Special Article

B12 Hypervitaminemia: Pathogenetic Pathways and Clinical Implications

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Abstract
Background. Pathological changes in vitamin B12 levels are a common laboratory finding. The increase in serum vitamin B12 levels is termed B12 hypervitaminemia, often perceived as a benign disorder, leading to its underestimation.
Objective. This paper aims to examine the potential pathogenetic mechanisms and the association of vitamin B12 excess with various diseases.
Methodology. A literature search was conducted using the PubMed, DOAJ, and Google Scholar databases. The search was focused on original articles, review papers, and reference lists published in English since 2000.
Results. Increased vitamin B12 levels are encountered in hematological disorders, solid tumors, renal and hepatic diseases, as well as inflammatory conditions. Vitamin B12 supplements increase the levels and can exacerbate underlying pathological conditions. The pathophysiology involves disturbances in the biology and metabolism of vitamin B12 and its carrier proteins. Falsely elevated values can arise due to interferences in quantitative assays. Elevated vitamin B12 may represent an incidental finding, accompany findings of underlying disease, and coexist with manifestations of functional deficiency.
Conclusion. Elevated vitamin B12 may serve as an early indicator for the diagnosis and monitoring of serious diseases. Understanding the underlying pathogenetic mechanisms and establishing diagnostic algorithms are crucial areas for future research.

Key Words: B12 hypervitaminemia, hematological malignancies, liver disease, renal failure, transcobalamins

Introduction

Vitamin B12 exists in various forms in the human body, which interconvert or are transformed into biologically inactive analogs in the liver or bowel by intestinal microorganisms (Ermens, Vlasveld & Lindemans, 2003). The main active forms are cytoplasmic methylcobalamin and mitochondrial adenosylcobalamin. Methylcobalamin is a coenzyme for methionine synthetase, facilitating the conversion of homocysteine to methionine. Methionine is converted to S-adenosylmethionine, essential for DNA and RNA methylation, and genomic stability. Adenosylcobalamin participates in the conversion of methylmalonic acid to succinyl-coenzyme A, an intermediate in the citric acid cycle. Vitamin B12 deficiency leads to elevated levels of homocysteine and methylmalonic acid (Ermens, Vlasveld & Lindemans, 2003; Arendt & Nexo, 2013). Vitamin B12 also plays a pivotal role in nervous tissue development and erythropoiesis.

The introduction of automatic analyzers in laboratory practice has increased vitamin B12 testing, especially in case of suspected vitamin B12 deficiency. The percentage of elevated levels ranges from 1.3% to 18%. High (600-1,000 pmol/L) and very high (>1,000 pmol/L) levels are observed in 13% and 7% of hospitalized patients, respectively (Arendt & Nexo, 2012; Andrès et al., 2013; Remacha et al., 2014). Despite often being considered benign, B12 hypervitaminemia has been linked to solid tumors, liver and kidney diseases, neuropsychiatric disorders, and autoimmune diseases. Therefore, vitamin B12 could serve as a useful parameter for the

early diagnosis or monitoring of various diseases. In clinical practice, there is no consensus on investigation and monitoring of B12 hypervitaminemia due to multifactorial pathogenesis. This review explores the pathophysiology and clinical significance of vitamin B12 excess across various disorders. It also delves into the assays used for quantifying vitamin B12 levels and outlines the diagnostic approach to B12 hypervitaminemia.

Material and methods

The literature search was conducted using PubMed, DOAJ, and Google Scholar databases, employing the keywords: B12 hypervitaminemia, hematological malignancies, liver disease, renal failure, transcobalamins. The search included review articles and original studies in humans, written in English since 2000. The reference lists of 83 retrieved journal articles were examined for additional pertinent studies. Fifty-nine articles deemed of greatest relevance are listed herein, comprising 38 clinical studies, 8 laboratory investigations, and 2 meta-analyses.

Results and discussion

Absorption and bioavailability of vitamin B12

Cobalamin, a water-soluble vitamin, is naturally found in foods of animal origin like meat, fish, and eggs. Nutritional supplements contain high doses of vitamin B12; however, only 1% is absorbed after oral administration. Therefore, a dose of 500 mcg is sufficient to meet daily needs (Arendt & Nexo, 2013). Vitamin B12 is released from food and binds to haptocorrin in saliva. Gastric acid facilitates vitamin B12 release from haptocorrin, and it then binds to intrinsic factor (IF), produced by gastric parietal cells. The vitamin B12-IF complex is absorbed via the cubam receptor in the terminal ileum (Ermens, Vlasveld & Lindemans, 2003). Vitamin B12 enters circulation by binding to transcobalamins (TC). TCI and TCIII belong to the haptocorrin family and are released from the secondary granules of granulocytes. They bind to 80-95% of vitamin B12 and are almost completely saturated by vitamin B12 and its analogs (Ermens, Vlasveld & Lindemans, 2003; Nielsen et al., 2012). Haptocorrins are metabolized in the liver, with a circulation half-life of 6-9 days (Zulfiqar & Andres, 2018). They are involved in immune defence and removal of inactive analogs, as 40% of them are saturated with analogs. Their biological role is of limited significance and their deficiency has no obvious effects (Ermens, Vlasveld & Lindemans, 2003).

TCII is mainly produced in hepatocytes and secondarily in endothelial cells, monocytes, and enterocytes (Andrès et al., 2013). In enterocytes, TCII binds to cobalamin in a 1:1 ratio and is the main carrier protein (Ermens, Vlasveld & Lindemans, 2003). TCII is bound to 5-20% of vitamin B12, representing the active fraction (Andrès et al., 2013). Congenital TCII deficiency is associated with neuropsychiatric disorders, pancytopenia, and megaloblastic anemia. The amount of free vitamin B12 is negligible. The bound forms with TCII and haptocorrins are holo-TCII and holo-HC, respectively, while the unconjugated forms are apo-TCII and apo-HC, respectively (Ermens, Vlasveld & Lindemans, 2003).

The cellular uptake of holo-TCII is mediated by the surface receptor CD320. Endocytosis of holo-TCII is followed by proteolysis. The liver stores the largest amount of vitamin B12 through receptors in hepatic endothelium. Vitamin B12 and its analogs are excreted in the intestine with bile. Around 90% of vitamin B12 is reabsorbed and binds to IF, while its analogs are excreted in the feces. The amount of vitamin B12 participating in the enterohepatic cycle is 2-5 times the normal intake (Ermens, Vlasveld & Lindemans, 2003; Arendt & Nexo, 2013). This supplies 5-7 mcg of vitamin B12 daily, maintaining normal stores for up to 5 years (Andrès et al., 2013). Free vitamin B12 is excreted in the urine, while holo-TCII and apo-TCII are filtered and reabsorbed in the kidney (Figure 1) (Ermens, Vlasveld & Lindemans, 2003).

Pathophysiology of high vitamin B12

The pathogenesis of B12 hypervitaminemia is often unclear and encompasses multiple mechanisms. Excessive intake is one aspect; but it often involves dysregulation of vitamin B12 biology and its carrier proteins. Associated pathology includes hematological malignancies, solid tumors, liver disorders, nephropathies, autoimmune diseases, infections, and neurodevelopmental

disorders. The underlying mechanisms involve increased hepatic release, increased production, or reduced haptocorrin clearance, and TCII deficiencies (Table 1) (Andrès et al., 2013).

Table 1.Clinical and pathogenetic aspects of B12 hypervitaminemia

	Pathogenetic mechanism	Clinical significance
Myeloproliferative disorders	– Production of haptocorrins by malignant myeloid cells	– Diagnostic criterion in polycythemia vera – Differential diagnosis from secondary hematological disorders – Indicator of tumor burden
Solid tumors	– Production of haptocorrins by malignant cells and reactive leukocytes – Reduced liver clearance of vitamin B12-haptocorrin complex	– Correlation with advanced tumor stage and high mortality rate
Renal disorders	– Vitamin B12 supplementation – Defects in transcobalamin II filtration and absorption in the kidneys	– Functional vitamin B12 deficiency – Association with reduced kidney function in hyperhomocysteinemia
Liver disorders	– Increased haptocorrin levels – Release of stored vitamin B12 – Reduced transcobalamin II synthesis	– Functional vitamin B12 deficiency – Correlation with disease severity
Inflammatory disorders	– Production of haptocorrins by reactive leukocytes and transcobalamin II by macrophages – Antibodies against transcobalamin II – Interfering antibodies in vitamin B12 quantification	– Correlation with acute inflammatory phase
Neurodevelopmental disorders	– Genetic polymorphisms affecting vitamin B12 binding proteins – Immune complexes affecting vitamin B12 metabolism – Respiratory and gastro-intestinal co-morbidities	– Correlation of high vitamin B12 levels in pregnancy with autism spectrum disorders

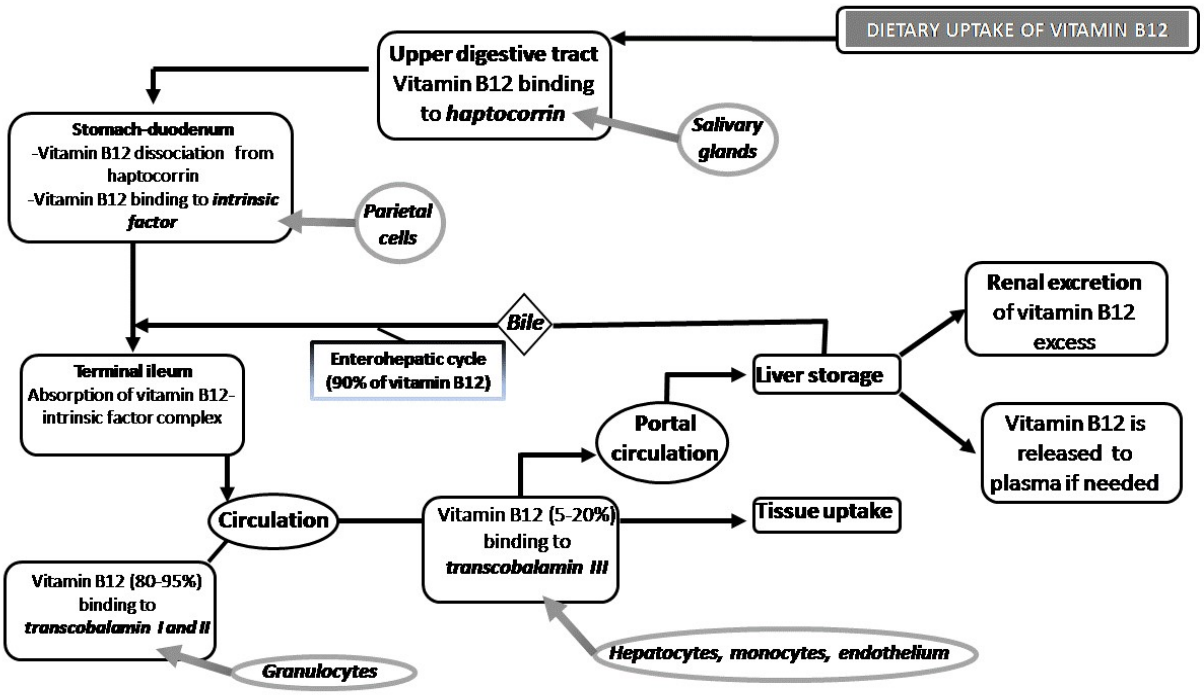

Figure 1. Vitamin B12 absorption and metabolism. Vitamin B12 is absorbed in the terminal ileum and transported to the liver via the portal circulation. Approximately 90% of the vitamin excreted into the intestine through bile is reabsorbed. A small amount of the vitamin is eliminated from the body through feces and urine.

Increased exogenous intake

Increased exogenous intake is mainly attributed to oral multivitamin supplements (Remacha et al., 2014). Diets rich in fat, vinegar, and pickles increase vitamin B12 synthesis by gut bacteria (Danchin & Braham S, 2017). In vegans, vitamin B12 levels are 33% and 57% lower than vegetarians and omnivores, respectively (Gilsing et al., 2010). Deficiency of iodine, selenium, and molybdenum can impair the activation of vitamin B12 from foods or supplements. Prolonged lack of active vitamin B12 leads to the accumulation of inactive forms, increasing serum levels (Russel-Jones, 2022). Vitamin B12 excess increases TCII saturation (Arendt & Nexo, 2013). Long-term parenterally administrated vitamin B12 was associated with anti-TCII autoantibodies in 30% of patients, reducing TCII clearance (Andrès et al., 2013).

Hematological malignancies

In chronic myelogenous leukemia (CML), vitamin B12 levels can increase up to 10 times the upper normal limit (Ermens, Vlasveld & Lindemans, 2003). Conversely, idiopathic B12 hypervitaminemia is linked to a 4-18 times higher risk of hematological disorders (Arendt & Nexo, 2012). The increased vitamin B12 is attributed to haptocorrin release from malignant granulocytes and myeloid progenitors. Serum vitamin B12 has been used in diagnosing and monitoring of CML, correlating with leukocytic count changes (Arendt & Nexo, 2013). The prognostic value of apo-HC in CML remains uncertain (Ermens, Vlasveld & Lindemans, 2003).

B12 hypervitaminemia occurs in 30-50% of polycythemia vera cases, where high levels serve as a minimal diagnostic criterion (Ermens, Vlasveld & Lindemans, 2003). Clonal eosinophilia showed vitamin B12 levels up to 30 times normal (Nanagas & Kovalszki, 2019). In myelofibrosis, increased apo-HC and/or apo-TC were observed in 50% of patients, and one-third of them had B12 hypervitaminemia (Zulfiqar & Andres, 2018). Similarly, an increase in apo-HC and apo-TC was reported in acute myeloid leukemia, with 30% of leukemia cases having high vitamin B12 (Ermens, Vlasveld & Lindemans, 2003;

Haghighat, Khajeh-Mehrizi & Ranjbar, 2023). In acute promyelocytic leukemia, vitamin B12 increased proportionally to disease burden (Ermens, Vlasveld & Lindemans, 2003).

B12 hypervitaminemia is rare in lymphoproliferative disorders. However, high vitamin B12 and apo-TC binding capacity were reported in multiple myeloma, lymphoma, and hairy cell leukemia due to enhanced macrophage activity (Ermens, Vlasveld & Lindemans, 2003; Arendt & Nexo, 2013). TCII increase mimics an acute-phase reaction to malignancy-related inflammation or may be due to pathological elimination in cancer patients (Obeid, 2022).

Solid tumors

The link between B12 hypervitaminemia and solid tumors is controversial. Associated malignancies include primary and secondary liver neoplasms, breast, prostate, colon, stomach, pancreas, lung, and kidney cancers (Andrès et al., 2013; Arendt & Nexo, 2013). The pathophysiology remains unclear, with relevant studies not investigating the intracellular vitamin B12 status (Nanagas & Kovalszki, 2019). Holo-HC is increased in more than 50% of patients with hepatocellular tumors (Ermens, Vlasveld & Lindemans, 2003). This is attributed to the reduced hepatic clearance of holo-HC due to decreased hepatic vascularity and haptocorrin receptors on malignant cells. Reactive leukocytosis and haptocorrin production by cancer increase the haptocorrins. High TCI is associated with advanced tumor stage and reduced treatment response. Released TCI binds to vitamin B12, increasing the levels in circulation (Obeid, 2022). Moreover, the destruction of hepatocytes in liver neoplasms and the immune response to renal tumors increase TCII (Ermens, Vlasveld & Lindemans, 2003; Andrès et al., 2013). A recent study showed an association between vitamin B12 and hepatocellular carcinoma due to liver damage. Higher levels were observed in advanced tumor stages. The incidence of vitamin B12 supplementation did not differ between cancer patients and the control group. Successful cancer treatment decreased vitamin B12, suggesting B12 hypervitaminemia may be a result rather than a cause of malignancy (Obeid, 2022).

In a study of 333,667 patients with vitamin B12 ≥200 pmol/L, 7% of them developed malignancy. The risk was high in the first year and at vitamin B12 levels >800 pmol/L. After the first year, the risk remained high, especially for alcohol- and smoking-related neoplasms and hematological malignancies in patients with vitamin B12 >800 pmol/L (Arendt et al., 2013). A study involving 25,783 patients revealed an increased risk for liver, pancreas, and bone marrow malignancies in those with high vitamin B12 in the first year (Arendt et al, 2019). A meta-analysis indicated a 26% increase in the risk of prostate cancer for every 100 pmol/L increase in vitamin B12 (Collin et al., 2010).

Elevated levels were associated with a higher incidence of metastases and one-month median survival. Vitamin B12 was up to 30-fold the upper normal limit in 30-40% of patients with liver metastases, and the tumor size was correlated with vitamin B12 levels (Zulfiqar & Andres E, 2018). An increased risk of both metastatic and non-metastatic cancers was found in 785 patients with vitamin B12 >738 pmol/L (odds ratio, OR 4.21) (Urbanski et al., 2020). Progression of gastric cancer was better associated with haptocorrins (Lacombe, Lenaers & Urbanski, 2022). In a study of 25,017 cancer patients, the survival rate was lower among those with high vitamin B12. The 30-day mortality rate was high in patients with vitamin B12 levels >601pmol/l (Arendt et al., 2016). The BCI index, derived from multiplying vitamin B12 by C-reactive protein (CRP), had a high prognostic value regarding the mortality rate in cancer patients under palliative care ($p<0.001$) (Geissbühler, Mermillod & Rapin, 2000).

A study of 621 elderly cancer outpatients highlighted BCI as a predictor for mortality in the first trimester and emergency hospitalization in the first month and trimester from diagnosis (Couderc et al., 2020). The first month mortality rate was 90% and 50% in patients with BCI >40,000 and <10,000, respectively (Geissbühler, Mermillod & Rapin, 2000). In a study of 329 patients with advanced and metastatic cancer, the median survival was 29 days in those with BCI >40,000 (Kelly, White & Stone, 2007).

Hepatic disorders

Alcoholic liver disease is well-studied regarding B12 hypervitaminemia, primarily due to increased haptocorrin binding to B12, reducing hepatic clearance (Arendt & Nexo, 2013). Vitamin B12 release from damaged cells is a complementary process. Reduced TCII synthesis leads to reduced vitamin's cellular entry, causing a functional deficiency with a concurrent increase in plasma (Andrès et al., 2013; Arendt & Nexo, 2013). B12 hypervitaminemia was observed in 25-40% of acute hepatitis cases, where cellular destruction due to inflammation results in vitamin B12 release into circulation. In liver cirrhosis, vitamin B12 correlated with disease severity and reached 4-5 times the upper reference values. Cellular damage in cirrhosis is milder compared to hepatitis, and the increased levels are mainly due to reduced cellular uptake. Liver biopsies of cirrhotic patients showed decreased intracellular vitamin B12 (Ermens, Vlasveld & Lindemans, 2003).

In acute heart failure, B12 hypervitaminemia is attributed to hepatic congestion and functional liver pathology (Zafarullah et al., 2008). Chronic heart failure may also affect vitamin B12 levels, associated with liver damage from right heart failure. Multivariate analysis in 129 patients with stable heart failure revealed a significant correlation with age and direct bilirubin. Vitamin B12 ≥205,8 pmol/L predicted all-cause mortality with sensitivity and specificity of 80% and 58%, respectively (Argan et al., 2018).

Renal disorders

In renal disorders, vitamin B12 deficiency is common due to impaired cubilin function and low-protein diets. Hyperhomocysteinemia is common among renal patients, even with normal vitamin B12 levels, necessitating vitamin B12 supplementation to maintain low levels. In chronic kidney disease (CKD), exogenous vitamin B12 intake saturates the reabsorption mechanisms in the proximal tubule, leading to increased serum concentrations. In this case, kidneys become a significant route of vitamin B12 excretion. Elevated vitamin B12 per se was not associated with reduced kidney function. However, in hyperhomocysteinemia, high vitamin B12 correlated with reduced kidney function (McMahon et al., 2015).

TCII is filtered at the glomerulus and reabsorbed in the proximal tubule by megalin. Defects in reabsorption process could lead to urinary TCII loss (Arendt & Nexo, 2012). In CKD, the functional vitamin B12 deficiency is due to increased urinary TCII loss, decreased TCII absorption, and reduced TCII cellular uptake (McMahon et al., 2015; Juszczak, Kupczak & Konecki, 2023). The association between B12 hypervitaminemia and interstitial nephropathy (OR 2.7) implicates impaired cellular uptake despite the excess of TCII receptors (Andrès et al., 2013).

Autoimmune diseases

In autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and Still's disease, vitamin B12 levels rise due to increased haptocorrin and TCII production by neutrophils and macrophages, especially during the acute inflammatory phase (Andrès et al., 2013; Arendt & Nexo, 2013). Antibodies against TCII and immune complexes were detected in 8% of patients and 25% of serum samples with high vitamin B12, usually in the context of autoimmune disorders, plasma cell dyscrasias, or other hematological disorders (Obeid et al., 2005). TCII-antibody complexes affected TCII clearance, prolonging its half-life in circulation. In autoimmune-related lymphoproliferative syndromes, persistently elevated vitamin B12 stems from lymphocyte-produced haprocorrins (Jeffery et al., 2010). Though antibodies have limited clinical significance since no decrease in intracellular vitamin B12 was observed, interference in quantitative assays should be considered when symptoms do not align with laboratory results (Obeid et al., 2005; Remacha et al., 2014).

Infectious diseases

Typhoid fever and malaria have been associated with elevated vitamin B12 and TCII levels. Similarly, 29% of HIV-positive adults and vertically infected children experienced B12 hypervitaminemia (Arendt & Nexo, 2012; Bowen et al., 2012). In 49 COVID-19 patients, 9 individuals with the worst prognosis had higher vitamin B12 compared to recovered ones (1,315±1,087 vs 583±295, p=0.02). Proposed mechanisms include increased carrier proteins, decreased hepatic clearance, and decreased peripheral

tissue uptake (Malik et al., 2009). In horses, known herbivore animals, intestinal colonization by Helicobacter pylori maintains normal vitamin B12. However, the association of Helicobacter pylori with vitamin B12 levels in humans remains unknown (Albayrak D & Albayrak C, 2021).

Neurodevelopmental disorders

Neurodevelopmental disorders, autism, and intellectual disabilities have been associated with B12 hypervitaminemia. A study involving 222 patients with neurodevelopmental disorders, 401 patients with schizophrenia, and 483 healthy controls demonstrated that those with neurodevelopmental disorders had 4.1 times more frequent high vitamin B12 (Dalbeni et al., 2021). High levels during pregnancy were associated with a two-fold risk of autism spectrum disorders (ASD) in childhood (Hope et al., 2020).

The pathophysiology implicates genetic factors, immune mechanisms, and co-existing respiratory and gastrointestinal diseases. High TCI expression was associated with poor verbal memory, a common finding in neurodevelopmental disorders (Raghavan et al., 2018). Increased binding of vitamin B12 to elevated TCI reduces cellular uptake (Akkouh et al., 2018). Variations in the CUBN gene, responsible for vitamin B12 uptake and secretion, are associated with high vitamin B12 and neurodevelopmental and psychiatric defects. Absence of TCII genes leads to reduced vitamin B12 efficiency and intracellular utilization (Schork et al., 2019).

In neurodevelopmental disorders, antibodies against vitamin B12 and immune complexes decrease renal secretion and cellular uptake, resulting in higher serum levels (Remacha et al., 2014; Dalbeni et al., 2021). Oxidative stress in hypoxic bronchopulmonary diseases increases vitamin B12 and exacerbates neurodevelopmental issues (Gunduz et al., 2017).

In 91 children with celiac disease and cerebral palsy, 19% had hypervitaminemia B12, and it was more frequent in those on antiepileptics. Children with high vitamin B12 weighed less than those with normal levels. The weak correlation between vitamin B12 and G anti-gliadin antibodies was attributed to subclinical intestinal inflammation, and altered intestinal microflora, especially in combination with valproic acid (Mirsa et al., 2017).

Clinical presentation

The clinical consequences of B12 hypervitaminemia are less studied compared to those of vitamin B12 deficiency. Vitamin B12, as a water-soluble vitamin, circulates without exhibiting toxicity in high concentrations (Stenberg, Böttiger & Nilsson, 2020). Therefore, vitamin B12 uptake in pernicious anemia is not associated with toxic, carcinogenic, or teratogenic effects (Borgannkar & Patil, 2021). However, doses exceeding therapeutic levels by 2,000-4,000% have been associated with dermatological issues like Rosacea fulminans (Molloy, 2021). Long-term intramuscular doses caused acne or worsened pre-existing skin lesions, which subsided upon vitamin cessation (Arendt & Nexo, 2013). Vitamin B12 supplements likely induce vitamin's synthesis by skin Propionibacterium, increasing the likelihood of acne. Additionally, they stimulate porphyrin synthesis, contributing to acne-related inflammatory changes (Koprivica & Bjelanovic, 2021).

Cyanocobalamin is metabolized to active methylcobalamin, releasing small amounts of cyanide. In CKD patients receiving high cyanocobalamin doses, the reduced glomerular filtration rate prevents the excretion of cyanide ions, leading to uremic nephropathy (Kang et al., 2015; Juszczak, Kupczak & Konecki, 2023).

An observational study of 105 osteoporotic patients revealed a link between excess B12 and spinal fractures, with quicker recurrence after recovery (Li et al., 2022). Daily doses ≥30 mcg led to a 25% higher fracture risk over 30 years. High vitamin B12 and B6 doses increased fracture risk in postmenopausal women (Meyer et al., 2019). Additionally, high vitamin B12 doses were associated with optic neuropathy and glaucoma (Liu et al., 2023).

Paradoxically, B12 hypervitaminemia may indicate functional vitamin B12 deficiency akin to low levels. Increased haptocorrins bind more vitamin B12, reducing its binding to TCII and cell transport. Conditions causing cell breakdown can elevate vitamin B12

alongside functional deficiency. Liver damage hinders vitamin B12 uptake and leads to stored vitamin's release (Andrès et al., 2013). Liver cirrhosis and CML patients may experience hematological and neurological symptoms as those deficient in vitamin B12 (Zulfiqar & Andres, 2018). Vitamin B12 levels >369pmol/L increased the thrombotic risk in elderly orthopedic and cancer patients, due to inflammation and hyperhomocysteinemia (Grossfeld et al., 2013; Arendt et al., 2017).

B12 hypervitaminemia was associated with a high mortality risk in both cancer and non-cancer patients. Oncology patients under palliative care with BCI >40,000 had a 90% three-month mortality rate (Zulfiqar & Andres, 2018). In advanced cancer patients, those with BCI >40,000 had a median survival of 29 days (Kelly, White & Stone, 2007). High vitamin B12 was associated with increased all-cause mortality in 5,571 patients without a history of vitamin B12 uptake, even after adjusting for various factors (Flores-Guerrero et al., 2020).

Despite the positive association between vitamin B12 and CRP during the first two days of hospitalization in the intensive care unit, elevated vitamin B12 was not a significant predictor of thirty- and ninety-day mortality (Stenberg, Böttiger & Nilsson, 2020). Vitamin B12 role as an acute-phase protein warrants further investigation (Romain et al., 2016). In 1,136 hospitalized elderly patients without malignancy or liver disease, mortality risk was 16.5% with vitamin B12 >481 pmol/L (Baztán et al., 2010). High levels (>400 pmol/L) in non-oncology geriatric patients were linked to a 4.48-fold risk of death within 90 days from admission (Salles et al., 2005). B12 hypervitaminemia in elderly patients is correlated with higher in-hospital mortality and two-year mortality, irrespective of comorbidities, performance status, or cognitive level (Grossfeld et al., 2013). In a study of 6,837 patients with ischemic heart disease, vitamin B12 and folate treatment was associated with increased malignancy and mortality risk (Ebbing et al., 2009).

Laboratory assessment

In laboratory, total vitamin B12, encompassing vitamin bound to haptocorrins and TCII after release from carrier proteins, is measured (Andrès et al., 2013). Competitive binding luminescence assays detect free vitamin B12 in serum or plasma by binding it to IF, calculating concentration from emitted light associated with remaining unbound IF. Modern analyzers like Roche Cobas E601, DXI 800 Unicel, Architect L200sr, ADVIA Centaur use electrochemiluminescence, chemiluminescence after enzymatic reaction, or plain chemiluminescence. Radio-immunometric methods (RIA and IRMA) are also sensitive to small vitamin amounts. These methods correlate well with methylmalonic acid and homocysteine levels (İspir et al., 2015).

Serum vitamin B12 has limited diagnostic accuracy for assessing tissue deficiency due to low specificity and sensitivity (Molloy, 2021). Measurement discrepancies result from lack of assay standardization and reference method determination (İspir et al., 2015). Reference values range from 200 to 600 pmol/L. Beckman DXI analyzer showed a lower reference limit (81-415 pmol/L) compared to Roche (140-500pmol/L) and Siemens (134-415pmol/L) (Jassam et al., 2023).

There is no consensus on high-level definition; some studies consider 700-1,500 pmol/L and >1,500 pmol/L as high and very high, respectively, while others use 601-1,000 pmol/L and >1,000 pmol/L (Stenberg, Böttiger & Nilsson, 2020). Pediatric reference levels vary due to maternal vitamin B12 uptake and fortified baby milk, with elevated serum vitamin B12 levels often from metabolically inactive haptocorrin complexes, not reflecting major defects (Mirsa et al., 2017).

Antibodies against IF, encountered in 70% of pernicious anemia cases, can interfere with assays, yielding falsely normal or high levels (Yang & Cook, 2012; .Iltar, Göçer & Kurtoğlu, 2019). Adding 25% polyethylene glycol (PEG) in a 1:1 ratio to pathological samples precipitates interfering immunoglobulins, revealing even undetectable vitamin B12 levels (Yang & Cook, 2012). Immune complexes with binding proteins can be detected by chromatography, often involving IgG, IgA, and/or IgM antibodies (Bowen et al., 2006;

Remacha et al., 2014). In low intracellular folate cases, inactive cobalamin analogs can also interfere with quantification (Nanagas & Kovalszki, 2019). Older methods reliant on microbial growth may yield falsely low levels due to serum antibiotics (Andrès et al., 2013).

Holo-TCII levels reflect active vitamin B12, with low holo-TCII serving as an early indicator of deficiency. Abnormal vitamin B12 levels should be confirmed with a second test and methylmalonic acid and homocysteine testing are essential for assessing cellular status (Ermens, Vlasveld & Lindemans, 2003; Andrès et al., 2013).

Diagnostic approach

Vitamin B12 lacks specificity as a diagnostic or monitoring marker, but levels >1,000 pmol/L may indicate underlying pathology (Arendt & Nexo, 2013). Confirming with a second test and checking medical history for supplements is essential (Andrès et al., 2013).

Exclusion of hematologic malignancies and solid tumors is critical, involving full blood count, peripheral blood smear microscopy, and imaging studies based on symptoms and signs. Creatinine, transaminases, and alkaline phosphatase help identify kidney and liver diseases. Erythrocyte sedimentation rate and CRP assess underlying inflammation. Skin lesion biopsy, coagulation tests, protein electrophoresis, ferritin, and folate levels are recommended. Direct Coombs reaction, autoantibodies testing, and lymphocyte subpopulations are recommended in cytopenic cases. Quantification of haptocorrins and autoantibody testing are used to rule out malignancy, liver or kidney pathology (Arendt & Nexo, 2013). Elevated soluble TC receptors (sCD320) can increase TC and holo-TC (Hoffmann-Lücke et al., 2013). Assessing methylmalonic acid, homocysteine, and holo-TC provides insights into intracellular vitamin B12 status. Elevated metabolite levels and low holo-HC suggest vitamin B12 deficiency.

Patients with negative initial work-up should have vitamin B12 rechecked, along with full blood count, and blood smear microscopy after 3-6 months. Follow-up for up to two years is advised to rule out hematological malignancies. Any new symptoms or changes in white blood cell counts require thorough assessment (Arendt & Nexo, 2013).

Conclusion: B12 hypervitaminemia is associated with serious and potentially fatal diseases, with a complex pathophysiology involving quantitative changes in cobalamin and its binding proteins. Clinical presentation often mirrors underlying conditions but can paradoxically resemble B12 deficiency due to functional deficiency. High vitamin B12 doses may exacerbate certain medical conditions, raising safety concerns. Diagnosis involves medical history, laboratory tests and imaging studies, with attention to method-specific reference intervals and antibody interference. Further research into metabolic pathways and population screening could deepen our understanding. Longitudinal studies on vitamin B12 levels and disease progression are also needed.

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