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Cobalamin and Folate Evaluation: Measurement of Methylmalonic Acid and Homocysteine vs Vitamin B_{12} and Folate

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Vitamin B_{12} and folate are two vitamins that have interdependent roles in nucleic acid synthesis. Deficiencies of either vitamin can cause megaloblastic anemia; however, inappropriate treatment of B_{12} deficiency with folate can cause irreversible nerve degeneration. Inadequate folate nutrition during early pregnancy can cause neural tube defects in the developing fetus. In addition, folate and vitamin B_{12} deficiency and the compensatory increase in homocysteine are a significant risk factor for cardiovascular disease. Laboratory support for the diagnosis and management of these multiple clinical entities is controversial and somewhat problematic. Automated ligand binding measurements of vitamin B_{12} and folate are easiest to perform and widely used. Unfortunately, these tests are not the most sensitive indicators of disease. Measurement of red cell folate is less dependent on dietary fluctuations, but these measurements may not be reliable. Homocysteine and methylmalonic acid are better metabolic indicators of deficiencies at the tissue level. There are no "gold standards" for the diagnosis of these disorders, and controversy exists regarding the best diagnostic approach. Healthcare strategies that consider the impact of laboratory tests on the overall costs and quality of care should consider the advantages of including methylmalonic acid and homocysteine in the early evaluation of patients with suspected deficiencies of vitamin B_{12} and folate.

Biochemical Features

The term vitamin B_{12} refers to a family of substances composed of tetrapyrrole rings surrounding a central cobalt atom with nucleotide side chains attached to the cobalt *(1)*. The overall group name is cobalamin, with each of the different cobalt-linked upper axial ligands conferring a different name: methyl (methycobalamin), hydroxyl (hydrocobalamin), H_2O (aquacobalamin), cyanide (cyanocobalamin), and 5-deoxyadenosine (deoxyadenosylcobalamin). Chemically, the term vitamin B_{12} refers to hydroxocobalamin or cyanocobalamin, although in general use this term applies to all cobalamin forms. The predominate form in serum is methylcobalamin, and the predominate form in the cytosol is deoxyadenosylcobalamin. Most immunoassays for vitamin B_{12} measure all of these forms, after conversion to cyanocobalamin.

Vitamin B_{12} has multiple binding proteins that facilitate its absorption and transport *(1, 2)*. Intrinsic factor is secreted by the parietal cells of the stomach and is required for the intestinal absorption of vitamin B_{12} in the distal ileum. There are three proteins called transcobalamin, subtyped I, II, III. Transcobalamin I (also called R or rapid protein) is ubiquitous in most body fluids, including gastric juice. Its major importance is the problem it may cause with falsely increased vitamin B_{12} measurements. When impure sources of intrinsic factor are used in competitive binding assays, it may contain R proteins, which bind to vitamin B_{12} analogs in addition to vitamin B_{12} and thereby cause falsely increased results. Manufacturers now are required to show that vitamin B_{12} reagents do not react with these vitamin B_{12} analogs. Transcobalamin II is found in plasma and transports vitamin B_{12} to receptors on cell membranes. Therefore, only the subcomponent of vitamin B_{12} that is bound to transcobalamin II is the biologically active form of the vitamin. Some investigators have advocated the measurement of serum holotranscobalamin II as a better measure of active vitamin B_{12}, but its clinically utility is not well established and assays are difficult *(3, 4)*. Transcobalamin III is produced by granulocytes, and increased concentrations of this protein in chronic myelogenous leukemia may cause high blood concentrations of "measured" vitamin B_{12}, whereas the concentrations of the active form of the vitamin may be within the reference interval for healthy subjects.

Folate is a general term related to a family of sub-

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stances containing a pteridine ring joined to both *p*-aminobenzoic acid and glutamic acid *(1, 5)*. Reduced forms of this molecule are called dihydrofolate and tetrahydrofolate. Multiple single-carbon moieties can cross-link between the amino group at position 5 of the pteridine ring and the amino group at position 10 of the *p*-aminobenzoic acid: methylene ($-CH_2-$), forminino ($-CHNH$), methyl ($-CH_3$), methenyl ($-CH-$), and formyl ($-CHO$). Each of these forms is involved in key metabolic functions: methylene in serine/glycine metabolism and thymidylate synthesis; forminino in histidine catabolism; methyl in methionine synthesis; and both methenyl and formyl in purine synthesis. Metabolic interconversion between these forms occurs via oxidation-reduction reactions. Multiple forms of folate are present in human sera, but the major form is methyltetrahydrofolate. Separation of these various forms can be achieved with chromatography systems, whereas most immunoassays measure a composite "blend" of these forms *(6)*. Both high- and low-affinity binders for folate are found in blood. The function of these binders is unknown. Increased concentrations of binders may be found in chronic myelogenous leukemia, hepatitis, and pregnancy.

Homocysteine is a four-carbon amino acid [$HS(CH_2)_2CHNH_2COOH$], resulting from the demethylation of methionine *(7)*. Homocystine is a dimer composed of two oxidized molecules of homocysteine linked by a disulfide bond. Multiple forms of homocysteine circulate in blood: the majority (65%) is disulfide linked to protein; ~30% is in a oxidized state, mostly as disulfide links to itself or cysteine; and ~1.5–4% is free reduced form *(8)*. Storage of plasma or serum causes redistribution of these forms with an increase in the protein-bound fraction. Storage of whole blood at room temperature causes significant increases in total homocysteine *(9, 10)*. Most analytic systems measure total homocysteine content after pretreatment with a reductant.

Methylmalonic acid (MMA)[1] is a four-carbon molecule [$COOH-CH(CH_3)COOH$] related to the catabolism of valine, isoleucine, and propionic acid. Serum MMA concentrations may be falsely increased in renal insufficiency. Urine concentrations of MMA are ~40-fold higher than serum concentrations *(11)*. Urine MMA values generally are normalized with the urine creatinine measurements *(12)*.

Metabolic Functions

There are two major metabolic roles for vitamin B_{12}: (*a*) synthesis of methionine from homocysteine; and (*b*) conversion of methylmalonyl coenzyme A to succinyl coenzyme A. There are five major metabolic roles for folate: (*a*) serine and glycine metabolism; (*b*) histidine catabolism; (*c*) thymidylate synthesis; (*d*) methionine synthesis; and (*e*) purine synthesis. A deficiency of either vitamin B_{12} or folate can lead to megaloblastic anemia. Folate and vitamin B_{12} metabolism is linked in transfer of a methyl group from N^5-methyltetrahydrofolate to cobalamin. In the absence of vitamin B_{12}, folate is "trapped" and cannot be recycled back into the folate pool. Eventually this leads to a reduction in thymidylic acid synthesis that produces megaloblastic anemia. Folate and vitamin B_{12} deficiencies also cause hyperhomocysteinemia, which is a risk factor for atherosclerosis. Folate deficiency in early pregnancy is associated with increased risks for neural tube defects.

Homocysteine is increased in the plasma of patients with deficiency of vitamin B_{12} or folate *(13, 14)*. Selected genetic defects also cause markedly increased homocysteine concentrations: methylene-tetrahydrofolate reductase deficiency, cystathionine-β-synthase deficiency, and methionine synthase deficiency *(15)*. Increased values also can be seen in end stage renal disease, carcinoma, methotrexate therapy, and phenytoin therapy. The effects of methotrexate and phenytoin therapy are related to changes in folate metabolism *(2)*.

Increased homocysteine concentrations also are associated with increased risk for cardiovascular disease. The Physicians Heart Study showed that homocysteine concentrations 12% above reference values conveyed a threefold increase in the risk of myocardial infarction *(16)*. The Framingham Heart Study showed an increasing prevalence of carotid-artery stenosis directly proportional to homocysteine concentrations *(17)*. Hyperhomocysteinemia also has been reported to increase the odds ratios for venous thrombosis 3.6- to 4.0-fold *(18, 19)*. The National Health and Nutrition Examination Survey (NHANES III) showed that participants in the highest quartile of homocysteine concentrations had a 2.9-fold increased odds ratio for stroke *(20)*.

The conversion of methylmalonyl coenzyme A to succinyl coenzyme A requires vitamin B_{12}; therefore, a deficiency of vitamin B_{12} causes increases in the concentration of MMA *(21)*. In fact, MMA concentrations often increase in early stages of vitamin B_{12} deficiency before measurable decreases in serum vitamin B_{12}. Increased MMA can be found with primary metabolic defects such as methylmalonyl CoA mutase deficiency. Increased concentrations also may be seen in renal insufficiency and hypovolemia *(2)*. Although many investigators regard increases in MMA to be early and specific indicators of functional vitamin B_{12} deficiency, this opinion is not unanimous. Chanarin and Metz *(22)* have emphasized that increases in MMA do not necessarily indicate pathology and may not require treatment. Because there is no "gold standard" for confirming vitamin B_{12} deficiency, the relative merits of these tests are dependent on indirect studies of clinical benefit.

Analytic Measurements

Both serum vitamin B_{12} and serum folate typically are measured by automated competitive displacement assays *(23)*. The bioassays that were the main measurement methods in the 1970s seldom are used today. Purified hog

[1] Nonstandard abbreviations: MMA, methylmalonic acid; CBC, complete blood cell count; and MCV, mean cell volume.

intrinsic factor often is used as the binder for vitamin B_{12} assays, and β-lactoglobulin (a milk folate binder) frequently is used in folate assays. Alkaline conditions or chemical reagents generally are used to break these vitamins away from their binding proteins before quantification. Serum vitamin B_{12} usually is converted to cyanocobalamin by potassium cyanide, and serum folate usually is reduced and stabilized with dithiothreitol before quantification.

The use of whole blood to measure the erythrocyte concentration of folate has theoretical advantages compared with the measurement of serum folate *(2, 24)*. The erythrocyte folate content represents the time average of the folate concentrations occurring at the genesis of each red cell. It therefore is much less dependent on dietary fluctuations. The concentration of folate is ~40- to 100-fold higher in erythrocytes than in serum. Therefore, it should be easier to measure. Unfortunately, the combination of preanalytical variation inherent in making and diluting the erythrocyte lysate and problems associated with measuring lysates rather than serum causes most erythrocyte folate assays to perform poorly *(25)*. Table 1 shows the CVs for six commercial assays for erythrocyte folate that have across-laboratory variations of 19.2–36.0%. On the same survey, serum vitamin B_{12} had CVs of 4.4–10.0% and serum folate had CVs of 12.6–18.6%.

Evaluation of Vitamin B_{12} Status

Several tests advocated for the diagnosis and subclassification of vitamin B_{12} deficiency are listed in Table 2. I assigned the frequencies according to the following rank order: rare, low, medium, moderate, and high. Measurement of the serum cobalamin concentration has been the cornerstone for assessing suspected cases of vitamin B_{12} deficiency. However, there are major limitations with this approach. Serum vitamin B_{12} concentrations are directly altered by the concentrations of the binding proteins. Falsely increased values are caused by myeloproliferative disorders. Falsely low values can be seen with folate deficiency, pregnancy, myelomatosis, and transcobalamin deficiencies *(26)*. Multiple groups have published about the limitations of serum vitamin B_{12} measurements *(2, 26–30)*, whereas others have urged caution in the interpretation of increased MMA concentrations in patients with vitamin B_{12} within the reference interval *(22, 31, 32)*.

Table 1. CVs on College of American Pathologist's Survey K-A, 1999.[a]

	Vitamin B_{12}, 250 ng/L	Folate, 2.5 μg/L	RBC[b] folate, 100 μg/L
Abbott AxSYM	10.0%	16.6%	19.5%
Abbott IMx	7.7%	12.6%	28.1%
Beckman Access	8.3%	13.2%	16.9%
Chiron ACS:180	9.8%	18.6%	36.0%
Chiron Centaur	7.7%	14.2%	19.2%
DPC Immulite	4.4%	18.0%	
Technicon-Immuno	6.3%	16.1%	27.1%
All methods	10.8%	24.9%	

[a] Reproduced with permission from College of American Pathologist's K-A, 1999 Summary Report.
[b] RBC, red blood cell.

Table 2. Vitamin B_{12} deficiency: diagnosis and subclassification tests.[a]

Procedure	Frequency of use	Diagnostic utility	Cost
Blood cell counts (CBC)	High	Low	Low
Serum cobalamin	High	Medium	Medium
Urine MMA	Low	High	Moderate
Serum MMA	Low	High	Moderate
Serum homocysteine	Medium	High	Moderate
Anti-parietal cell antibody	Low	Medium	Medium
Anti-intrinsic factor antibody	Low	High	Medium
Serum gastrin	Low	Moderate	Medium
Schilling test	Low	Moderate	High
Neutrophil lobe count	Rare	Medium	Medium
Serum pepsinogen	Rare	Moderate	Moderate
Bone marrow examination	Low	Medium	High

[a] Tests below the line are rarely used and seldom necessary.

Examination of peripheral blood smear by experienced personnel, combined with the complete blood cell count (CBC) have long been the traditional methods for evaluating vitamin B_{12} deficiency anemia. The classical findings of florid pernicious anemia are readily identifiable by most hematologists, but the subtle changes associated with early vitamin B_{12} deficiency are more difficult to identify. In a case-control study, Metz et al. *(28)* found neutrophil hypersegmentation in two-thirds of the patients with low vitamin B_{12} compared with only 4% of controls. This was the only hematologic change that correlated well with vitamin B_{12} deficiency. They did not find appreciable changes in the mean cell volume (MCV) until the vitamin B_{12} concentration was below 200 ng/L. Chanarin and Metz *(22)* attribute part of the reported insensitivity of the blood smear and MCV to the wide ranges of normality accepted by many laboratories. They recommended that MCVs >94 fL be considered suspicious for vitamin B_{12} deficiency; however, this represents a major proportion of the patients having CBCs at most institutions, so the specificity of following up these cases would be low. In addition, patients with neurologic symptoms caused by vitamin B_{12} deficiency may not have any hematologic abnormalities.

Many groups now recognize MMA and homocysteine tests as the most sensitive and specific indicators of functional vitamin B_{12} deficiency *(2, 11, 12, 14, 15, 21, 27, 29, 30, 33–36)*. MMA and homocysteine concentrations are increased in many patients with "normal" vitamin B_{12} concentrations. Fig. 1 shows MMA concentrations from a stratified sample of 72 patients measured at the Mayo Clinic. Increased MMA was found even with vitamin B_{12} concentrations as high as 400 ng/L. Similarly, Holleland et al. *(37)* found increased MMA or homocysteine concentrations (see Fig. 2) in >20% of patients with serum vitamin B_{12} concentrations within the reference interval. Some laboratory-based

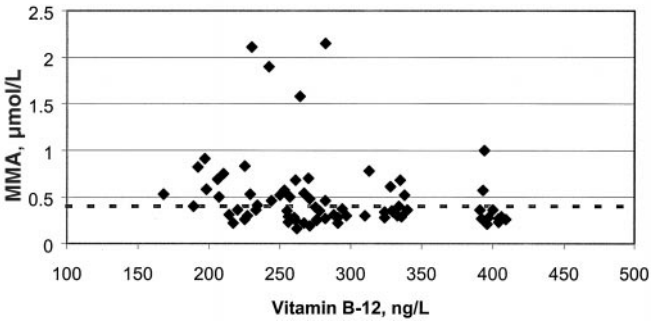

Fig. 1. MMA concentrations for specimens selected from vitamin B_{12} assay according to stratified vitamin B_{12} concentrations.

Reference range for MMA is ≤0.4 µmol/L. Reference range for vitamin B_{12} is 200–650 ng/L.

algorithms recommend initially testing serum vitamin B_{12} and following up low values with MMA measurements *(2)*. The choice of the threshold vitamin B_{12} concentration for triggering follow-up is controversial. If the lower limit of normal (200 ng/L) is used, multiple patients with increased MMA would be missed. If higher values, such as 500 ng/L, are used (as advocated by some), the majority of the patients having vitamin B_{12} tests would have follow-up MMA tests *(38)*. Fig. 3 shows the distribution of vitamin B_{12} results for the assay used at the Mayo Clinic with a reference range of 200–650 ng/L.

Evaluation of Causes of Vitamin B_{12} Deficiency

The vitamin B_{12} absorption (Schilling) test is the classical procedure for determining whether a patient can absorb vitamin B_{12} *(2)*. This is a two-stage procedure: in stage 1, radioactive vitamin B_{12} is given by mouth, followed by a flushing dose of nonradioactive vitamin B_{12}. The percentage of radiolabel excreted in a 24-h urine is measured. Stage 2 is like stage 1 except that intrinsic factor is given with the labeled vitamin B_{12}. An abnormal stage 1 followed by a normal stage 2 test is consistent with pernicious anemia. If both stages are abnormal, other causes of low vitamin B_{12} (such as ileal malabsorption) should be considered. The Schilling test seldom is used at present, mainly because of the difficulties in using radioisotopes and the inconvenience of the test. In addition, the absorption of crystalline vitamin B_{12} may differ from the absorption of vitamin B_{12} in food. Lindgren et al. *(39)* have found that MMA and homocysteine measurements are more sensitive tests of early pernicious anemia and recommend them as better tests. Other procedures such as serum gastrin, serum pepsinogen, and upper gastrointestinal endoscopy are alternative mechanisms for evaluating gastric atrophy.

Pernicious anemia, a condition associated with chronic gastric atrophy, is the most common cause of vitamin B_{12} deficiency *(40)*. There are multiple immunologic causes of chronic gastritis that can be detected by serologic assays. Anti-parietal cell antibodies are present in ~85% of the cases, but they are nonspecific because they are present in 3–10% of healthy persons *(2, 28, 40)*. Anti-intrinsic factor antibodies are present in only approximately one-half the cases of pernicious anemia, but they are quite specific for this disease *(2, 28, 40)*. Serum gastrin and serum pepsinogen A and C are sensitive indicators of gastric atrophy *(2, 40, 41)*. Approximately 80% of cases of pernicious anemia have increased gastrin, and combinations of the three markers can identify most cases *(41)*.

Bone marrow examination by a competent hematopathologist can provide valuable information, but this procedure seldom is necessary for evaluating vitamin B_{12} deficiency *(2, 36)*. The deoxyuridine suppression test is a sensitive indicator of cobalamin and folate deficiency *(42)*. The test is only rarely used because it requires bone marrow specimens, uses a radiolabel, and is difficult to control *(22, 33, 42)*.

Several years ago, a cascade for automatically scheduling a series of tests for patients with suspected pernicious anemia was introduced by Mayo Medical Laboratories (see Fig. 4). The cascade begins with measurement of serum vitamin B_{12}. Specimens with test values below 150 ng/L are examined for intrinsic factor blocking antibodies. Specimens with positive antibodies in this setting are considered "consistent with pernicious anemia". Specimens negative for antibodies have follow-up gastrin measurements. Increased gastrin values >200 ng/L are considered "consistent with pernicious anemia". Specimens with vitamin B_{12} concentrations of 150–300 ng/L have follow-up MMA tests. Those with MMA concentrations >0.4 µmol/L are subjected to intrinsic factor antibody testing, and those negative for antibodies have gastrin testing.

This cascade has many advantages for accelerating laboratory investigation, but in light of recent studies it also has some inadequacies. The cascade begins with vitamin B_{12}, which could miss patients with significant pathology that would be detected by MMA and homocysteine. In addition, this algorithm focuses predominately on the subset of deficiency caused by pernicious anemia. Other forms of vitamin B_{12} and folate deficiency also can cause significant pathology. The upper limit of vitamin B_{12} for this cascade to proceed to MMA was set at 300 ng/L; however, some patients with vitamin B_{12} concentrations above this may have abnormal MMA values (as shown in Figs. 1 and 2). An alternative diagnostic

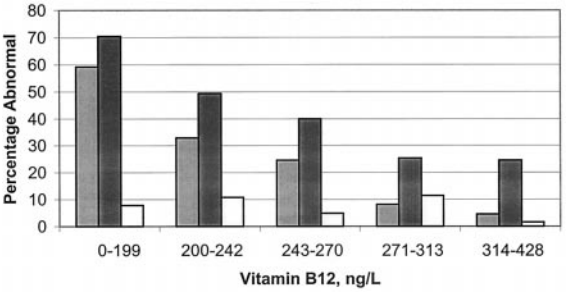

Fig. 2. Abnormalities in MMA (▨), homocysteine (▩), and folate (□) concentrations found with various concentrations of vitamin B_{12} plotted from data presented by Holleland et al. *(37)*.

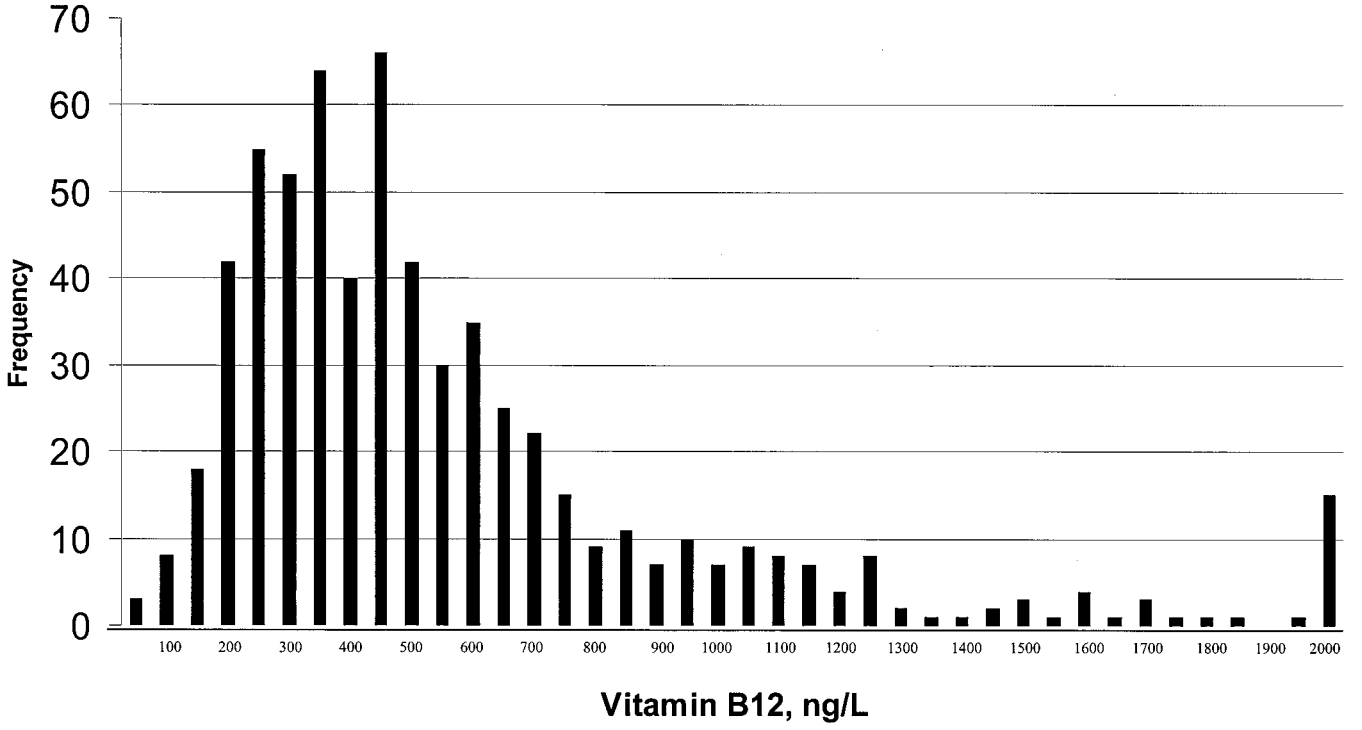

Fig. 3. Distribution of vitamin B_{12} concentrations for clinical specimens analyzed on the Bayer ACS:180 immunoanalyzer. The reference range is 200–650 ng/L.

strategy would be to begin by measuring all four components (vitamin B_{12}, folate, MMA, and homocysteine), and then follow up with specific tests to subclassify the disorders in accordance with the clinical presentation (i.e., anemia, neurologic deficiency, neuropsychiatric disturbances, and cardiovascular risks). Different "cascades" would be utilized for each of these clinical presentations, but each cascade would begin with the four-test panel.

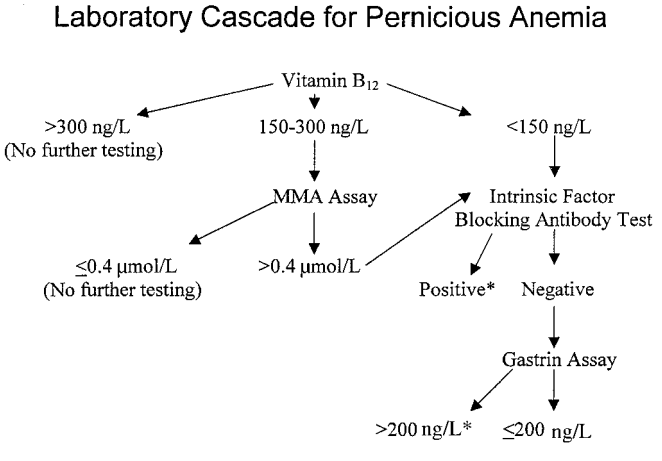

Fig. 4. Algorithm for laboratory evaluation of patients suspected of having pernicious anemia, beginning with vitamin B_{12} measurements and cascading to tests for MMA, intrinsic factor blocking antibodies, and serum gastrin.

* indicates consistent with pernicious anemia.

Evaluation of Folate Status

Fewer test procedures are available to investigate folate status compared with vitamin B_{12} status (see Table 3). There are potential interdependencies between folate and vitamin B_{12}. Patients may need to be evaluated for deficiencies of both vitamins to fully investigate their differential of diagnosis. For example, deficiency of either vitamin B_{12} or folate can cause increased homocysteine concentrations. In addition, decreased folate may cause low vitamin B_{12} concentrations because of metabolic blocks. Treatment with folate may normalize the vitamin B_{12} concentrations. A common clinical dilemma occurs when both vitamin B_{12} and folate concentrations are low and it is not known whether a clinical deficiency is present for both vitamins or for one or the other.

There is no consensus for the laboratory evaluation of folate status. The same limitations regarding minimal

Table 3. Folate deficiency: diagnostic tests.[a]

Procedure	Frequency of use	Diagnostic utility	Cost
Blood cell counts (CBC)	High	Low	Low
Serum folate	High	Medium	Medium
Erythrocyte folate	Low	Medium	Moderate
Serum homocysteine	Medium	High	Moderate
Neutrophil lobe count	Rare	Moderate	Medium
Bone marrow examination	Low	Medium	High

[a] Tests below the line are rarely used and seldom necessary.

changes in the CBC and MCV described for vitamin B_{12} also relate to folate. Erythrocyte folate may be considered instead of serum folate if there have been recent dietary changes, but one should be aware of the analytic limitations of erythrocyte folate assays. In addition, decreases in erythrocyte folate are not specific for folate deficiency in that they also occur in vitamin B_{12} deficiency.

The US government mandated the fortification of grain products with folic acid beginning in the fall of 1997 *(43, 44)*. These programs target an increase in the dietary folate of ~100 μg per person per day depending on diet. This process was implemented to reduce the incidence of neural tube defects. The amount of supplement was chosen to reduce neural tube defects without masking occult vitamin B_{12} deficiency. In subjects not previously taking vitamins, the mean serum folate concentration increased from 4.6 to 10.0 μg/L, whereas the mean homocysteine concentration decreased from 10.1 to 9.4 μmol/L *(44)*. This change in dietary folate will significantly alter test values in the US. The effect of this change on "normal" reference ranges for folate and homocysteine is not fully known, but laboratorians and clinicians should be aware that these changes have occurred when interpreting test values.

Other Implications of Vitamin B_{12} and Folate Deficiency Beyond Megaloblastic Anemia

Both vitamin B_{12} and folate deficiency are associated with neuropsychiatric disorders *(45–50)*. The mechanisms of these disturbances are not known. Both folate and vitamin B_{12} deficiency may cause depression and dementia, whereas only vitamin B_{12} deficiency causes demyelinating neuropathy *(46)*. Some nonfocal neuropsychiatric abnormalities are found more frequently in vitamin B_{12} deficiency, suggesting a cobalamin-dependent enzyme defect *(45)*. Other studies have suggested that elderly patients with cognitive and depression changes can benefit from folate supplementation *(47)*. In addition, folate may be a key variable for identifying patients likely to respond to antidepressant treatment *(48)*. The hematologic indices for many of these patients with neuropsychiatric disorders are within reference values, so one should not role out the possibility of vitamin deficiency based only on normal hematology tests *(49)*.

In addition to neuropsychiatric disorders, folate deficiency and hyperhomocysteinemia have significant relationships with occlusive vascular disease, spinal degeneration, and immunologic tolerance for neoplasia *(50)*.

Future Directions for Laboratory Testing

The development of rapid, widely available, automated assays has led to a large number of requests for serum vitamin B_{12} and folate measurements *(23)*. Currently, many clinicians request these assays whenever they consider vitamin B_{12} and folate deficiencies or hyperhomocysteinemia as part of their differential. Many clinicians are not aware of the problems with binding proteins and the inconsistencies between the concentrations of vitamins and metabolic products. It is assumed that convenience and tradition rather than scientific evidence has led to the increased number of orders for these serum vitamin measurements. Many clinicians may consider tests for MMA and homocysteine to be esoteric procedures that are reserved for special investigations, and therefore, they use the simpler and more readily available vitamin B_{12} and folate assays for routine investigations.

A new technology, electrospray tandem mass spectrometry, may make MMA and homocysteine assays more attractive. Recently, the Mayo Clinic Laboratories implemented a tandem mass spectrometry procedure for measuring homocysteine *(51)*. The procedure requires no immunodiagnostic reagents and no expensive chromatographic columns. This procedure has a retention time of 1.5 min and a throughput of 2.5 min per analysis. The labor time is less than that required for most automated immunoassays. A similar tandem mass spectrometry procedure is being developed for MMA. The main impediment to widespread implementation of these procedures in most clinical laboratories is the cost of the equipment. However, these instruments can be used to measure multiple analytes, including drugs, and if the equipment cost is amortized over many assays, the technique may become cost competitive and more readily available. Even with the current limitations on assay convenience and laboratory costs, quality issues related to correct diagnoses and the downstream clinical costs of multiple patient visits justify the wider use of MMA and homocysteine measurements.

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