Case Report

Unexplained Hypercobalaminemia: A Rare Diagnostic Pitfall

Anil K Chokkalla[1,2*], Kafi B Thomas[3], Arun Rathinam[4]

[1]*Laboratory Medicine, Sanford Health, Fargo, ND, USA*
[2]*Pathology, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND, USA*
[3]*Hematology and Medical Oncology, Sanford Cancer Center, Bismarck, ND, USA*
[4]*Hematology and Oncology, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND, USA*

Article Info

[*]***Corresponding Author:***
Anil Chokkalla, PhD, D(ABCC), NRCC
Clinical Chemist, Sanford Laboratories
Medical Director, Sanford Fargo Point-of-Care Testing
Clinical Assistant Professor, University of North Dakota
Office: 4C156, Sanford Broadway Medical Center
801 Broadway North, Fargo, ND-58102
E-mail: anilkiran.chokkalla@sanfordhealth.org

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Abstract

Persistently elevated serum vitamin B12 may suggest underlying pathology once analytical interferences are excluded. Here, we report a case of 56-year-old female with high vitamin B12 levels incidentally noted by primary care provider. Repeat testing with different assay method over time demonstrated consistently high values, prompting further evaluation. Hematology-oncology provider initiates malignancy workup due to well-documented association between neoplastic process and elevated vitamin B12 levels. Concurrently, clinical laboratory was contacted to rule out potential assay interference related to macro-vitamin B12 complexes. A polyethylene glycol precipitation study demonstrated markedly low vitamin B12 recovery, supporting the presence of a macro-vitamin B12 complex as the cause of spurious elevation. This case report highlights the importance of excluding macro–vitamin B12 interference prior to initiating extensive malignancy workup and the lack of evidence-based guidelines for the laboratory assessment of macro-complexes.

Case description

A 56-year-old female presents to the hematology-oncology clinic for the follow-up of elevated vitamin B12 levels. Past medical history is significant for hypothyroidism and being managed with levothyroxine (100 µg). At the time of initial encounter, patient was undergoing evaluation for fatigue, unintentional weight loss of 7 pounds over last couple months and headache without fever, vision changes and vomiting. Laboratory workup included complete blood count, peripheral smear examination, vitamin B12, and thyroid function panel. Patient was euthyroid with a free T4 of 1.4 ng/dL (reference interval: 0.7-1.5 ng/dL) and TSH of 2.71 µIU/mL (reference interval: 0.35-4.94 µIU/mL). Furthermore, complete blood count showed normal hemoglobin, white blood cells, absolute neutrophil and platelet counts. Patient's vitamin B12 levels are elevated at >1,500 pg/mL (reference interval: 180-914 pg/mL) in September 2025 at an outside facility using Siemens Atellica method. Repeat testing using same method yielded exact same result i.e. >1,500 pg/mL in October 2025. Primary care provider subjected this patient for vitamin B12 testing at our laboratory, which is based on Abbott Alinity method. Consistently, vitamin B12 levels are reported over upper limit of reportable range i.e. >2,000 pg/mL (reference interval: 213-816 pg/mL) on two different days that are one week apart. Patient denied taking supplements containing vitamin B12. Due to persistently elevated vitamin B12, hematologic malignancy was suspected by the hematology-oncology provider. As a part of malignancy workup, age-appropriate cancer screenings were reviewed. Patient's first colonoscopy at the age of 50 years showed a 2 mm sensile polyp in the cecum and 8 mm polyp in the ascending colon. Histopathology on resected tissue was notable for tubular adenoma without high-grade dysplasia. Patient's Esophagogastroduodenoscopy biopsy from 2020 indicated eosinophilic esophagitis. Patient's mammogram was benign with BI-RADS score of 1. Serum protein electrophoresis results were unremarkable with gamma fraction of 0.6 g/dL (reference interval: 0.5-1.3 g/dL). Physical exam did not reveal palpable lymphadenopathy or hepatosplenomegaly. Hepatic and renal function tests are all within normal limits. Evidence from literature shows association between elevated vitamin B12 levels and an underlying solid or hematologic malignancy such as hepatocellular, breast and colon carcinoma, chronic myeloid leukemia and polycythemia vera as well as renal and liver disease [1]. Patient's white blood count and absolute neutrophil count were normal, and differential with absence of basophilia and immature granulocytes. Review of peripheral blood smear by hematopathologist did not reveal any other abnormalities. CT scan (chest/abdomen/pelvis) was ordered for further evaluation. Collectively, the laboratory and clinical features do not support the presence of an underlying myeloproliferative neoplasm. Previous studies linked falsely elevated vitamin B12 levels due to assay interference secondary to macro-complex [2]. Provider contacted the clinical laboratory to assess the macro-vitamin B12 status

Case discussion

Vitamin B12 deficiency has been implicated in diverse pathological states such as neuropathy, cognitive dysfunction, anemia, cardiovascular disease, age-related macular degeneration, and gastrointestinal disorders. However, the clinical significance of elevated serum B12 levels is not well understood [3]. Elevated vitamin B12 levels often reflect recent supplementation, as serum concentrations typically peak about 6–8 hours after ingestion [4]. An estimated 10% laboratory specimens show vitamin B12 levels above the upper limit of normal [5]. Several preanalytical factors like 1) macro complexes, 2) heterophile antibodies and 3) autoantibodies against intrinsic factor are implicated in the literature [4]. Additionally, piecemeal evidence links elevated vitamin B12 levels to hematologic or solid-organ malignancies. High association was noted between hypercobalaminemia and neoplastic disease with a odds ratio of 1.8 [1]. Hence, Hematology/Oncology providers are presented with a dilemma to rule out preanalytical vs. clinical causes of elevated vitamin B12. The former is challenging due to lack of analytical methods, whereas the latter requires extensive workup. Circulating macro-complexes form predominantly due to binding of analyte or its carrier protein with immunoglobulins. Best known examples include macro-TSH, macro-amylase, macro-troponin, macro-prolactin and macro-creatine kinase. The key features of these macro-complexes are slow clearance from the bloodstream and lack of biological activity [5]. In the bloodstream, ~ 4/5th of vitamin B12 is bound to haptocorrin, while the 1/5th is carried by transcobalamin, the transport protein responsible for delivering vitamin B12 to tissues. Macro-vitamin B12 complexes are thought to originate from aberrant binding of carrier proteins with immunoglobulins or soluble receptors and in rare instances, from direct binding of free vitamin B12 with γ-globulins [4]. Polyethylene glycol (PEG) precipitation is a widely used non-specific method for the removal of macromolecules from plasma samples. Of note, gel filtration chromatography is the reference method but often limited to specialty reference laboratories. Currently, there are no evidence-based guidelines for troubleshooting macro-complexes.

To rule out interference due to macro B12 complex, we subjected the patient's specimen to PEG precipitation, followed by measurement of monomeric vitamin B12 in the supernatant as described previously [6]. Briefly, 100 µL of PEG 8000 solution (250 g/L) was mixed with 100 µL of patient plasma and incubated at 370C for 30 mins. The macro-vitamin B12 complex is precipitated by centrifuging at 13,000 rpm for 3 mins, and the supernatant is analyzed in duplicates along with neat sample. Vitamin B12 concentration of the pre-PEG treated sample was 1894 pg/mL, whereas the post-PEG treated sample was 169 pg/mL. Importantly, the recovery of vitamin B12 [2*(post-PEG B12/pre-PEG B12)x100] after PEG treatment was only 18% (Table 1). Previous studies recommend that a recovery of ≤50% after PEG precipitation is consistent with macro-vitamin B12 interference [3]. Hemotology-oncology

provider showed interest in elucidating the components of this macro-vitamin B12 complex. To further investigate the constituents of macro-vitamin B12 complex, the plasma levels of rheumatoid factor, immunoglobulin's G, A and M were measured. Interestingly, we did not notice any abnormality of these protein levels in the neat specimen (Table 1). It will be interesting to subject these specimens to confirmatory testing with gel filtration chromatography, but we did not have access to this method within our geographical location. It is plausible that soluble transcobalamin receptor is responsible for the macro-vitamin B12 complex formation [7]. Altogether, these findings are suggestive of potential interference with macro-vitamin B12 complex.

Table 1: PEG precipitation of macro-vitamin B12 complex.

Analyte	Result	Reference Interval
Vitamin B12 Neat (pg/mL)	>2000	213-816
	>2000	
Vitamin B12 Saline (pg/mL)	1853	
	1935	
Vitamin B12 PEG (pg/mL)	167	
	170	
Rheumatoid Factor (IU/mL)	<13	<30
Immunoglobulin G (mg/dL)	795	552-1631
Immunoglobulin M (mg/dL)	107	33-393
Immunoglobulin A (mg/dL)	314	65-421

Vitamin B12 is quantified on the Abbott Alinity platform using a two-step competitive chemiluminescent microparticle immunoassay. In this method, pre-treatment step releases vitamin B12 from carrier proteins which then competes with a labeled vitamin B12 analog for binding to intrinsic factor–coated paramagnetic microparticles, producing a chemiluminescent signal that decreases as the analyte concentration increases. Importantly, the assay package insert does not list macro-vitamin B12 interference as potential limitation. Clinical Laboratory Standards Institute guidelines do not currently provide specific recommendations for evaluating assay interference caused by macro-complexes. This gap leaves laboratories to rely on local protocols and published case reports when assessing potential macro-vitamin B12 interference. Even though vitamin B12 levels are consistently high due to macro-vitamin B12 complex, it is important to rule out functional deficiency using adjunct biomarkers such as methylmalonic acid, holo-transcobalamin and homocysteine, a notable limitation of the present case.

Ethical approval

Patient consent submitted to Sanford Health Information Management.

Declaration of conflicts

The authors declare no conflict of interest.

Funding and Data Availability

Not applicable.

CRediT author statement

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