Spurious Elevations of Vitamin B_{12} with Pernicious Anemia

TO THE EDITOR: Within a 3-week period, two women, 46 and 48 years of age, presented with peripheral neuropathy and associated pancytopenia with macrocytic anemia. Clinical suspicion for pernicious anemia was high, but vitamin B_{12} levels were 1644 pg per milliliter (1228 pmol per liter) and 1321 pg per milliliter (975 pmol per liter), respectively (reference range, 246 to 1320 pg per milliliter [181 to 974 pmol per liter]). On subsequent bone-marrow evaluation, specimens from both patients showed profound megaloblastic features. Additional findings on laboratory tests included elevated levels of homocysteine and methylmalonic acid combined with detection of intrinsic factor–blocking antibodies and antiparietal-cell antibodies, which further supported the diagnosis of pernicious anemia. In both patients the blood counts responded to treatment

with vitamin B_{12}, but both continue to have symptoms of peripheral neuropathy.

There have been reports of false normal results for vitamin B_{12} levels generated by automated analyzers when the serum of patients with megaloblastic anemia is evaluated. The results have been attributed to the possibility that high levels of intrinsic factor–blocking antibodies interfere with the assay.[1,2] Today, vitamin B_{12} assays are primarily performed on automated analyzers that apply a method based on the competitive binding of serum vitamin B_{12} with reagent intrinsic factor. Many of these platforms have also been found to be inaccurate when serum containing intrinsic factor–blocking antibodies is analyzed.[2] Disconcertingly, pernicious anemia is the most common cause of vitamin B_{12} deficiency, and up to 70% of patients with pernicious anemia have intrinsic factor–blocking antibodies.[3]

To investigate further, we precipitated serum immunoglobulins by adding 25% polyethylene glycol (PEG) by volume in a 1:1 dilution with serum. Using unmodified and PEG-treated samples of serum from the two patients and from three controls (patients without macrocytic anemia), we then ran tests for vitamin B_{12} levels (Fig. 1). In the PEG-treated samples from the two patients, vitamin B_{12} levels decreased to below the limit of detection; the PEG-treated samples from the controls showed a decrease compatible with the 1:1 dilution.

We have been performing vitamin B_{12} assays on the Siemens Dimension Vista system at our institution. A review of the package insert shows that the manufacturers are aware of this issue and recommend testing for intrinsic factor–blocking antibodies if test results are in conflict with the clinical diagnosis. We are in the midst of evaluating other platforms for this assay and have notified our clinicians of the issues described. However, we are concerned that there is insufficient awareness in the medical community of the possibility of spuriously high vitamin B_{12} levels; we urge pathologists to review their methods and clinicians to incorporate the information presented here into their diagnostic evaluations.

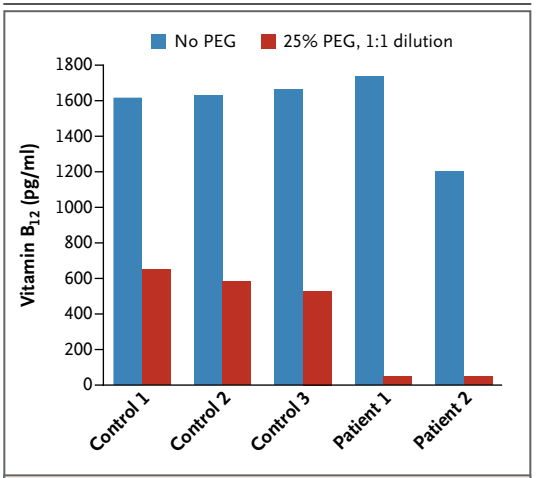

Figure 1. Spuriously Elevated Vitamin B_{12} Levels in Two Patients with Pernicious Anemia.

Vitamin B_{12} levels in serum samples to which polyethylene glycol (PEG) had been added and serum samples to which PEG had not been added are shown for two patients with pernicious anemia and three controls without anemia who had normocytic red cells. To convert the values for vitamin B_{12} to picomoles per liter, multiply by 0.7378.

David T. Yang, M.D.
Rachel J. Cook, M.D.

University of Wisconsin School of Medicine and Public Health
Madison, WI
dtyang@wisc.edu

Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

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