

To the editor:

Cobalamin-responsive disorders and unreliability of cobalamin, methylmalonic acid, and homocysteine testing

Solomon¹ claims that many patients with clinical features suggestive of cobalamin (Cbl) deficiency who have normal serum Cbl, methylmalonic acid (MMA), and homocysteine (HCys) levels may respond to Cbl therapy, while many patients with low serum Cbl, high serum MMA, or high serum HCys levels may fail to respond to Cbl therapy. He concludes that these tests are unreliable for the diagnosis of Cbl deficiency. I do not think that there is much support for his conclusion.

First of all, Solomon writes that resolution of signs and symptoms consistent with Cbl deficiency in 12 patients occurred prior to any Cbl therapy. How can he then attribute to Cbl therapy the resolution of signs and symptoms of those who received Cbl?

Second, the response to pharmacologic doses of Cbl does not support the diagnosis of Cbl deficiency. The dose of Cbl for a therapeutic trial of Cbl deficiency is 1 μ g Cbl daily for 10 days. This dose would produce optimal reticulocyte response within 7 to 10 days and, if continued, would produce a complete hematologic response.² Patients who do not respond to small doses of Cbl but respond to pharmacologic doses of Cbl are not Cbl deficient.

Third, the criteria that Solomon has used for demonstrating a response to Cbl therapy (5 fL reduction of mean corpuscular volume [MCV] or an increase in hematocrit of 0.05 point within 3 months of Cbl therapy) are nonspecific. Such a response can be seen in a patient who has folate-deficiency anemia treated with high doses of Cbl.² Similar hematologic changes may be seen in any patient with alcoholic liver disease or ethanol abuse who stops drinking ethanol; in patients recovering from acute hemolytic anemia, anemia associated with acute infection; or in patients with

hypothyroidism treated with thyroid extract, irrespective of whether they are given Cbl.

Fourth, of 8 patients who met Solomon's criteria for hematologic response, only 2 demonstrated an increase in hematocrit within 3 months of Cbl therapy. The other 6 patients showed only reduction of MCV. If the reduction of MCV were due to Cbl therapy, why did the hematocrit not increase?

Finally, Solomon reports that low or intermediate Cbl levels were present in 46% of the responders and 56% of the nonresponders, and increased MMA values were present in 73% of responders and 88% of nonresponders. Consequently, he claims that these tests are unreliable. In the presence of low serum Cbl or high serum MMA, failure of response to Cbl does not exclude Cbl deficiency, but indicates that the anemia or neurologic abnormalities were not due to Cbl deficiency.

Serum Cbl, MMA, and HCys, like any other laboratory tests, may give false-positive or false-negative results in certain situations. One has to be familiar with these situations in order to interpret the laboratory results properly rather than claiming that these tests are unreliable.

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To the editor:

Is testing for clinical cobalamin deficiency truly unreliable?

Cobalamin and metabolite assays' sensitivities depend greatly on whether the tested patients have clinical abnormalities (95%-99% sensitivities) or not (< 70% for cobalamin, but data and criteria vary).^{1,2} In his report of massive diagnostic failures in even symptomatic cobalamin deficiency, Solomon misperceives what failed.³

The likeliest initial culprit is a defective cobalamin assay. Overlapping the study's time period, we reported that the ADVIA Centaur (Bayer Diagnostics, Tarrytown, NY) assay produced falsely normal results in 16 of 22 cobalamin-deficient sera that had consistently subnormal levels by 2 radioisotopic assays.⁴ Assay error best explains Solomon's truly unprecedented claims that just 5 (14%!) of 37 patients with symptomatic deficiency had cobalamin levels less than 200 ng/L or that as many as 20 (54%) of 37 could have had levels more than 300 ng/L.

As to metabolite data, the pretreatment values were very inconsistent and frequently jumped the divide between normal and abnormal. Left unexamined were whether assay or patient fluctuation was responsible and if variability rather than therapy

explained the (unreplicated) posttreatment improvements. Equally puzzling was the tendency of results to be less, not more, abnormal in presumably deficient patients than in nondeficient ones.

The clinical and therapeutic diagnoses raise concerns too. Hematologic findings, which lacked individual complete blood count (CBC) details, were surprisingly insubstantial. Only 2 of 37 patients had anemia, 1 of whom incongruously "responded" to cobalamin without changing mean corpuscular volume; 6 others (75% of hematologically abnormal patients) had mild macrocytosis without anemia, something that more often reflects alcoholism,⁵ which, being often both unrecognized and fluctuating, can confound therapeutic assessments; and blood smears were not examined for hypersegmentation, a major failing given the study's goals and controversial findings. Neurologic information was sketchy, and the appendices suggest diagnostic alternatives; patient 3, for example, closely mimics a reported patient whose alcohol-induced neurologic and other abnormalities were mistakenly deemed

cobalamin responsive.⁶ Further suggesting subclinical or no deficiency rather than clinical deficiency in many patients was the near-absence of the characteristically extreme metabolite elevations of clinical deficiency¹ (eg, methylmalonic acid > 1000 nM) and the rarity of abnormal Schilling test results⁷ (only 4 of 12 were abnormal, while the 5 positive intrinsic factor antibody results seem artifacts of cobalamin injection⁸ because, among other things, the 2 patients also given Schilling tests had incompatibly normal absorption).

The proposal of overwhelming, structural diagnostic inadequacy in symptomatically cobalamin-deficient patients, therefore, seems attributable instead to the more prosaic and remediable failures of manufacturers and clinical laboratories, compounded by an uncertain diagnostic mix of study patients. An important subtext, too, is the confusing cobalamin cutpoint inflation peppering the data and that the paper promotes. As discussed elsewhere,⁹ the growing impact on clinical diagnosis and management of this inflation, paradoxically derived from data in subclinical deficiency, has drawbacks that have not been thoroughly examined. Clinicians may minimize potential diagnostic confusion by carefully distinguishing clinical from subclinical cobalamin deficiency, despite their overlaps.^{9(Tab1)} Clinical deficiency is a relatively uncommon medical disease, usually easily diagnosed, typically relentless, and requiring urgent treatment; subclinical deficiency is a common biochemical abnormality, often less easily diagnosed and usually having a still-undefined course and therapeutic imperatives.^{7,9}

Response:

Tests for cobalamin-responsive disorders are unreliable

Carmel and Shojania are critical of my conclusion that laboratory testing is not predictive of clinical responses to *pharmacologic* doses of cobalamin.¹ However, they fail to distinguish the more inclusive concept of “cobalamin-responsive” disorders from the classic concept of “cobalamin deficiency” (Table 5 of the original article). Thus, Shojania’s statement that responses to “pharmacologic” doses of cobalamin (in contrast to “physiologic” doses) do not support the diagnosis of cobalamin deficiency is exactly the point of the article. Similarly, Carmel’s concern that results were less abnormal “in presumably deficient patients than in nondeficient ones” also confuses cobalamin deficiency with cobalamin responsiveness.

Carmel suggests that the Advia Centaur assay results for cobalamin were misleading since this method gave normal results in 17 patients who had low values with a radioisotope dilution assay.² However, none of these 17 patients had values higher than 400 pg/mL with the Advia Centaur method, while 15 (41%) of the 37 responders in my study had levels higher than 400 pg/mL (Appendix 2 of the original article). Moreover, even if cobalamin deficiency is defined by elevated methylmalonic acid values, 23% of responders had normal values, and only 37% of responders had values more than 3 standard deviations above the normal mean, the cut-off suggested for identifying patients with symptomatic cobalamin deficiency (Table 3).³

Carmel also notes that metabolite values in individual patients were inconsistent. Indeed, this variability is a major point of the article and significantly impacts clinical practice.

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Both Carmel and Shojania note the infrequency of anemia and the presence of mild macrocytosis. This is not surprising in an outpatient study where problems were identified during routine clinic visits. Nonetheless, “isolated” macrocytosis and “isolated” anemia are well recognized even in hospitalized patients with cobalamin deficiency.⁴ The neurologic abnormalities described (“Results” in the original article) and their dissociation from anemia and macrocytosis are also consistent with previous reports.^{5,6}

The role of “diagnostic alternatives” concerns both Carmel and Shojania. Most prior reports were retrospective reviews of hospitalized patients with limited direct knowledge of medical problems in individual patients. In contrast, patients in my study were followed for several years, allowing more familiarity with their medical disorders, including possible alcohol abuse. Confounding disorders in responders (Appendix 1 of the original article) were unlikely to affect the results since clinical responses occurred without other changes in therapy. Indeed, responses to cobalamin have been reported in diabetes and other diseases (references 43-48 of the original article). Patients with confounding disorders were purposefully excluded from the nonresponder group. Similarly, in studies of neurologic abnormalities associated with frank cobalamin deficiency, patients with an alternative diagnosis were excluded *only* if follow-up was inadequate or if they did not clearly respond to cobalamin therapy. Thus, it is possible that some patients with confounding disorders responded despite the presence of cobalamin deficiency rather than because of it.^{5,6}

Shojania noted that 12 patients with clinical abnormalities were excluded because they improved without therapy. However, clinical manifestations in all 37 responders were persistent for at least one month prior to beginning treatment.

Less relevant are Carmel's comments regarding hypersegmented neutrophils, Schilling test results, and intrinsic factor antibody data. Hypersegmented neutrophils have limited value, and their presence or absence would not detract from the therapeutic effects of cobalamin on the clinical parameters monitored.⁷ While intrinsic factor antibody and Schilling test abnormalities address mechanisms of cobalamin *deficiency*, they neither establish nor exclude the presence of cobalamin *responsive* disorders. Similarly, Carmel's comments on subclinical cobalamin deficiency are not applicable to my study of patients with clinical abnormalities.

Finally, Shojani misread Table 4. Actually, 45% of patients with either low and intermediate serum cobalamin levels *or* moderately increased and elevated methylmalonic acid levels were nonresponders. Thus, testing is about a 50-50 proposition that fully supports the conclusion that serum cobalamin and metabolite measurements often fail to predict whether a patient will respond to pharmacologic doses of cobalamin.

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