Neutrophil Myeloperoxidase Measurement Uncovers Masked Megaloblastic Anemia

By Margaret L. Gulley, Stuart A. Bentley, and Dennis W. Ross

We report the observation of a high neutrophil myeloperoxidase activity (MPXI) in patients with megaloblastic anemia. MPXI is rapidly measured as part of an automated complete blood count (Technicon H*1, Technicon Instruments Corp, Tarrytown NY). We describe the range of MPXI levels in healthy and patient populations and in 10 cases of megaloblastic anemia, including five with elevated mean cell volume (MCV) and five without macrocytosis. Regardless of the MCV, our megaloblastic patients had hypersegmented neutrophils and elevated MPXI levels without visible alteration of granule content. MPXI measurement may be particularly useful in identifying cases of "masked megaloblastic anemia" where the MCV is below 100 fl. The advantage of the MPXI over other methods of uncovering masked megaloblastic anemia is its simplicity when performed as part of a routine complete blood count on an automated hematology instrument.

THE CHARACTERISTIC blood findings of megaloblastic anemia (macroovalocytes and neutrophil hypersegmentation) result from inadequate folate or defective folate utilization as occurs in vitamin B12 deficiency. The macrocytosis can be obscured by concomitant disorders that in themselves cause microcytosis, resulting in masked megaloblastic anemia. For example, iron deficiency,1,2 hereditary elliptocytosis,3 α thalassemia,2 β thalassemia,4,5 hemoglobin H disease,6 or fragmentation7 coexisting with megaloblastosis have resulted in masked megaloblastic anemia. Transfusion can cause spurious masked megaloblastosis (unpublished observation). Although the macrocytosis of megaloblastic anemia may be obscured, the leukocyte abnormalities are preserved.

We report an abnormally high neutrophil myeloperoxidase activity in 10 patients with megaloblastic anemia, including five patients with masked megaloblastosis. Myeloperoxidase measurement by flow cytometry may be particularly useful in identifying megaloblastosis complicated by other erythrocyte abnormalities.

MATERIALS AND METHODS

Megaloblastic anemia without macrocytosis (ie, masked megaloblastic anemia) was diagnosed in five patients by the University of North Carolina Hospital's hematopathology service from 1986 through 1989. During the same period, five cases of typical macrocytic megaloblastic anemia were seen by the hematopathology service, four with B12 deficiency and one with combined B12 and folate deficiencies. All megaloblastic anemia patients had the following abnormalities: low folate or vitamin B12 level, low hemoglobin, hypersegmented neutrophils, and elevated lactate dehydrogenase (LDH). The marrow (examined in 8 of 10 cases) was hypercellular with giant granulocyte precursors and enlarged erythroid precursors with open nuclear chromatin for the degree of cytoplasmic maturation. Clinical and laboratory data were retrieved by reviewing patient and laboratory records.

Blood counts including myeloperoxidase measurements were done on a Technicon H*1 instrument (Technicon Instruments Corp, Tarrytown, NY) on whole blood collected in EDTA. Quality-control procedures included the use of the manufacturer's reference material as a primary standard, whole blood calibration, and data monitoring by a moving average algorithm.

A normal value study was performed on 111 medical student blood samples obtained as part of routine health care screening. A patient value study was performed on 205 consecutive unselected blood samples from both clinic and hospitalized patients. Mean and standard deviation (SD) were calculated using Statgraphics (STSC, Rockville, MD). Means were compared by the Wilcoxon sign rank test.

CASE REPORTS

Detailed case histories are provided for the five patients with masked megaloblastic anemia, since it is these patients who are least suspected of having megaloblastosis on initial examination and in whom the myeloperoxidase activity (MPXI) test may provide a clue to the correct diagnosis.

Case 1. Masked pernicious anemia. A 16-year-old black teenager underwent a partial thyroidectomy for Grave's disease. His white blood cell (WBC) count was 3.0 (normal 4 to 12 × 10^3/μL), hemoglobin (Hb) 12.4 (normal 12.3 to 17.4 g/dL), mean cell volume (MCV) 67 (normal 81 to 97 fl) and platelets 245 (normal 150 to 444 × 10^3/μL). At age 19 he presented with fever, progressive fatigue, and weight loss. On physical examination he had splenomegaly and trace guaiac positive stool. Neurologic examination was unremarkable and thyroid function tests were normal. His WBC count was 1.6; Hb 4.1 with a reticulocyte count of 0.8% (normal 0.8% to 2.5%); MCV 64; platelet count 34; and LDH 9,600 (normal 31 to 261 U/L). The MPXI was elevated to 20.2 (manufacturer's normal value range 10 to 10). The blood smear showed leukocytoblastosis, hypersegmented neutrophils, dacrocytes, oval macrocytes, and red blood cell (RBC) fragments. The marrow was 100% cellular with marked erythroid hyperplasia, megaloblastic erythropoiesis, and giant granulocytic precursors. Storage and erythroid iron were adequate. Focal necrosis was found on marrow biopsy sections. Hb electrophoresis showed a normal migration with Hb A2 of 2.7% (normal 1.6% to 3.5%) and mildly elevated Hb F of 3.2 (normal less than 2%). B12 was below 50 (normal 200 to 963 pg/mL); folate 8.6 (normal 1.9 to 13.6 ng/mL); iron 38 (normal 35 to 165 μg/dL); and total iron binding capacity (TIBC) 190 (normal 320 to 550 μg/dL). He was treated with 5 U of RBCs, oral folate (for 3 days), and vitamin B12 injections. Five days later the reticulocyte count was 5%. Eight weeks later the WBC count was 3.9, Hb 13.6, MCV 73, and platelets 256. Neutrophil hypersegmentation resolved and the MPXI returned to normal at 4.4. The blood smear contained numerous spherocytes, teardrop cells, and frag-
ments. Hb A2 was still normal at 2.2% and Hb F remained elevated to 3.5%. A year later the WBC count was 3.2, Hb 14.3, MCV 75, and platelets 208 with persistent microcytosis and rare ovalocytes. Iron studies were not repeated. Further work-up of his erythrocyte abnormality was not performed.

The low vitamin B12 level combined with his response to vitamin B12 administration suggests that vitamin B12 deficiency was the cause of his megaloblastic anemia, presumably pernicious anemia in light of his previous autoimmune thyroid disease. The coexisting erythrocyte defect cannot be defined due to limited follow-up.

**Case 2. Masked vitamin B12 deficiency.** A 28-year-old black woman presented with fatigue and abdominal pain. Her hematocrit was 25%, and she was treated with oral ferrous sulfate. Nine months later she developed pelvic inflammatory disease, and at that time her WBC count was 3.5, Hb 5.4, and MCV 97. The blood smear showed poikilocytosis with many teardrops. The marrow was hypercellular with megaloblastic erythropoiesis, giant granulocytic precursors, and hypersegmented neutrophils. Storage iron was mildly decreased. She was treated with blood transfusion but no vitamin therapy. Three months later she had a WBC count of 3.1, Hb 3.5 with 1.0% reticulocytes, MCV 88, platelets 171, and LDH 9,700. MPXI was elevated to 23.7. The smear showed numerous erythrocyte fragments, teardrops, macroovalocytes, and nucleated RBCs, as well as neutrophil hypersegmentation. The marrow had megaloblastic features with adequate storage iron. Serum iron was 237, TIBC 207, and ferritin 295 (normal 11 to 120 ng/mL). B12 was less than 50, folate 6.8, osmotic fragility normal, and Hb electrophoresis normal. She was administered 2 U of RBCs, a brief course of oral folate, and monthly intramuscular vitamin B12 therapy. One week later her WBC count was 3.7, Hb 8.5 with 7.9% reticulocytes, MCV 93, and platelet count 313.

Three years later she presented with an acute subarachnoid hemorrhage. WBC count was 22.9, Hb 15.9, MCV 82, platelets 374, and LDH 400. MPXI was normal at 2.2. She died the next day and postmortem examination revealed a 40% cellular marrow with mild granulocytic hyperplasia, left-shifted granulopoiesis, and unremarkable erythropoiesis. Storage iron was mildly decreased and erythrocytoid iron was adequate. There was no morphologic evidence of macrocytosis.

The patient recovered from vitamin B12 deficiency, presumably pernicious anemia. The lack of macrocytosis remains inexplicable.

**Case 3. Masked folate deficiency.** A 36-year-old obese black woman underwent gastric stapling and cholecystectomy 10 years previously, at which time her blood counts and smear morphology were unremarkable (WBC 9.4, Hb 13.4, MCV 89, platelet 310, and LDH 185). Three years ago she presented with an acute febrile illness. Her WBC count was 6.4, Hb 6.3 with 0.4% reticulocytes, MCV 96, platelets 185, folate less than 0.7, B12 203, iron 190, and TIBC 304. MPXI was elevated to 21.1. She was diagnosed and treated for folate deficiency and lost to follow-up until 1 year ago when she presented with a 5-month history of fatigue, nausea, and amenorrhea. Additional history included a poor diet (jello, fruit juice, and occasional fruit, no vegetables in at least 6 months), pica (cornstarch and red clay), and ethanol abuse. Her WBC count was 2.7, Hb 3.2 with 9.7% reticulocytes, MCV 88, platelets 90, LDH 6,138, AST 77 (normal 6 to 17), ALT 13 (normal 4 to 24), alk phos 45 (normal 9 to 90), folate 0.7, B12 198, iron 284, and TIBC 355. MPXI was elevated to 32.8. The blood smear contained hypersegmented neutrophils, teardrops, macroovalocytes, and RBC fragments. The marrow showed erythroid hyperplasia with megaloblastic erythropoiesis and giant granulocytic precursors. Storage iron was adequate and erythrocytoid iron was increased with numerous ringed sideroblasts. Hb electrophoresis was normal including Hb A2 of 2.7 and Hb F of 0.2. She was treated with supplemental folic and vitamin B12 and responded with a reticulocytosis of 7.5% after 4 days. Her WBC count was 11.0, Hb 3.5, MCV 92, and platelet count 192. The MPXI declined to 28.5. Three weeks later her WBC count was 7.1, Hb 9.1 with continued reticulocytosis of 4.4%, MCV 86, and platelet count 422.

She failed to renew her folic acid prescription and was lost to follow-up until this year when she presented with weakness and shortness of breath. Her WBC count was 6.2, Hb 5.1 with 1.6% reticulocytes, MCV 95, platelets 227, LDH 5576, folate less than 0.7, B12 219, iron 343, TIBC 420, and ferritin 524. Liver function tests were essentially unchanged from the previous values. MPXI was normal at 6.5. The blood smear and marrow were unchanged except that ringed sideroblasts were rare. She was treated with folic acid but response to therapy could not be monitored due to patient noncompliance.

The low serum folate levels indicate that the primary cause of megaloblastic anemia was folate deficiency due to gastric surgery, poor dietary intake, and/or alcoholism. The lack of macrocytosis could be due to erythrocyte fragmentation, sideroblastic anemia, and/or chronic disease (alcoholic liver disease). Although the patient presented with masked folate deficiency on three separate occasions, only in the first two instances was the MPXI elevated. A possible explanation for the lack of elevated MPXI on her third presentation could be that she was partially folate-repleted as evidenced by the relatively high reticulocyte count (1.6%) compared with prior presentations (0.4 and 0.7%).

**Case 4. Folate deficiency masked by hereditary elliptocytosis.** A 23-year-old gravida III para II black woman gave a history of "abnormally shaped red cells." During the current and previous pregnancies, she took supplemental iron but no folic acid. Just before the delivery of her first child she had an Hb of 10.0 and MCV of 87, and just before her second delivery her Hb was 11.9 and MCV 86. Early in her third pregnancy she had an Hb of 9.4, MCV 89, Fe 77, and TIBC 279. The blood smear showed marked ovalocytosis without neutrophilic hypoparepsis. Later in pregnancy there was progressive anemia and reticulocytopenia with normal MCV, and she was referred for hematologic consultation at 32 weeks of gestation. The WBC count was 6.2, Hb 5.6 with 0.9% reticulocytes, MCV 88, platelet count 398, and LDH 2,586. The MPXI was elevated to 15.8. The blood smear revealed hypersegmented neutrophils and marked ovalocytosis. The bone marrow was 90% cellular with marked erythroid hyperplasia, megaloblastic erythropoiesis, and giant granulocytic precursors. Storage and erythrocytoid iron were adequate. Folic acid was 1.2 and vitamin B12 was 193. Two months after folate therapy and just before childbirth, the WBC count was 17.1, Hb 11, MCV 86, and platelet count 276. Two months after delivery, the WBC count was 6.5, Hb 11, MCV 83, and platelet count 433. The patient had folate deficiency associated with pregnancy. Macrocytosis was apparently masked by underlying hereditary elliptocytosis.

**Case 5. Masked vitamin B12 deficiency.** A 78-year-old white woman had a 6-month history of progressive low-back pain, weight loss, and weakness. On physical examination she was cachectic with guiac negative stools. WBC count was 3.8, Hb 3.8, MCV 95, platelets 139, LDH 745, and MPXI 8.9. The blood smear contained anisocytic RBCs and hypersegmented neutrophils. Vitamin B12 was 59, folate 3.5, iron 24, TIBC 314, and ferritin 9. She was transfused 4 U of RBCs, and the next day her Hb was 9.8, MCV 89, and MPXI 12.8. Her bone marrow was 70% cellular with erythroid hyperplasia, megaloblastic erythropoiesis, and giant metamyelocytes and bands. Iron stores were absent, although erythroid iron was adequate. No tumor was identified. Despite treatment with intramuscular vitamin B12 and oral iron supplements, her Hb remained around 9 with an MCV in the mid 80s. Five months later she was found to have disseminated adenocarcinoma, presumably of ovarian origin, and she died soon thereafter.
In addition to occult tumor and vitamin B₁₂ deficiency, the patient probably had iron deficiency and/or anemia of chronic disease to account for the lack of macrocytosis. An initial MPXI of 8.9 inexplicably increased to 12.8 after transfusion, but further MPXI measurements were not performed.

RESULTS

In 111 healthy medical students the mean MPXI was -2.1 with an SD of 5.8, while our general patient population had a mean of -2.8 with an SD of 7.4 (see Fig 1). The means of the student and patient populations were not statistically different from each other (P > .56).

In the study group of 10 consecutive megaloblastic anemia patients, average MPXI levels ranged from 8.9 to 34.1. Table 1 shows the Hb and MPXI levels of each patient, grouped by MCV values. The first group had masked megaloblastic anemia (MCV < 99) while the second group had typical macrocytic megaloblastic anemia (MCV ≥ 100). There was no significant difference between the two groups with respect to Hb (P > .30) or MPXI (P > .35), while the MCV was expectedly different (P < .01). All 10 patients had hyposegmented neutrophils but there was no morphologic alteration in primary granule content or size.

DISCUSSION

The initial work-up of patients with anemia should include a blood smear search for macroovalocytes and neutrophil hypersegmentation to screen for megaloblastosis. It is generally accepted that six-lobed neutrophils, significant numbers of five-lobed cells, or a lobe average over 3.5 is suggestive of megaloblastic hypersegmentation. When the erythrocyte volume changes are masked by concomitant microcytic conditions, changes in neutrophil appearance may be the only morphologic clue to a megaloblastic etiology of anemia. Heretofore, automated instruments have failed to detect the neutrophil-related manifestations of megaloblastic anemia.

We have observed that neutrophil myeloperoxidase is elevated in patients with megaloblastosis, and we have identified a simple automated method of detecting the elevation. This simple test, the MPXI, can be used as an auxiliary test for megaloblastosis.

The biologic basis for an elevated MPXI in megaloblastosis appears to be the presence of an increased number of myeloperoxidase-laden granules in neutrophils due to skipped cellular divisions during maturation. Myeloperoxidase is synthesized in the promelocyte where it is packaged into azurophilic granules. During each of three subsequent cellular divisions, the number of azurophilic granules is halved so that the granules are divided among four progeny. In megaloblastosis, mitosis is inhibited due to lack of nucleotide substrate, so maturation occurs without sufficient cell division and, consequently, neutrophil myeloperoxidase levels are high.

Myeloperoxidase measurement is only one method of detecting the biochemical inadequacy of patients with megaloblastosis. Serum methylmalonic acid and total homocysteine measurements are helpful in diagnosing and monitoring vitamin B₁₂ deficiency. An older method still used in some laboratories is the deoxyuridine suppression test (dUst). The dUst, like the myeloperoxidase method, detects slowed cell division in the bone marrow. However, the dUst is a much more cumbersome procedure performed by culturing marrow cells or blood lymphocytes in the presence of radiolabeled thymidine. When deoxyuridine is added, normal cells preferentially convert it to deoxythymidine and hence suppress their uptake of thymidine, whereas cells from patients with megaloblastosis continue to use exogenous thymidine.

A much simpler method is the neutrophil myeloperoxidase index, a parameter produced as part of the automated cytochemical differential of the Technicon H₄⁺¹. Peroxidase activity and cell size are measured by light absorbance and light scatter as each leukocyte flows through a mercury arc light beam. The mean peroxidase activity of the neutrophil
population is calculated and reported as a relative deviation from the mean of a normal population. The manufacturer's normal range for MPXI is $-10$ to $+10$. In a healthy person studied 92 times over a period of 2 years, the coefficient of variation of peroxidase activity, including both analytical and biologic variation, is 2.8%.

As illustrated by the five cases reported here, the MPXI test is useful in uncovering masked megaloblastic anemia. In cases 1, 2, and 3, an elevated MPXI was associated with masked megaloblastic anemia due to vitamin $B_12$ deficiency, while cases 3 and 4 demonstrate an elevated MPXI in masked megaloblastic anemia due to folic deficiency. In all five cases, the degree of megaloblastic anemia was severe as judged by parameters other than MCV. In only two patients (cases 1 and 2) could we obtain MPXI values after therapy to confirm normalization.

Elevated MPXI is not specific for megaloblastosis. We and others have reported elevated MPXI in some myeloproliferative and myelodysplastic disorders, most uniformly in chronic myelogenous leukemia and acute nonlymphocytic leukemia (FAB-M3) where the high MPXI mirrors the presence of immature granulocytes in the blood. However, these malignancies are readily distinguished from megaloblastosis by further testing.

Prospective studies should be done to evaluate sensitivity, specificity, and normalization of MPXI during treatment of megaloblastosis. Also, it would be interesting to evaluate patients with early vitamin depletion or variant clinical manifestations of vitamin $B_12$ depletion, such as the neuropsychiatric disorders described by Lindenbaum et al. in which both macrocytosis and anemia may be absent.

In the initial work-up of anemia, we found the MPXI to be a useful parameter in conjunction with RBC indices and blood smear review in identifying patients having megaloblastosis. The advantage of the MPXI over other methods of uncovering masked megaloblastosis is its simplicity when performed as part of a routine complete blood count on an automated hematology instrument.

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REFERENCES

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ML Gulley, SA Bentley and DW Ross