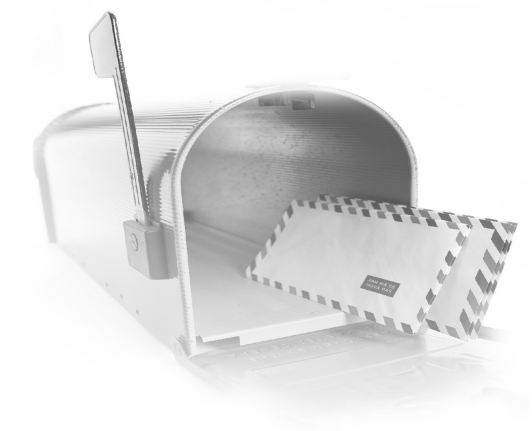

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Letters to the Editor

False elevations of vitamin B12 levels due to assay errors in a patient with pernicious anemia

TO THE EDITOR: Measurement of vitamin B12 levels is the gold standard for the diagnosis of vitamin B12 deficiency. In current practice, total serum vitamin B12 measurements are performed in the clinical laboratory with competitive binding luminescence assays, the results of which may not always accurately reflect actual vitamin B12 stores [1]. Here, we report a case in which a competitive binding luminescence assay led to a falsely increased vitamin B12 result for a patient presenting with classic hematologic and biochemical features of pernicious anemia.

Case

A 45-year-old woman presented with complaints of nausea, weight loss, fatigue, and dizziness that was present for 1 month without any known systemic disease. She ate a good, well-balanced diet and was not taking any medication. On clinical examination, pallor was the only significant finding. Laboratory examination results are shown in Table 1. In the peripheral blood smear, anisopoikilocytosis, macroovalocytes, rare schistocytes, teardrop forms, microspherocytes, and hypersegmented neutrophils were observed. She received four units of red blood cells within a 1-month period. Bone marrow examination showed markedly hypercellular marrow with marked erythroid hyperplasia and megaloblastic hemopoiesis. The hematologic and biochemical features of the blood test results were inconsistent with the diagnosis of any disease.

The assays for vitamin B12 were performed in our laboratory using the UniCelR DxI 800 Cbl assay (Beckman Coulter, Brea, CA, USA), and another assay was performed in a different laboratory using the Elecsys E170 Cbl assay (Roche Diagnostics Corp, Indianapolis, IN, USA). Despite high vitamin B12 levels in repeated assays owing to a very strong suspicion of pernicious anemia, further investigation was performed to establish vitamin B12 deficiency (Table 1), and parenteral vitamin B12 replacement was initiated.

Table 1. Laboratory findings of the patient before injection of vitamin B12.

Hemoglobin (g/dL)	6.4 (NR: 11.7–15.5)
Hematocrit (%)	19.3 (NR: 34.5–46.3)
MCV (fL)	122 (NR: 80–102)
White blood cell (10^3/μL)	4.1 (NR: 4.5–11)
Neutrophil (10^3/μL)	2.2 (NR: 1.8–6.4)
Platelet (10^3/μL)	86 (NR: 159–388)
Serum creatinine (mg/dL)	0.8 (NR: 0.66–1)
Alanine aminotransferase (U/L)	17 (NR: 10–50)
Lactate dehydrogenase (U/L)	2,182 (NR: <248)
Bilirubin (total/direct) (mg/dL)	1.9/1.5 (NR: 0.3–1.2/<0.2)
Prothrombin time (sec)	14 (NR: 9.5–13.2)
Activated partial thromboplastin time (sec)	23 (NR: 25–37)
D-dimer (mg/L)	0.4 (NR: 0–0.55)
Direct Coombs	Negative
Reticulocyte (%)	2.3 (NA: 0.5–1.5)
Haptoglobin (mg/dL)	<7.5 (NA: 30–200)
Folic acid (ng/mL)	8.3 (NR: 3.1–20)
Vitamin B12[a] (pg/mL) (1. Assay)	>1,500 (NR: 126–505)
Vitamin B12[a] (pg/mL) (2. Assay)	>1,500 (NR: 126–505)
Vitamin[b] B12 (pg/mL) (in a different laboratory)	992 (NR: 200–950)
Vitamin[a] B12 (pg/mL) (in a third laboratory)	282 (NR: 211–911)
Glucose 6 phosphate dehydrogenase (U/g Hb)	28 (NR: 6.9–20.5)
Additional laboratory examination as second step diagnostic scheme	
Homocysteine (μmol/L)	81.5 (4.9–15)
Methylmalonic acid	Not available
Anti-intrinsic factor antibody[c]	Positive
Holotranscobalamin (pmol/L)	<5 (NR: 25–165)
Gastrin (pg/mL)	2,760 (NR: 13–115)

[a]The assay carried out in laboratory using UniCelR DxI 800 Cbl assay (Beckman Coulter, Brea, CA, USA). [b]The assay carried out in laboratory using Elecsys E170 Cbl assay (Roche Diagnostics Corp, Indianapolis, IN, USA). [c]Intrinsic factor antibodies were detected by a solid phase enzyme immunoassay with highly purified intrinsic factor purified from porcine gastric mucosa as the antigen and performed as per the manufacturer's instructions (EuroImmun, Lübeck, Germany).
Abbreviations: NR, normal range; MCV, mean cell volume.

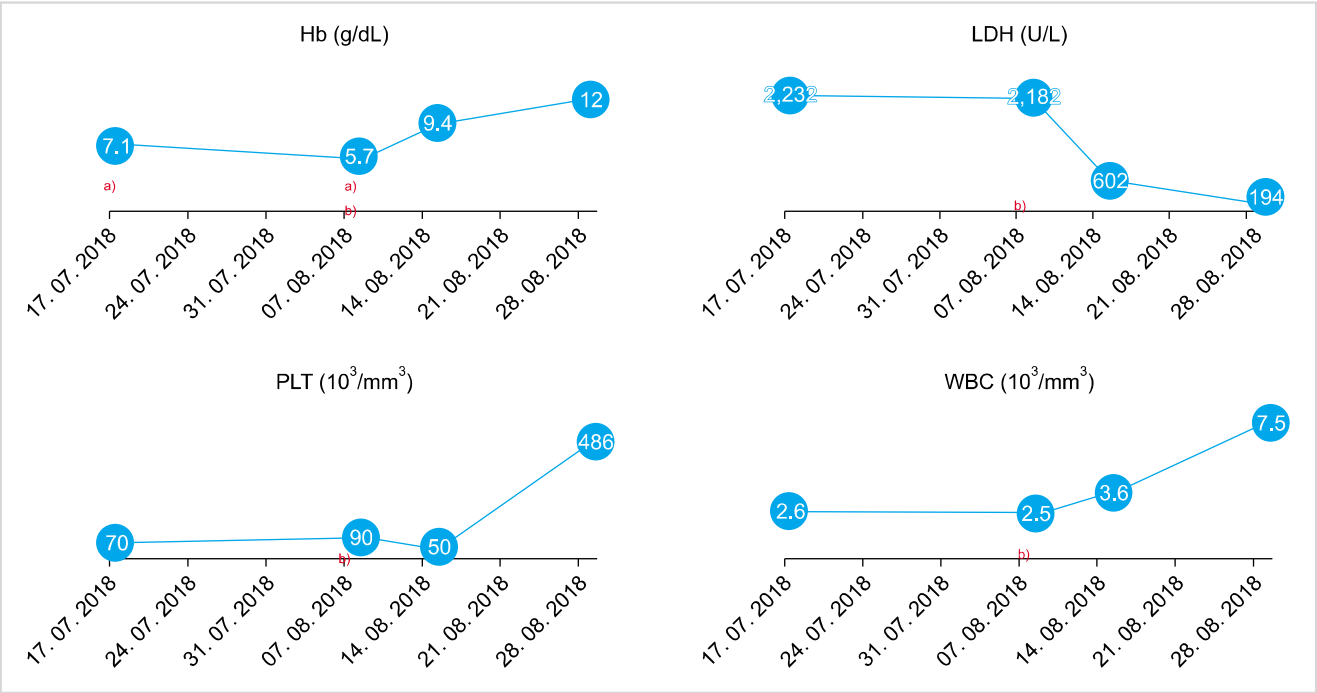

Fig. 1. Hematological recovery with supplementation of vitamin B12. [a]2U of red blood cells replacement. [b]Supplementation of vitamin B12 initiated.

Cyanocobalamin was administered by intramuscular injection at an initial dose of 1,000 mcg once per day for 1 week and followed by 1,000 mcg once per week. Approximately 2 weeks after the supplementation was initiated, clinical and hematological recovery was observed (Fig. 1).

Discussion

Holotranscobalamin (holo-TC), also known as active B12, is the only form of vitamin B12 that is taken up and used by the cells in the body. It accounts for approximately 10% of the circulating vitamin B12 and is the earliest marker showing vitamin B12 depletion [1, 2].

Vitamin B12 deficiency is generally suspected based on related symptoms, clinical findings, and laboratory results and is confirmed by measuring vitamin B12 levels. However, current vitamin B12 measurement methods may miss the lack of vitamin B12 in some cases. These methods, based on competitive binding luminescence assays, have been used since 1990. The assay uses binding to intrinsic factor (IF) following dissociation from the binding proteins, with a readout based on the remaining amount of unbound IF. The main problem with these assays is caused by the presence of IF antibodies in the test sample. IF antibodies may bind the test IF reagent and if there is a failure in the denaturation step intended to denature IF-blocking antibodies, spuriously normal or increased vitamin B12 levels can be measured [3, 4]. Low vitamin B12 levels can be measured as false normal or false high, especially in pernicious anemia, due to excessive amounts of anti-intrinsic factor antibodies present in the serum [5-7].

In the light of data from the available literature, a normal or high vitamin B12 measurement does not exclude vitamin B12 deficiency in cases when vitamin B12 deficiency is suspected. In such an instance, holo-TC and/or metabolic tests, such as homocysteine or methylmalonic acid, may be considered for patients for whom there is a high suspicion of pernicious anemia in the absence of a low vitamin B12 level. Additionally, an alternate approach involves providing vitamin B12 treatment and confirming or eliminating vitamin B12 deficiency according to the response status.

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No potential conflicts of interest relevant to this article were reported.

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First case report of latent tuberculosis reactivation complicating treatment with nilotinib in chronic myeloid leukemia

TO THE EDITOR: Development of tyrosine kinase inhibitors (TKIs) targeting the *BCR-ABL* fusion gene has greatly increased overall survival and major molecular response rates in chronic myeloid leukemia (CML). However, atypical infections such as tuberculosis (TB), hepatitis B virus reactivation, and varicella zoster infection, among others, have been reported after treatment with TKIs [1-6]. Furthermore, several preclinical studies have shown that *BCR-ABL*-targeting TKIs, such as imatinib, dasatinib, and nilotinib, inhibit CD4+ and CD8+ T-cell activity and proliferation [7-9]. Besides their effects on T cells, recent data have shown that TKIs impair B-cell immune responses in CML through off-target inhibition of kinases important for B-cell signaling [10].

It has been reported that nilotinib does not significantly increase infection compared to imatinib and dasatinib [1, 11], but herein, we report the first case in the literature of TB expressed in the form of atypical pneumonia during nilotinib treatment.

A 45-year-old man was referred to our hospital on account of leukocytosis and splenomegaly. He was diagnosed with chronic phase (CP) CML in March 2011. Treatment was started with imatinib but stopped in May 2011 because of hyperbilirubinemia and pericardial/pleural effusion. Subsequently, imatinib was switched to dasatinib, but after 1 year of dasatinib administration, he developed grade 3–4 pleural effusion and thrombocytopenia. Thus, dasatinib was changed to nilotinib 400 mg twice a day (standard dose) on May 2, 2012.

In December 2014, the patient visited the hospital on account of cough, fever, and dyspnea on exertion that had worsened over the prior 2 weeks. Computed tomography (CT) scan of the chest showed diffuse subtle ground glass opacities in both hemithoraces, which was suspicious of atypical pneumonia such as viral infection, pneumocystis pneumonia, miliary TB, or drug-induced pneumonitis (Fig. 1). There was no other specific finding in the lung parenchyma, no lymphadenopathy, and the amount of pleural effusion observed was similar to that observed on the CT scan taken 2 years prior to the event, which had been caused by dasatinib. The initial sputum acid-fast bacilli (AFB) smear stain yielded negative findings, but the interferon-gamma release assay (IGRA) results were positive although the patient had no history of TB. Serum cytomegalovirus and Epstein-Barr virus real-time polymerase chain reaction (PCR) results, and consecutive blood and sputum culture results were all negative.

The bronchoalveolar fluid white blood cell count was 200/μL and was lymphocyte-predominant, comprising 62% lymphocytes and 34% macrophages. Bronchoalveolar fluid AFB stain and TB PCR results were negative.

Initially, intravenous methylprednisolone and intravenous piperacillin/sulbactam were administered based on our suspicion of interstitial lung disease and superimposed

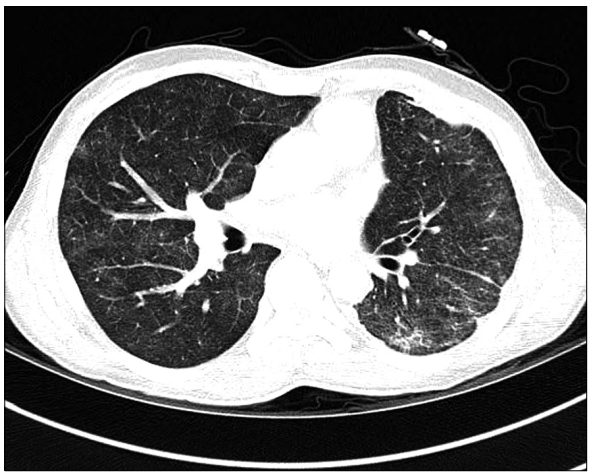

Fig. 1. Computed tomography scan of the chest. Lung setting view image showed diffuse subtle ground glass opacities in the hemithoraces, and atypical pneumonia such as viral infection, pneumocystis pneumonia, miliary TB, or drug-induced pneumonitis was suspected.