

Pitfalls in the Diagnosis of Vitamin B₁₂ Deficiency

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ABSTRACT

Vitamin B₁₂ deficiency is a well-described disorder with a spectrum of manifestations ranging from macrocytic anemia to neuropsychiatric disorders including depression, dementia, and subacute combined degeneration of the spinal cord. Lack of vitamin B₁₂ arises from insufficient intake or malabsorption. In clinical practice, serum total cobalamin (Cbl) levels are the initial test of choice for detecting B₁₂ deficiency as they are widely available and cost-effective. However, this test is limited in specificity and sensitivity, missing many patients within the laboratory "gray zone" of deficiency. Measurements of serum methylmalonic acid (MMA) and homocysteine (Hcy) levels, which accumulate in B₁₂ deficiency, become useful when Cbl levels are equivocal but clinical suspicion remains high. Early vitamin B₁₂ replacement is important in preventing potentially irreversible neurologic damage.

We report a case of a 75 year-old man presenting with symptomatic anemia, neuropsychiatric findings, and repeatedly normal serum cobalamin levels, eventually diagnosed with vitamin B₁₂ deficiency due to pernicious anemia. This case highlights the potential difficulty in establishing this common diagnosis due to false-negative Cbl assay results. Given its high prevalence, vitamin B₁₂ deficiency must be included in the differential diagnosis of patients with progressive neuropsychiatric findings and/or hematologic derangements as rapid diagnosis and supplementation may prevent permanent complications.

INTRODUCTION

Vitamin B₁₂ deficiency is a well-described disorder with a spectrum of manifestations ranging from macrocytic anemia to neuropsychiatric disorders including depression, dementia, and subacute combined degeneration of the spinal cord [1]. Lack of vitamin B₁₂ arises from insufficient intake or malabsorption [2]. At-risk populations include the elderly, alcoholics,

strict vegetarians, as well as patients with intestinal inflammatory diseases, autoimmune conditions, post-bariatric surgery, and users of certain medications including proton pump inhibitors, histamine receptor antagonists, and biguanides [2, 3]. There is also an association between primary hypothyroidism and B₁₂ deficiency [4]. In clinical practice, diagnosis is typically established by measurement of serum cobalamin (Cbl) levels. Vitamin B₁₂ deficiency can

be reflected in elevated methylmalonic acid (MMA) and homocysteine (Hcy) levels, but these tests are not routinely obtained unless the initial Cbl levels are equivocal [1].

CASE

A 75-year-old male presented to the hospital with generalized weakness, dizziness, and mild confusion for three weeks. His medical history significant for colon cancer treated by resection 13 years ago, hypertension, and benign prostatic hyperplasia (BPH). He denied fevers, chills, dyspnea, chest pain, bleeding, bruising, melanotic stools, diarrhea, vomiting, or dysuria. Three weeks prior to presentation, silodosin was started for treatment of BPH. The patient had been on ramipril and atenolol for hypertension which he continued to take as prescribed. He lived an active life prior to symptom onset (Table 1) (Figure 1).

Table 1.

White cell count	5,900/ μ L (normal differential)
Hemoglobin	8.9g/dL
Mean corpuscular volume	121.4 fL
Platelets	79,000/ μ L
Lactate dehydrogenase	527 U/L
Total bilirubin	1.8 mg/dl
Direct bilirubin	0.4 mg/dl
Haptoglobin	<8 mg/dl
D-dimer	436 ng/ml
Fibrinogen	248 mg/dl
INR	1.14
Reticulocyte count	1.6%
TSH	1.13 mIU/ml
Urine hemosiderin	Negative
Direct antiglobulin test	Negative
Peripheral smear	3+schistocytes, moderate spherocytes and ovalocytes, and hypersegmented neutrophils.

On admission, vital signs were notable for orthostatic hypotension with a blood pressure of 106/82 supine and 80/52 standing. Physical exam was unremarkable and rectal exam revealed heme-negative stool. See Table 1 and Figure 1 for admission labs and peripheral smear findings.

The patient's anti-hypertensive and BPH medications were held and he was transfused one unit of packed red cells. Deficiency in folate and/or vitamin B₁₂ levels was suspected, however, serum folate level returned at 12.3 ng/ml [normal, 3.1 – 17.5 ng/ml] and vitamin B₁₂ level at 489

pg/ml [normal, 254 – 1320 pg/ml], both sent pre-transfusion. Repeat vitamin B₁₂ level was 321 pg/ml. A bone marrow biopsy showed erythroid hyperplasia with a left shift, megaloblastic changes, hypersegmented neutrophils, increased iron stores, and normal flow cytometry/cytogenetics (Figures 2 and 3).

Figure 1. Peripheral smear showing hypersegmented neutrophils.

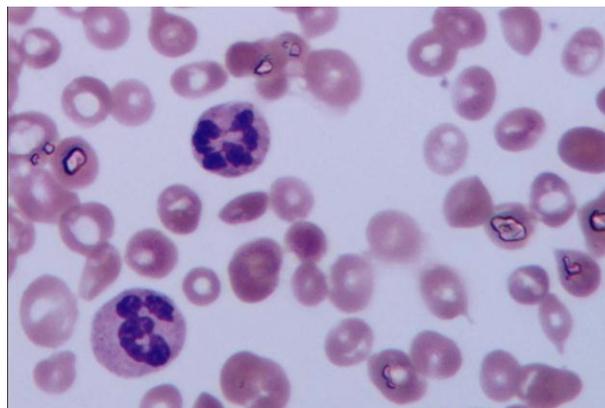


Figure 2. Bone marrow biopsy showing erythroid hyperplasia, megaloblastic changes, and absence of maturation.

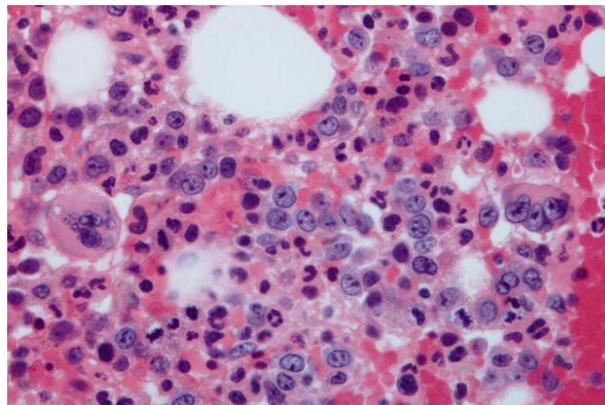
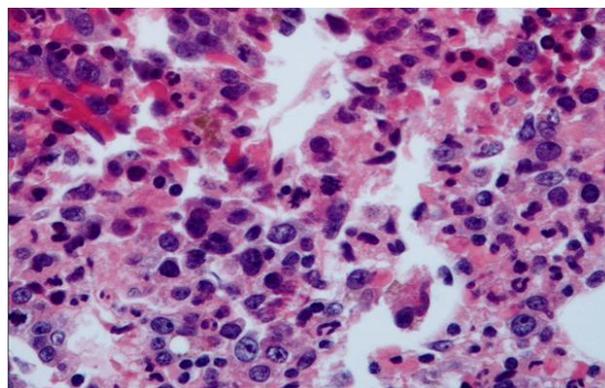


Figure 3. Bone marrow biopsy showing increased erythroid to myeloid ratio with leftward shift of erythropoiesis.



Despite a normal serum cobalamin level, the clinical picture was suspicious for vitamin B₁₂ deficiency, and empiric therapy with cyanocobalamin (1 mg intramuscularly daily) was initiated. Soon after, his labs revealed the following: methylmalonic acid, 28 nmol/ml [normal, <0.4 nmol/ml]; homocysteine, 72.4 μmol/L [normal, 3.2 - 10.7 μmol/L], and presence of both anti-parietal cell and intrinsic factor antibody titers, leading to the diagnosis of pernicious anemia. He was continued on cyanocobalamin injections, administered weekly for four weeks and then monthly injections thereafter, eventually achieving a hemoglobin of 12.8 g/dL, MCV of 97.2 fL, and platelets of 130,000/μL, and a durable resolution of his dizziness and confusion at his two month follow-up.

DISCUSSION

Serum cobalamin levels can be misleading in the workup of vitamin B₁₂ deficiency. In this case, compelling evidence to suggest clinical B₁₂ deficiency was apparent on presentation by a marked macrocytic anemia with evidence of hemolysis. The peripheral smear demonstrated oval macrocytes and hypersegmented neutrophils, commonly seen in folate/B₁₂ deficiency [5]. When the anemia is more severe, there may be marked poikilocytosis with teardrop cells and red-cell fragments, also observed in this case [5]. Folate/B₁₂ deficiency can further lead to thrombocytopenia and morphologic aberrations resembling a variety of hematologic disorders such as myelodysplastic syndrome and acute leukemia [6]. The decision to perform a bone marrow biopsy was based on the discrepancy between the clinical presentation and normal folate/Cbl levels as well as presence of thrombocytopenia, with biopsy results further supporting B₁₂ deficiency as the etiology of his anemia. The diagnosis confirmed by elevated MMA/Hcy levels and increased anti-parietal cell/intrinsic factor antibody titers, leading to pernicious anemia.

In clinical practice, serum total Cbl levels is the initial test of choice for detecting B₁₂ deficiency as it is widely available and cost-effective. This test is limited in specificity and sensitivity, often missing many patients within the laboratory "gray zone" of deficiency [1, 2, 3]. Such

patients usually have Cbl levels ranging from 125 pg/ml to 250 pg/ml, although these cut-offs vary based on the assay type [2]. In plasma, vitamin B₁₂ is bound to two proteins - haptocorrin and transcobalamin. The Cbl assay predominantly reflects B₁₂ bound to haptocorrin (metabolically inactive), and transcobalamin-bound B₁₂ (metabolically active) which contributes much less to the total serum Cbl level [5]. Conditions such as chronic myeloid leukemia can cause an increase in serum haptocorrin levels leading to a false-negative result in true vitamin B₁₂ deficiency [7]. Other factors causing false-normal results include intrinsic assay error, assay variability among manufacturers, and intra-patient variability [8, 9, 10].

This case highlights the difficulty in establishing a diagnosis of B₁₂ deficiency with a Cbl assay within normal limits on repeated studies. Measurements of serum MMA and Hcy levels, which accumulate in B₁₂ deficiency, become useful when Cbl levels are equivocal but clinical suspicion remains high [1, 2]. Although generally considered more sensitive indicators of B₁₂ deficiency than total Cbl levels (i.e. "confirmatory tests") [2, 10], the utility of these metabolites may be limited. Renal insufficiency may cause elevation of both MMA and Hcy, while increased Hcy may also represent lack of folate or vitamin B6 [2, 11]. Metabolite assay cost and slow turnaround time are further limitations to these tests [2]. Accumulating evidence indicates that serum holotranscobalamin (holoTC), an earlier marker of B₁₂ deficiency that decreases before total Cbl, may be superior to other assays and has been proposed as a first-line diagnostic test, but is not yet routinely available in the clinical setting [2, 12]. Clinicians should rely on metabolite assays combined with clinical judgment in establishing the diagnosis of B₁₂ deficiency when Cbl assay results are equivocal.

Early vitamin B₁₂ replacement is important in preventing potentially irreversible neurologic damage. Treatment should be started expeditiously for severe neurologic symptoms with their risk of irreversibility [14]. Treatment should be continued until clinical improvement is evident or a definitive diagnosis can be made [1, 2]. In patients with pernicious anemia, vitamin B₁₂ supplementation may begin with daily injections of cyanocobalamin at a dose of 1000 μg for the

first week, followed by weekly injections for the first month, and maintenance monthly injections thereafter [2, 13]. Oral dosing is acceptable in patients with intact gastrointestinal absorption and absence of severe neurologic manifestations [13]. Iron levels should be monitored and corrected as replacement therapy may cause increased demand on iron stores. Folate deficiency may be unmasked with repletion of vitamin B₁₂ [2]. Treatment response should occur with reticulocytosis within one week as well as eventual reduction in MCV and resolution in the anemia within 1-2 months [2].

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CONCLUSION

Given its high prevalence, vitamin B₁₂ deficiency must be considered in the differential diagnosis of patients presenting with progressive neuropsychiatric findings and/or hematologic derangements, as rapid diagnosis and supplementation may prevent permanent complications.

CONFLICT OF INTEREST

The authors have no conflicts of interest or financial disclosures to report.

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