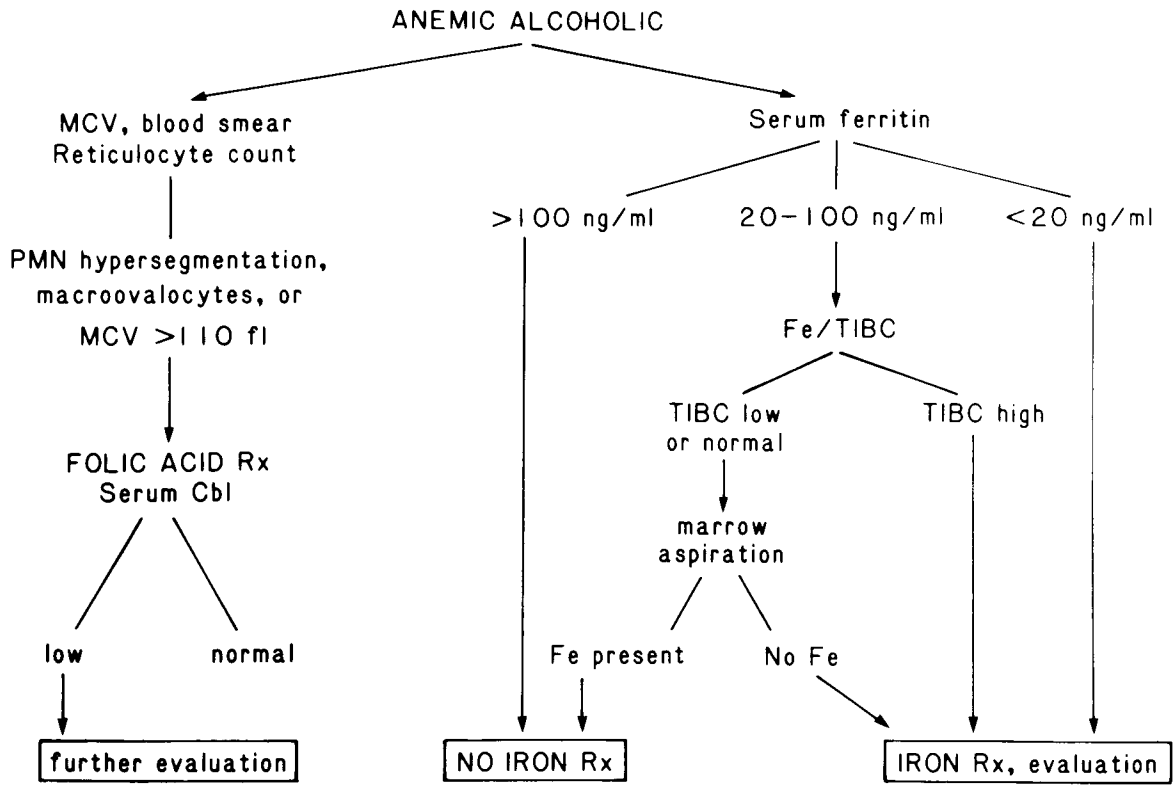

FIG. 4. Diagnostic and therapeutic approach to anemia in alcoholics (cbl = cobalamin, Fe = iron, fl = femtoliters, MCV = mean corpuscular volume, PMN = polymorphonuclear leukocytes, Rx = therapy, TIBC = total iron binding capacity).

phasis on possible sources of blood loss) would be indicated.

These recommendations have been designed with an eye on reducing the number of costly diagnostic tests without sacrificing useful diagnostic information. We do not include routine determination of serum or erythrocyte folate levels or of iron/TIBC concentrations. These tests, when viewed critically in our series of patients, were as often misleading as they were helpful. Had these recommendations been applied prospectively to our series of 121 patients, cobalamin and serum iron/TIBC levels would have been obtained in only 41 and 13 patients, respectively, and bone marrow examination would have been required in 9. We favor the selective therapeutic use of folic acid in patients with a high likelihood of megaloblastic change while simultaneously ruling out cobalamin deficiency rather than the indiscriminate use of folate in all patients, since we have encountered several alcoholics with combined systems disease who were treated at New York City hospitals with folic acid only.

Two points should be emphasized concerning these recommendations. One is that they are meant as guidelines for the use of laboratory tests rather than rigid algorithms to be applied in all patients; they cannot substitute for the findings of a careful history and physical examination, which may lead to modifications in diagnostic approach. Secondly, they are tentative guidelines, based on currently available data. To our knowledge, this is the first study to evaluate the sensitivity, specificity, and predictive value of standard tests in the diagnosis of anemia in alcoholics. The incidence and etiology of anemia may vary considerably with the selection of patients for study (7) and our findings may not necessarily apply universally. Since the data have been obtained on a group of acutely ill, city hospital alcoholics with a high incidence of malnutrition, further studies are needed to test the general validity of our recommendations in the evaluation of this commonly encountered clinical problem.

Summary

In order to develop a diagnostic approach to the common problem of anemia associated with alcoholism, 121 chronic alcoholics admitted to a general medical service with a low hematocrit were evaluated. Multiple contributing causes of anemia were present in most patients. Megaloblastic marrow change was found in 33.9% of patients, sideroblastic

change in 23.1%, absent iron stores in 13.2%, aggregated macrophage iron in 81.0%, and acute blood loss in 24.8%.

The MCV was of little value in predicting the presence of megaloblastic change unless markedly elevated (>110 fl). In 15 of 41 patients with megaloblastic marrow morphology (36.6%) the MCV was normal or low. Among 40 patients with MCV values between 100 and 110 fl, megaloblastic change was not present in the bone marrow smears of 24 (60.0%). Neutrophil hypersegmentation was 95% specific but only 78% sensitive for megaloblastic change; in contrast, the presence of macroovalocytosis was 90% sensitive but only 68% specific. Serum lactic dehydrogenase, plasma folate, and erythrocyte folate levels had such low sensitivities and specificities for megaloblastic change as to be of little predictive value. Hematologic responses to folic acid were often inadequate in patients with megaloblastic morphologic changes, apparently because of associated acute and chronic illness.

Our findings are consistent with the hypothesis that 2 mechanisms account for the development of megaloblastic hematopoiesis in alcoholics: 1) induction of folate deficiency and 2) a direct toxic effect of alcohol on erythroid precursors independent of folate depletion, as reflected by the presence of normal plasma and erythrocyte folate levels in several patients with megaloblastic change.

In no patient was sideroblastic change the sole apparent cause of anemia. Megaloblastic hematopoiesis and aggregated macrophage iron frequently accompanied sideroblastic change. Examination of the blood smear revealed siderocytes in one-third of patients with sideroblastic marrows and dimorphic erythrocyte morphology in the majority. Dimorphic blood smears, however, were neither sensitive nor specific for sideroblastic change. Serum iron concentrations were usually not elevated in the group with sideroblastic abnormalities.

In predicting marrow iron stores, serum iron and iron-binding capacity concentrations were often non-diagnostic or misleading. Serum ferritin levels <100 ng/ml, however, showed 100% sensitivity and 95% specificity for absent marrow iron stores despite the frequent presence of abnormal liver function.

On the basis of our findings, practical guidelines have been formulated for the evaluation and therapy of anemia in alcoholics.

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