Masked macrocytosis and the use of serum vitamin B_{12} and folate assays[1]

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A case of "masked" macrocytosis in an iron and folate deficient male alcoholic is presented. Reliance on algorithms based on mean corpuscular volume (MCV) values for decision-making in the workup of anemic patients may, occasionally, produce diagnostic difficulties, and combination deficiencies or concomitant disease states may affect test interpretation. Appropriate use of radioisotopic dilution assays for vitamin B_{12} and folate depends on careful clinical observation, blood count, and screening examination of the peripheral smear before confirmatory tests are ordered.

Index terms: Anemia, macrocytic • Folic acid deficiency • Vitamin B_{12} deficiency

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Appropriate laboratory investigations of anemia have been extensively discussed,[1–3] and various algorithms have been created to indicate appropriate strategies for evaluation of treatable deficiencies of vitamin B_{12} and folate. These algorithms, although carefully designed, are not always reflective of the realities of patient evaluation. We illustrate some problems which may arise in the interpretation of Coulter mean corpuscular volume (MCV) values and pitfalls in the appropriate use and interpretation of radioisotopic assays for serum vitamin B_{12} and folate. The logical progression of diagnosing macrocytic anemia from a Coulter MCV greater than 100 fl and then ordering vitamin B_{12} and folate levels may, under certain circumstances, be a dangerous oversimplification which creates diagnostic confusion.

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1 A, B

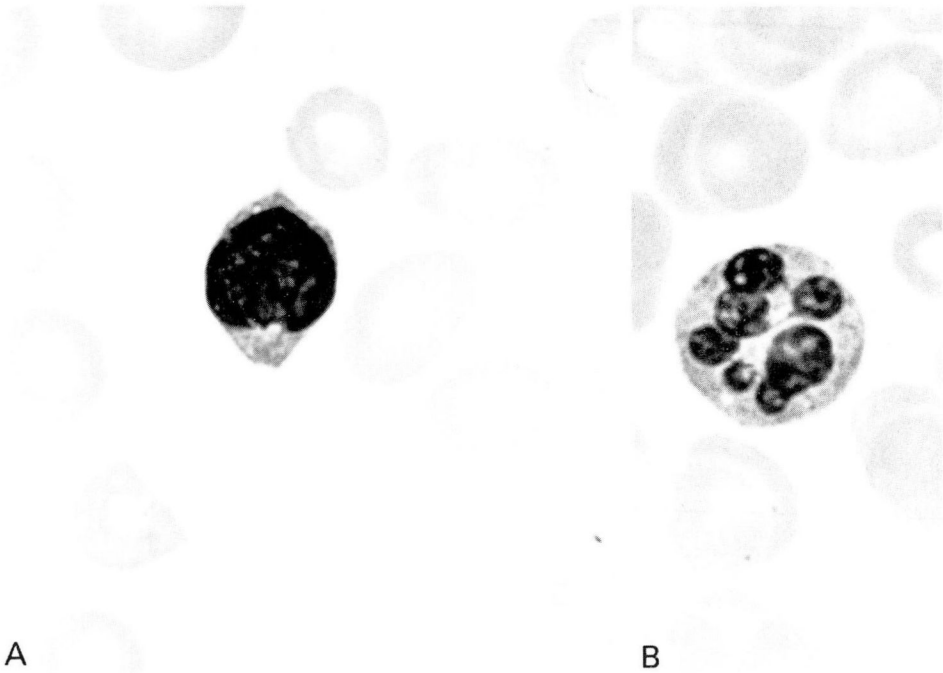

Fig. 1. A. Oval macrocytes surround this normal small lymphocyte (Wright's stain, original ×800).
B. Hypersegmented (six-lobed) neutrophil in patient's peripheral blood (Wright's stain, ×800).

Case report

A 26-year-old black male student with a history of alcohol and drug abuse was admitted for investigation of weakness and dizziness. On admission his complete blood count (CBC) values showed severe anemia, with minimal neutropenia and thrombocytopenia (*Table 1, Count A*). Despite no obvious macrocytosis based on red cell indices (MCV of 97.9 fl), peripheral smear examination demonstrated oval macrocytes and hypersegmented polymorphonuclear leukocytes. (*Fig. 1*); anisocytosis and poikilocytosis were also noted. Bone marrow aspiration showed intense megaloblastic erythroid hyperplasia with marked changes in granulocytic precursors (*Fig. 2*). Serum folate was less than 1 ng/ml (normal > 2.3 ng/ml), and vitamin B_{12} was 350 pg/ml (normal 170–700 pg/ml) by radioisotopic assay. Serum iron and iron binding capacity were 71 μg/dl and 360 μg/dl, respectively, with a transferrin saturation of 20%; bone marrow iron stain showed 4+ iron stores. The patient was transfused with 2 units of packed cells, and therapy with folate 1 mg daily by mouth without iron supplementation was begun; he displayed a prompt reticulocyte response which peaked on the eighth day at 16%. He was discharged from the hospital with the CBC values labeled as Count B in *Table 1*.

Approximately 6 months later he was seen as an outpatient complaining once again of weakness and found to have a moderate microcytic hypochromic anemia (*Table 1, Count C*). Bone marrow aspiration at this time showed unremarkable normoblastic erythroid hyperplasia but a total absence of stainable iron. Serum iron was 33 μg/dl and iron binding capacity 513 μg/dl, with 6% transferrin saturation. Iron deficiency anemia was diagnosed, and he was treated with ferrous sulfate tablets 20 mg bid; he again displayed a reticulocyte response. He has experienced no further problems with anemia.

Discussion

The problems encountered in the diagnosis and management of this patient's anemia illustrate interesting points in the differential diagnosis of macrocytic anemia. The algorithm approach for anemia evaluation usually consists of a flow diagram beginning with evaluation of the red cell indices generated by a Coulter S series analyzer or similar laser counter. These are used to select further "appropriate" diagnostic laboratory tests. Most such algorithms[1–3] establish an MCV of 100 fl as the cutoff point for the diagnosis of pernicious anemia and folate deficiency; one authority states: "MCV. . .less than 100 fl is *not* pernicious anemia."[2] Indeed , profound anemia without abnormalities in the Coulter MCV may be the only manifestation in the CBC values of severe underlying vitamin B_{12} or folate deficiency—so-called "masked macrocytosis." Masked macrocytosis is not rare, occurring in as many as 9% of patients with megaloblastic anemia.[4,5] Masked macrocytosis with Coulter MCV values as low as 85 fl may occur at presentation in patients with pernicious anemia or dietary folate deficiency.[6,7] Masked macrocytosis due to pernicious anemia or folate deficiency is most likely to occur as a result of concomitant deficiencies of both iron and folate in chronic alcoholics,[7]

2 A, B

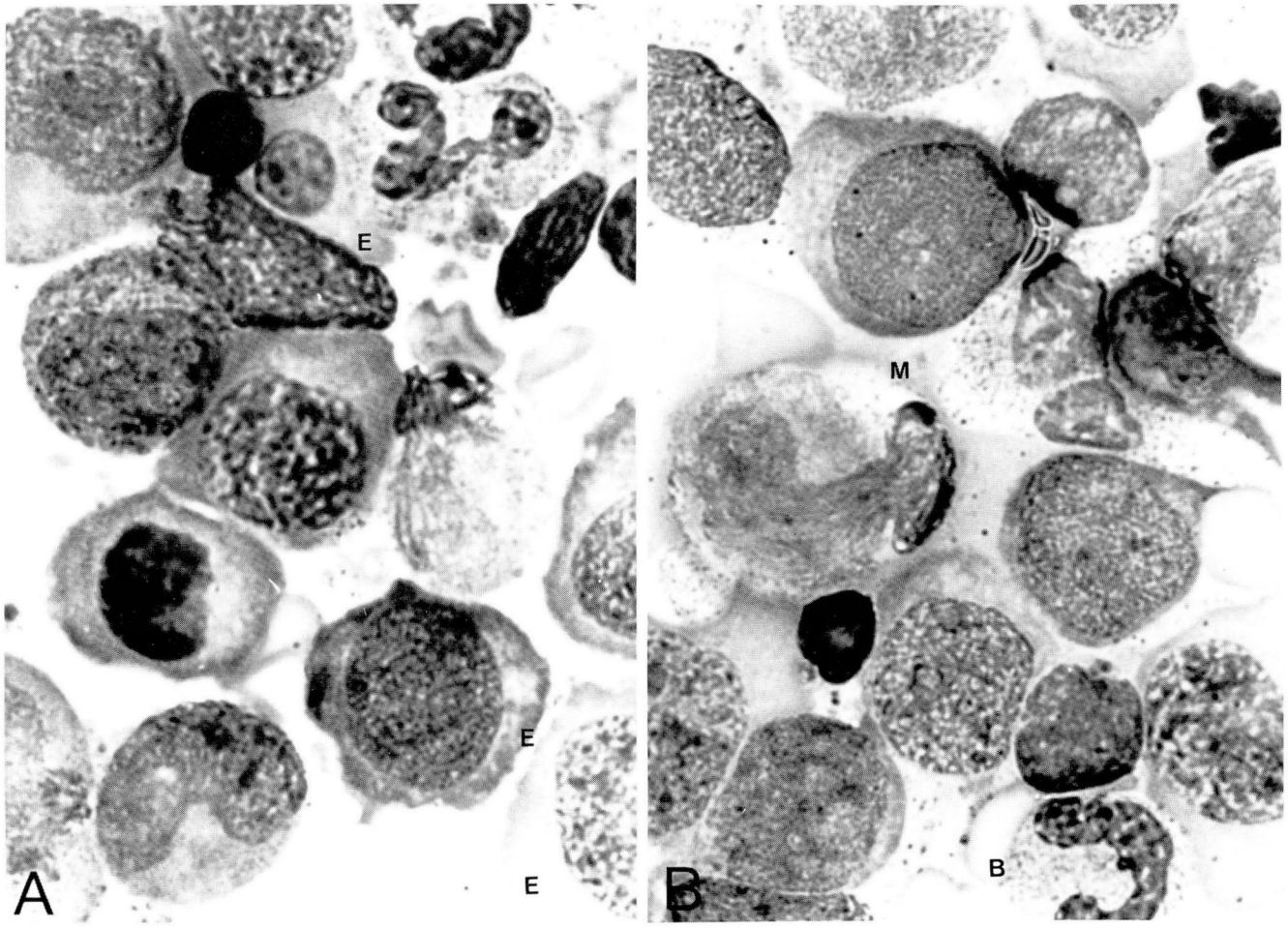

Fig. 2. A. Markedly megaloblastic erythroid precursors (*E*) from bone marrow aspirate (Wright's stain, ×800). **B.** Giant metamyelocytes (*M*) or band forms (*B*) in marrow (Wright's stain, ×800).

and, as in the present patient, the assessment of iron stores by serum iron or ferritin levels, iron binding capacity, or examination of a marrow smear stained for iron can all yield normal or misleading results.[7,8] Iron stores may appear adequate or increased, but following replacement of the missing vitamin, rapid incorporation of iron into new red cells leads to exhaustion of limited available iron and to rapid transition from megaloblastic to iron deficient erythropoiesis with consequent development of microcytic hypochromic anemia.

Another cause of masked macrocytosis, especially in blacks, Asians, and other affected ethnic groups, is coexisting folate deficiency or pernicious anemia and thalassemia.[6–9] How may such patients be detected? In the masked macrocytosis cases reported by Spivak,[7] smear evaluation disclosed abnormalities limited primarily to the white cell series—abnormal giant bands or hypersegmented neutrophils. Macrocytosis was difficult to detect by smear evaluation in these patients, but Howell-Jolly bodies, though prolonged searching was required to locate them, were seen in occasional red cells in 5 of the 8 patients

Table. CBC values

Test time	Count A admission	Count B discharge	Count C follow-up	Units
WBC	2.8	14.8	10.2	$\times 10^9$/L
RBC	1.32	5.57	5.43	$\times 10^9$/L
Hgb	4.41	13.5	10.7	g/dl
Hct	0.129	0.414	0.353	....
MCV	97.9	74.2	64.9	fl
MCH	33.6	24.2	19.7	pg
MCHC	34.3	32.7	30.4	%
PLT	141	376	403	$\times 10^9$/L
RDW	16.3	19.2	18.4	U.

Abbreviations: WBC = white blood count; RBC = red blood count; Hgb = hemoglobin; Hct = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelet count; RDW = red cell distribution width.

studied. Herbert[10] and Lindenbaum[11] also stress the value of smear examination for the detection of granulocytic maturation abnormalities indicative of masked macrocytosis. Another form of masked macrocytosis readily seen on smear is a mixed population of schistocytes (red cell fragments) and macrocytes resulting in an overall normal MCV. This combination is particularly likely to occur in patients with megaloblastic anemia[5,6] who are also septic.

What of the interpretation of an elevated MCV generated by Coulter S series analyzers? In only a minority of patients with an elevated MCV will abnormally low values for serum vitamin B_{12} or folate be found indicating treatable vitamin deficiencies. The Coulter MCV is a very sensitive indicator for minor degrees of macrocytosis that would be overlooked by simple smear examination. Thus, since the introduction of these instruments, investigators have found that a minor degree of macrocytosis may be seen in a variety of clinical situations that do not reflect treatable deficiencies of any vitamin and which will not respond to vitamin therapy. In a study by Davidson et al[12] of 68 patients with an MCV in the 100–110 fl range, only 17 had decreased vitamin B_{12} or folate levels (25%); of 8 patients with an MCV over 110 fl, 7 had abnormal levels and treatable vitamin deficiencies. The patients with an MCV of 100–110 fl had round, not oval, macrocytes on smear, and neutrophils were not abnormal. A combination of round macrocytes without hypersegmented neutrophils or giant bands is commonly seen in patients who consume excess alcohol,[13,14] following chemotherapy,[14] and, on occasion, in individuals with aplastic anemia or myelodysplastic syndromes.[15] Thus, the likelihood of megaloblastic anemia due to treatable vitamin deficiencies is very small in a patient with an MCV between 100 and 110 fl and with round macrocytes and normal neutrophils seen on smears. Vitamin assays in this situation are unlikely to yield useful diagnostic information.[16]

The assay of serum vitamin B_{12} and folate has been criticized for a variety of technical reasons. The most serious technical problem has received wide attention as the result of the studies by Kolhouse et al[17] and Cooper and Whitehead.[18] These authors reported that several commonly used kit radioassays for serum vitamin B_{12} determination evaluated between 1977 and 1979 yielded false-normal vitamin B_{12} levels in patients with low or borderline low vitamin B_{12} levels as determined by microbiologic assays. These patients actually had treatable pernicious anemia. Kolhouse et al[17] showed that the radioassay kits contained cobalamin binders capable of binding so-called B_{12} analogues or biologically inactive cobalamins. These nonspecific binders made up 50% to 80% of the stated B_{12} binding capacity. The presence of such cobalamin analogues in normal and B_{12}-deficient human serum remains controversial.[19–21] However, since publication of these articles, the manufacturers of kit radioassays for vitamin B_{12} have adopted two strategies to prevent false-normal levels from being reported. Pure intrinsic factor, usually prepared from hog intestinal mucosa, may be used, which will not bind serum analogues. However, since pure intrinsic factor is expensive and difficult to prepare, other manufacturers have elected to retain their impure binders but to block any nonspecific binding of analogues by addition of an excess of synthetic unlabeled analogues to the binder. This allows for determinations of the so-called "true" rather than "total" (true plus analogue) cobalamin level. In 1982, Oxley reevaluated the nonspecific analogue–binding of currently available radioassay kits for serum vitamin B_{12} (personal communication). He found that all test kits evaluated had less than 10% analogue-binding capacity—a significant improvement from the 50%–80% observed in the Kolhouse study. Finally, in 1983, field evaluation of vitamin B_{12} determinations suggests that, if anything, the frequency of low vitamin B_{12} radioassay levels exceeds the frequency of pernicious anemia or true vitamin B_{12} deficiency in the general population.[22] The serum vitamin B_{12} assay may also give misleading results in some clinical situations.[23] Normal serum vitamin B_{12} levels in the presence of true tissue deficiency can be found in congenital transcobalamin II deficiency,[24] following prolonged inhalation of nitrous oxide,[25] and following the recent injection of vitamin B_{12} such as that given for the Schilling test. The converse situation, low serum vitamin B_{12} levels without tissue deficiency, is seen in pregnancy,[26,27] in pure folate deficiency,[26,28] veganism,[26] and in congenital or acquired transcobalmin I deficiency.[29] With the exception of folate deficiency and pregnancy, these situations are rare.

The assay of serum folate by either microbiologic (*Lactobacillus casei*) or radioisotopic dilution methods is beset with both physiologic and tech-

nical artifacts.[12] Tissue folate deficiency may be present despite normal serum folate levels obtained by radioassays, especially in alcoholics[30] and anorexics.[12] Conversely, falsely low levels of folate by microbiologic assay may be found in patients taking antibiotics[30] or hydantoin derivatives[31] and during the third trimester of pregnancy.[12]

Both gallium and technetium scans reportedly have major effects on radioisotopically determined folate and B_{12} levels.[32] A falsely low serum folate level usually results when these isotopes are present, but in vitamin B_{12} measurement either falsely high or falsely low values may occur depending on whether the isotope is separated in the bound or free fraction. Effects may persist for as long as 2 weeks after the scan is performed.[32]

Appropriate interpretation of Coulter MCVs and appropriate use of radioisotopic vitamin assays in the investigation of anemia depend on judicious clinical evaluation. Vitamin assays should not be ordered indiscriminately.[16] If the Coulter printout indicates macrocytosis, smear evaluation is mandatory to assess the degree of macrocytosis, the shape of the macrocytes, and the presence of morphologic abnormalities in granulocytes. The efficiency of vitamin assays, either microbiologic or radioisotopic, for detection of treatable vitamin deficiencies is very low in a patient whose MCV is 100–110 fl without oval macrocytes or changes in granulocytic maturation. Assays should not be used in these situations unless other symptoms, such as neurologic changes, suggest vitamin deficiency. If the MCV is normal or slightly elevated and smear evaluation shows oval macrocytes or neutrophilic maturation abnormalities, masked macrocytosis may be present, and serum vitamin B_{12} and folate measurement may be useful for diagnosis and early treatment, with possible prevention of neurologic damage.[33] However, the presence of masked macrocytosis in such situations should alert the physician to the possibility of complicating disorders such as iron deficiency or thalassemia; the presence of a vitamin deficiency may affect the result of the tests for iron deficiency.[34] In combined vitamin and iron deficiency, little change may be seen in the red cell indices or morphology[6]; giant metamyelocytes or hypersegmented polymorphonuclear leukocytes may be the only manifestation of a severe treatable vitamin deficiency. Finally, improvement since 1979 in the technique of radioisotopic dilution assays for serum vitamin B_{12} makes it most unlikely that a patient with treatable pernicious anemia would give a falsely normal vitamin B_{12} assay; the same statement cannot yet be made for serum folate assays. The basic guideline in using laboratory data for the investigation of macrocytic anemia is that if clinical and laboratory observations are discordant, the physician should be aware of the conditions that can give rise to this situation and should seek a rational explanation for the discrepancy.

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