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Review

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Unexpected high plasma cobalamin

Proposal for a diagnostic strategy

Abstract

It is well-established that more than 8% of patients examined for vitamin B12 deficiency unexpectedly have increased plasma levels of the vitamin, but so far there are no guidelines for the clinical interpretation of such findings. In this review, we summarise known associations between high plasma cobalamin and diseases. We report associations mainly with cancer, liver and kidney diseases, but also with a number of other diagnostic entities. The pathogenic background is poorly understood and is likely to be multi-factorial, involving increased concentrations of one or both of the circulating cobalamin binding proteins, transcobalamin and haptocorrin. Based on current knowledge, we suggest a strategy for the clinical interpretation of unexpected high plasma cobalamin. Since a number of the associated diseases are critical and life-threatening, the strategy promotes the concept of 'think the worst first'. It is important to realise that high cobalamin levels can be an unspecific marker for cancer. If this can be ruled out, diseases of the liver and kidney should be considered.

Keywords: cobalamin-binding proteins; diagnostic strategy; high plasma cobalamin; vitamin B12.

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Introduction

More than 8% of patients referred for vitamin B12 (cobalamin, Cbl) measurement have high plasma levels [1–4]. This is somewhat contradictory, since it is the deficiency state and thereby low Cbl levels that physicians intend to find when requesting measurement of plasma Cbl for their patients.

The high levels are well explained if the patient is on treatment with pharmacological doses of Cbl. However, if this is not the case, no consensus exists on how to interpret such a finding.

In this review, we present a brief summary of the dynamics of plasma Cbl. We describe the various pathological conditions that have been linked to elevated plasma Cbl, with an emphasis on more recent studies, and we suggest a diagnostic strategy for the interpretation of unexpected high levels of Cbl.

Plasma cobalamin

Food of animal origin is the source for dietary Cbl. These include dairy products, meat, fish and eggs. Upon ingestion, Cbl is released from the food and bound to salivary haptocorrin (HC). In the upper small intestine, HC is degraded by intestinal enzymes thereby allowing Cbl to associate with gastric intrinsic factor (IF). The IF-Cbl complex is absorbed across the luminal membrane on ileal cells after binding to its receptor, cubam. The intestinal uptake is saturable, and in healthy individuals the maximal capacity is reached on a daily intake of around 6 μg. Approximately 1% of an oral dose is passively absorbed, making a daily dose of around 500 μg sufficient to ensure adequate uptake even in individuals with defective Cbl absorption.

After absorption, Cbl is released to the portal blood and is bound to either transcobalamin (TC) or HC (previously referred to as transcobalamin I, transcobalamin III or R-binder). Free Cbl is excreted in the urine; hence, Cbl remains in plasma only if it is bound to TC or HC.

The Cbl-saturated fraction of TC (holoTC, active B12) mediates the cellular uptake of Cbl from the circulation by binding to its cell-surface receptor CD320. In addition, both holoTC and unsaturated TC (apoTC) are filtered and reabsorbed in the kidney where it is recognised by the multifunctional receptor megalin. While TC mediates a daily uptake of around 4 nmol of Cbl to all cells of the body,

HC only promotes a daily uptake of around 0.1 nmol Cbl delivered exclusively to the liver through binding to the asialoglycoprotein receptor. The inactive forms of Cbl, the so-called analogues, and Cbl are both recognised by HC, and in healthy individuals around 40% of circulating HC is saturated with such analogues. The major part of TC circulates as apoTC, while HC is virtually fully saturated with Cbl or analogues (Figure 1). For recent reviews see [8, 9].

The presented features explain the dynamics of plasma Cbl. The turnover for saturated HC is around 40 times slower than for holoTC [10], which explains why the major part of circulating Cbl is bound to HC. But since the bulk of unsaturated binding capacity ($UB_{12}BC$) is apoTC, most newly absorbed Cbl binds to TC.

Measurement of Cbl, holoTC, apoTC and HC has been used in the research laboratory for more than 50 years. A substantial amount of the older literature has combined estimates of $UB_{12}BC$ with various separation techniques in order to get an estimate of pathological concentrations of one or the other of the two binding proteins. Today, the total concentration of both TC and HC can be measured by immunological methods, and such methods have also been developed for quantification of the Cbl saturated proteins [5, 6, 11]. Based on these methods our currently employed reference intervals are: total TC: 600–1500 pmol/L [6]; total HC: 240–680 pmol/L [5] and holoTC: 40–150 pmol/L [11]. Measuring the total concentrations of the Cbl binding proteins is mainly applicable for research purposes, while methods for measurement of holoTC are commercially available [12]. Still, measurement of total plasma Cbl is widely used in the clinical setting, and remains the first choice for assessing Cbl status [13].

Routine assays for Cbl estimate the sum of Cbl bound to TC and HC, and Cbl is measured after liberation of the vitamin from the two binding proteins. The first assays to be developed depended on growth of microorganisms. Later acid denaturation at 100°C combined with protein-binding assays that recognised both Cbl and its analogues came into use. Today most assays combine an alkaline denaturation of HC and TC with a protein-binding assay that employs a binding protein specific for Cbl, thus not recognising the analogues [14]. Due to variations in assay design it is important to use method-dependent reference intervals when interpreting the results. In our laboratory, we employ an interval of 200–600 pmol/L for total Cbl [7], and thus levels above 600 pmol/L are considered unexpectedly high.

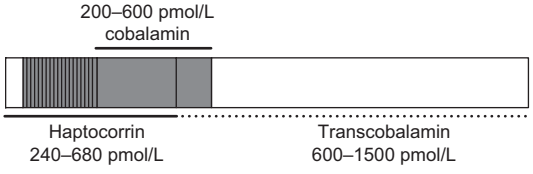

Figure 1 Cobalamin and its binding proteins in plasma.
The figure indicates representative values for the various fractions of Cbl and its binding proteins, TC and HC. Reference intervals are indicated for Cbl, total TC and total HC [5–7]. The plasma Cbl level is the sum of Cbl bound to HC and TC (grey bars and line in top of figure). In addition, Cbl analogues are attached to HC (grey bar with vertical lines). TC and HC not saturated with Cbl are indicated as white bars. Together the white bars represent the $UB_{12}BC$. Total TC (dotted line) and HC (full line) is indicated in the bottom of the figure. Cbl, cobalamin, vitamin B12; HC, haptocorrin; TC, transcobalamin; $UB_{12}BC$, unsaturated cobalamin binding capacity.

As evident from the above, high plasma Cbl levels can occur in otherwise healthy individuals that are treated with pharmacological doses of the vitamin, since such treatment will lead to increased TC saturation (see also Figure 1). However, if Cbl treatment can be excluded as the underlying cause, high plasma Cbl denotes an alteration in Cbl metabolism. The alterations are either increased synthesis or decreased clearance of TC and/or HC. In addition, release of Cbl from body stores may cause high levels.

The disease associations and the suggested underlying pathological mechanisms leading to elevated Cbl levels are outlined below.

Haematological malignancies

The associations between high Cbl levels and haematological diseases are well documented and the pathogenesis involves release of HC from proliferating leukocytes.

Chronic myeloid leukaemia (CML) is the most thoroughly studied disease entity. Already in the 1950s, researchers showed that patients with CML had elevated Cbl levels, sometimes exceeding several thousand pmol/L [15]. The patients also had high $UB_{12}BC$ caused by a protein similar to HC, originating from leukocytes (transcobalamin III) [16]. Several later studies confirmed that high $UB_{12}BC$ and levels of Cbl support the diagnosis of CML in patients suspected for this disease. Further, measurement of these parameters could be applied to follow the course of disease [17, 18].

High Cbl and HC levels have also been described in other haematological diseases, such as polycythaemia vera [18], myeloprofilerative syndrome [18], acute leukaemia [15, 18], and eosinophilia and eosinophilic leukaemia [19]. As for CML, it is hypothesised that the high levels are caused by HC release from proliferating leukocytes, although the current evidence is not as comprehensive. In addition, the diagnostic and/or prognostic values of Cbl and HC levels have yet to be recognised for these conditions.

High Cbl and $UB_{12}BC$ levels have been observed in lymphoproliferative diseases, such as multiple myeloma and lymphoma [18, 20, 21]. Here, the alterations were caused by either high TC levels [18, 20, 21] or high HC levels [21]. The possible sources for the high TC levels are unknown, but may relate to macrophage activity [22].

In accordance with the above, we recently documented that patients with unexpected high Cbl levels had 4- to 18-fold higher risk of suffering from an underlying haematological disease [1].

Liver diseases

Etiologically different liver diseases are associated with high Cbl levels [1]. The most widely studied is alcoholic liver disease [23–25]. In this condition, the high plasma Cbl is associated with high HC levels, thus, possibly caused by decreased hepatic clearance [24]. An increased release of Cbl from damaged hepatocytes may also contribute [23, 26]. A few studies showed that Cbl levels in alcoholics without manifest liver disease correlated with hepatic enzymes [26, 27], and that a high Cbl/folate-ratio could help to discriminate between alcoholic and other liver diseases [25].

Several studies have confirmed an association between liver cancer and elevated levels of Cbl [3, 28, 29], and the plasma Cbl level has been suggested as a prognostic marker in patients with hepatocellular carcinoma (HCC) [29]. The underlying pathogenesis may not be explained solely by a release of the vitamin from damaged hepatocytes. Also, an increased HC production and/or decreased HC uptake could be involved [28]. Interestingly, a rare form of primary liver cancer, fibrolaminar HCC, is known to synthesise HC [30], and patients with this disease have shown very high levels of both Cbl, HC and $UB_{12}BC$. Recently, we presented a case story that showed plasma levels of HC to be a promising marker for disease progression, an important observation since other biomarkers perform inadequately in patients with this rare form of liver cancer [30].

Solid tumours

In addition to liver cancer, high levels of plasma Cbl has been reported sporadically in patients with lung [31], breast [31], gastrointestinal [3, 31, 32] and renal cancer [33]. Since HC is synthesised by all of these tissues [34], a plausible explanation for the elevated Cbl levels is an increased release of HC to the circulation. In this context, it is interesting that in patients with gastric cancer, HC levels correlated better to disease progression than Cbl levels or $UB_{12}BC$ [32].

The relation between plasma Cbl and prostate cancer has been studied to some extent. A meta-analysis of studies performed up to September 2009 reported an increase of up to 26% in prostate cancer risk for every 100 pmol/L increase in Cbl [35]. Again, high levels of Cbl were caused by high HC levels [35].

Although cancer is associated with unexpected high Cbl levels [1, 3] the associations between solid tumours and high plasma Cbl have not been uniformly confirmed in epidemiological studies [36–40].

Autoimmune disorders

In autoimmune disorders both production of TC and HC may lead to high Cbl levels [41]. A third mechanism may also be involved – decreased TC clearance due to auto-antibodies impairing renal filtration and possibly cellular uptake.

The occurrence of antibodies against TC was first described in the late 1960s in Cbl-treated patients [42]. Later, similar types of antibodies were found in non-treated patients [43], and recently, the presence of such antibodies has been reported in at least 8% of patients with unexplained high Cbl levels [4]. Apparently, the occurrence of auto-antibodies have few clinical implications and in most cases the only observation is elevated Cbl levels [4, 43].

High levels of Cbl caused by increased HC or TC have been reported in patients with rheumatoid arthritis [41, 44], and high TC levels in patients with adult-onset Still's disease [45]. The sources were suggested to be polymorphonuclear granulocytes for HC [41, 44] and macrophages for TC [41, 44, 45]. This supports involvement of macrophages in diseases associated with high Cbl caused by high TC levels [22].

Patients suffering from autoimmune lymphoproliferative syndrome (ALPS) consistently present high Cbl levels and currently, this feature is one of the diagnostic parameters for this disease [46]. ALPS is dominated by lymphatic proliferation, and the high Cbl levels are caused by HC production from lymphocytes [47].

Renal diseases

In the early 1960s, Matthews and Beckett found elevated Cbl levels in diabetic patients with renal disease [48] and

later expanded their studies to show high plasma Cbl also in other patients with renal diseases [49]. They suggested their findings to be caused by a decreased renal Cbl clearance [49], and both HC and TC levels were reported elevated in recent studies [1, 2]. TC has a molecular mass of 38 kDa [8] and is filtered in the kidney. This in turn may explain the high Cbl levels in patients with an impaired kidney function. The apparent size of the highly glycosylated HC is much larger (>70 kDa), therefore it is not filtered in the kidney [8]. Another possible explanation has been offered by the observation that the transport of Cbl into the cells is impaired in patients with renal diseases [50], leading to cellular Cbl deficiency [51].

Infectious diseases

The associations between infectious diseases and elevated plasma Cbl are probably multifactorial and the evidence of any underlying pathogenesis is sparse.

Both malarial infection and typhus has been related to high Cbl and TC levels [52, 53].

Most intriguingly, studies on Cbl metabolism in HIV have shown results, revealing the presence of both low [54] and high levels of Cbl as a common feature. High Cbl levels were found in up to 29% of HIV-infected patients [55] and also in vertically HIV-infected children [56]. Though not with extremely high levels, both high TC and HC have been reported for HIV-infected patients [57]. Extreme changes have also been encountered. We recently reported an HIV-infected patient with high Cbl levels (1450 pmol/L) along with extremely high levels of both TC (83,500 pmol/L) and HC (23,700 pmol/L) [1]. These contradictory results have not been understood pathogenically.

High plasma Cbl and mortality

The use of Cbl levels as a prognostic marker of mortality has been explored in different patient groups.

In five independent cohorts of cancer patients, high Cbl levels were positively associated with mortality risk [58–62], mainly in patients with HCC [61] or with hepatic metastases [58, 60]. These observations led to the introduction of a new index, the Cbl levels times the C-reactive protein levels. This index has shown to be of some value as a predictor of mortality [58, 60, 62], although it has not been widely introduced in the clinical setting.

High plasma Cbl has also been described as a predictor for mortality in non-cancer patients [59, 63–65], even after adjusting for multiple factors such as co-morbidity or after excluding patients with liver disease.

Interestingly, the cut-offs for high mortality risk were between 350 and 480 pmol/L [63–65], values well within the reference interval.

Diagnostic strategy for high plasma Cbl levels

As outlined in this review, numerous diseases have been associated with high plasma Cbl, but the diagnostic performance of elevated Cbl levels in any of these diseases have not been established. Hence, our strategy for interpretation of unexpected high levels of Cbl is focused on 'think the worst first'. So far, the level of Cbl that should give rise to concern has not been determined. Based on our previous study [1], we suggest a cut-off of 1000 pmol/L when employing a method for plasma Cbl measurement with 600 pmol/L as the upper reference limit [7]. It is important to stress that the strategy presented below does not suggest using high Cbl levels as a diagnostic test and

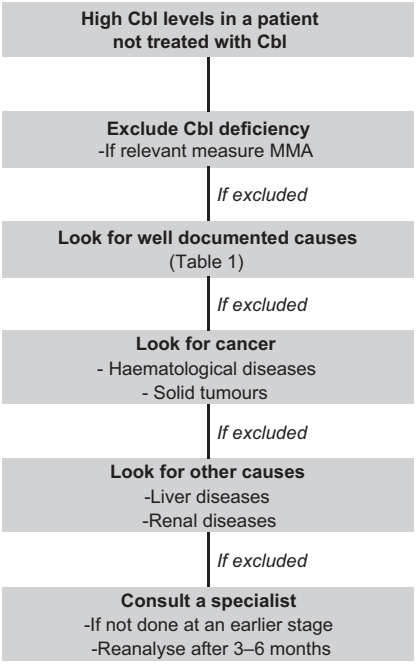

Figure 2 Diagnostic strategy for interpreting unexpected high cobalamin levels.
The proposed diagnostic strategy when interpreting high Cbl levels, notably levels above 1000 pmol/L, in a patient in whom plasma Cbl measurement is requested in order to diagnose Cbl deficiency. The strategy does not suggest using high Cbl levels routinely in the diagnosis of the relevant diseases. Cbl, cobalamin, vitamin B12; MMA, methylmalonic acid.

Well-documented associations
Fibrolaminar hepatocellular carcinoma
Autoimmune lymphoproliferative syndrome
Chronic myeloid leukaemia
Possible associations
Haematological diseases and malignancies
Unknown cancer and metastases
Liver disease (not aetiology specific)
Renal disease
Anti-transcobalamin auto-antibodies (not disease-related)
Debatable associations
Rheumatoid arthritis
Infectious diseases
HIV/AIDS

Table 1 Diseases associated with elevated plasma Cbl levels.

that it should not replace other established biomarkers relevant for a particular disease. The strategy is focused on what to consider when unexpectedly encountering elevated Cbl levels in a patient evaluated for vitamin B12 deficiency.

Figure 2 presents our suggested strategy. Plasma measurement of Cbl is requested to diagnose or rule out Cbl deficiency, and therefore the first consideration is whether the patient is Cbl deficient despite the high levels of Cbl, which may be encountered in patients with renal diseases [50, 51].

If the patient has definite signs of Cbl deficiency, the metabolic markers methylmalonic acid (MMA) and/or homocysteine should be measured. Including the measurement of holoTC levels can also provide additional information if Cbl deficiency is sustainably suspected on clinical grounds. High values of the metabolites and/or low levels of holoTC support the notion of a deficiency state, although correct interpretation of holoTC and MMA levels in renal disease patients can be difficult [66].

Table 1 lists a number of relatively rare diseases that should be excluded in patients with unexpected high Cbl levels. If the patient is young, ALPS and fibrolaminar HCC should be considered. Next in line are CML and other malignant conditions. It is important to realise that elevated levels of Cbl may prove to be an unspecific sign of malignant disease. If malignant disease can be excluded, then evaluate possible liver disease and subsequently renal disease. If all of this does not lead to a clarified cause for the high plasma Cbl, consider consulting a specialist in order to measure the concentrations of TC and HC, and perhaps the presence of auto-antibodies. This is advisable because it may help to clarify the condition of the patient, and it may lead to new knowledge concerning the metabolism of Cbl. As an example, we recently discovered a family with elevated plasma Cbl caused by high levels of the soluble TC receptor, sCD320 and a complex formation between TC and sCD320 that in turn led to elevated levels of TC and holoTC [67].

If all diagnostic evaluation fails and the end result is an unexplained high level of Cbl, we suggest re-examination within 3–6 months. If the patient still presents with high plasma Cbl, we suggest that the strategy should be followed once more.

Perspectives

High Cbl levels have been associated with many different conditions including severe and life-threatening diseases, but current knowledge gives rise to numerous unanswered questions and challenges. In general, the pathogenic backgrounds leading to high Cbl levels in the specific disease entities are yet to be scrutinised. Moreover, the performance of plasma Cbl as a marker for diseases other than Cbl deficiency has not been thoroughly evaluated. Studying Cbl metabolism in specific diseases is of obvious importance in order to improve the use of high plasma Cbl levels in the clinical setting and also in order to explore the possible usefulness of measuring TC and/or HC.

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