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Review

Significance of elevated cobalamin (vitamin B12) levels in blood

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Abstract

Elevated levels of serum cobalamin may be a sign of a serious, even life-threatening, disease. Hematologic disorders like chronic myelogeneous leukemia, promyelocytic leukemia, polycythemia vera and also the hypereosinophilic syndrome can result in elevated levels of cobalamin. Not surprisingly, a rise of the cobalamin concentration in serum is one of the diagnostic criteria for the latter two diseases. The increase in circulating cobalamin levels is predominantly caused by enhanced production of haptocorrin. Several liver diseases like acute hepatitis, cirrhosis, hepatocellular carcinoma and metastatic liver disease can also be accompanied by an increase in circulating cobalamin. This phenomenon is predominantly caused by cobalamin release during hepatic cytolysis and/or decreased cobalamin clearance by the affected liver. Altogether it can be concluded that an observed elevation of cobalamin in blood merits the a full diagnostic work up to assess the presence of disease.

1. Function of cobalamin

Initially, vitamin B12 only referred to cyanocobalamin, which is the first form of cobalamin that was purified. Presently the terms vitamin B12 and cobalamin are used interchangeably, although the more general term cobalamin is preferred. In the human body, cobalamin exists in multiple forms, of which only two are biologically active as coenzyme [1]. Methylcobalamin acts as a coenzyme with methionine synthase, which is a key enzyme in the folic acid-dependent synthesis of pyrimidines and purines (see Fig. 1). Adenosylcobalamin is involved in the enzymatic degradation of fatty acids by methylmalonyl CoA mutase. In vivo the various forms of cobalamin can be converted from one into the other. In addition, they can be converted into cobalamin analogues by microorganisms of the liver and gut. Most likely these analogues are not biologically active. However, for some forms an inhibiting activity on normal (coenzymatic) activity of cobalamin has been reported [1].

2. Uptake of cobalamin in the intestine

Normal daily intake of cobalamin is approximately 4 micrograms. Cobalamin derived from (animal-derived) nutrition is released from its protein environment in the stomach (see Fig. 2). Coupling to haptocorrin (HC) is a process that starts quickly after food ingestion because of the presence of HC in saliva. Subsequently, it is transported to the duodenum, where HC becomes enzymatically degraded. Free cobalamin is subsequently bound to Intrinsic Factor (IF). Finally, intestinal uptake of this complex occurs in the terminal ileum by a saturable, receptor-mediated process. The maximal uptake amounts approximately 3 μg per day. In addition 5 - 7 μg cobalamine are reabsorbed by enterohepatic recirculation.

3. Transport in blood

Several hours after uptake by the ileal mucosa, cobalamin bound to transcobalamin II (TC II), appears in the portal circulation (see Fig. 2). TC II is a physiologic transport protein (MW $\pm$ 38,000) that is synthesized by hepatocytes, endothelial cells, and by enterocytes. One cobalamin molecule is bound to one molecule of TC II, probably in the enterocyte, and is transported as such to the tissues.

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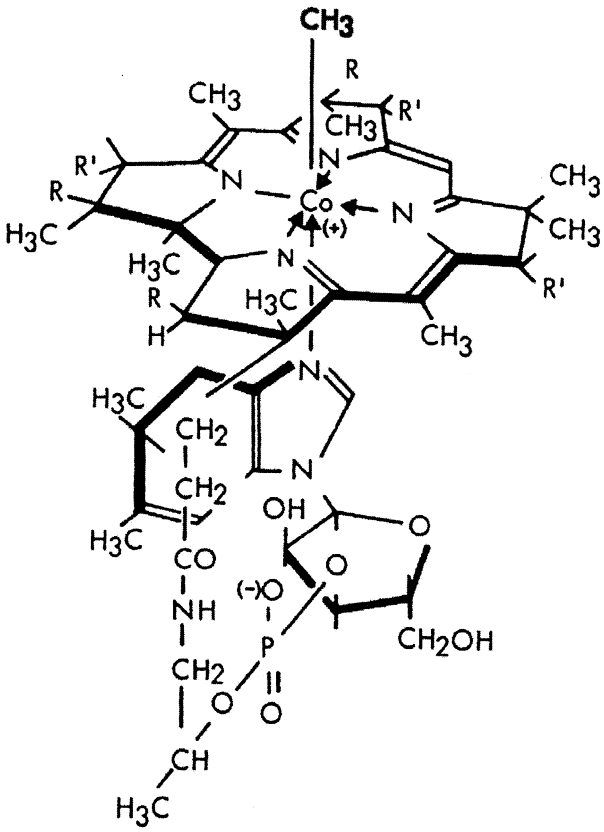

Fig. 1. Structure of methylcobalamin. The upper CH_3 -group is involved in the conversion of homocysteine to methionine.

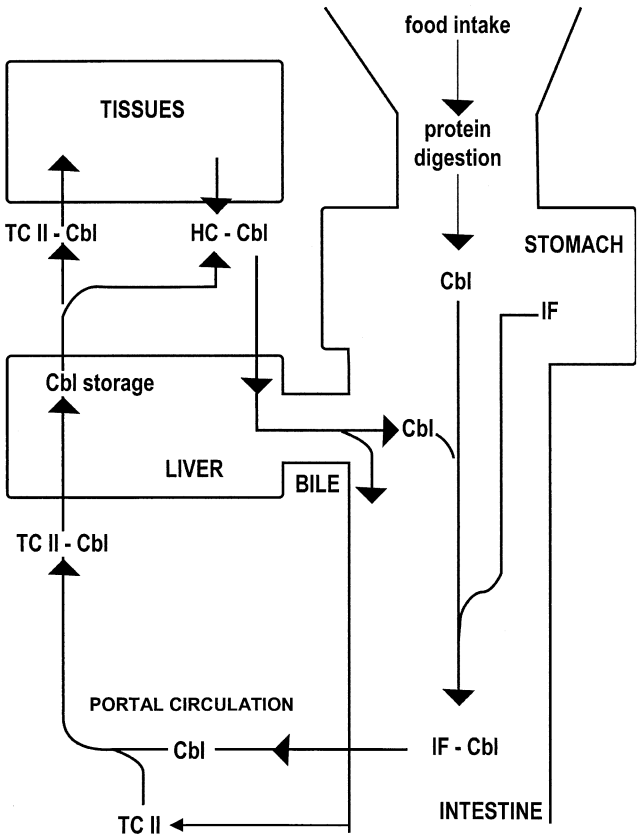

Fig. 2. Scheme of the most import pathways of cobalamin uptake, transport and accumulation in the human body.

Only 5 to 20% of plasma cobalamin is bound to TC II. Most of the cobalamin in blood is bound to circulating HC. A summary of the most important properties of the cobalamin binding proteins in plasma is presented in Table 1. The amount of free cobalamin in the circulation is negligible. The nomenclature of this glycoprotein (MW ± 60,000) is confusing because it is occasionally also indicated as cobalophilin, R-binder, or transcobalamin I/III. The cobalamin-saturated forms of HC and TC II are indicated as holo-HC and holo-TC II, and the unoccupied binding proteins as apo-HC and apo TC-II, respectively. Molecular biologic research has shown that the genes coding for HC, TC II and IF may have originated from a precursor gene by duplication [3]. Although many tissues produce mRNA for HC, circulating HC is predominantly synthesized by myeloid cells. There are two forms: the sialic acid-rich form, and the sialic acid-poor form. There are indications that the former is produced by myeloid precursor cells, whereas the latter is produced by the more mature granulocytes [1,4]. Because the sialic acid-poor form has a relatively short half life, the sialic acid-rich form predominates in the circulation. Most cobalamin in blood is bound to the sialic acid-rich HC. The role of this binding protein is not yet clear. Analogous to lactoferrin and transferrin, HC may play a role in the defense agains microorganisms by the capture of cobalamin. In addition, a role of HC in the clearance of cobalamin analogues from the circulation is suspected [5]. However, the physiologic role of HC may be limited as a deficiency of HC has no obvious adverse effects [6].

4. Uptake and storage in tissues

The uptake of holo-TC II by the tissues is mediated by specific transcobalamin receptors at the cell surface. This is a rapid process. Upon IV injection of ^{57}Co. holo-TC II, the

Table 1
Summary of the most important properties of the plasma cobalamin binding proteins

	TCII	HC
Site of synthesis	liver, enterocytes endothelium, monocytes	salivary glands, gastric mucosa, myeloid cells
MW	38 kD	60 kD
Affinity constant*	$1{,}1 \times 10^{11}$/M	$0{,}8 \times 10^{11}$/M
T_{half} in plasma	40 min.–5 hrs	6–9 days
TBBC**	380–1130 pmol/L	145–860 pmol/L
UBBC***	350–970 pmol/L	55–370 pmol/L

* For adenosyl-Cbl at 20 °C, pH 7,4
**Total cobalamin-binding capacity in plasma
***Unsaturated cobalamin-binding capacity in plasma

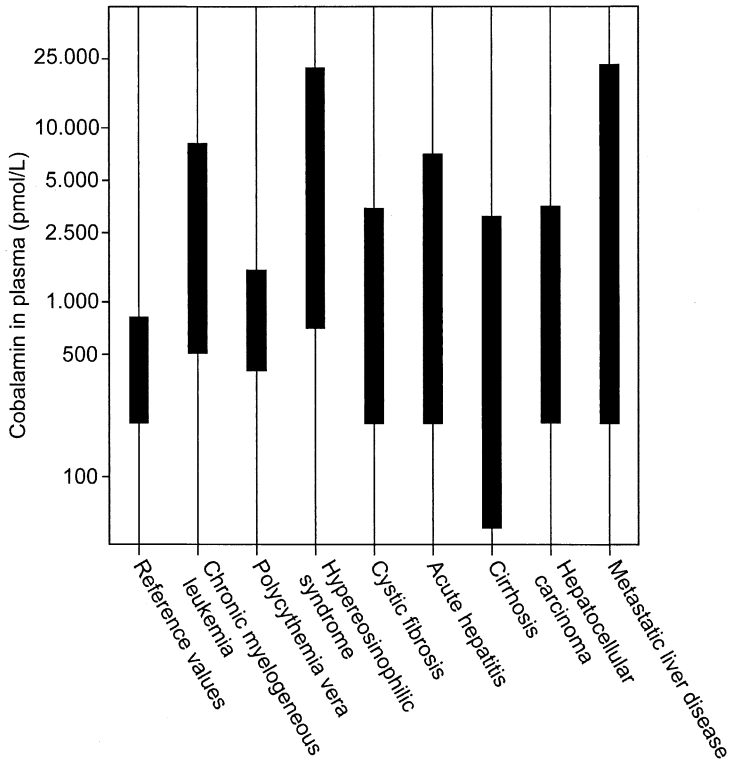

Fig. 3. Schematic overview of the observed cobalamin concentrations in various diseases.

complex is almost completely cleared after its first passage through the tissues, in particular the liver [7]. Cobalamin is released by proteolysis after endocytosis of the receptor-holo-TC II complex. The number of receptors on the cell surface varies depending on the intracellular requirement of the tissues [8]. The majority of cobalamin that enters the body *via* nutrition is stored in the liver. It is remarkable that hepatocytes do not carry TC II receptors, and that uptake of cobalamin by the liver is mediated by endothelial cells [9]. Normally the liver stores a cobalamin supply of several milligrams, which is sufficient to cover the daily need for several years. Unbound cobalamin can also penetrate the tissues by means of passive diffusion. However, this non-specific process normally has little significance.

Cobalamin and cobalamin analogues are secreted by the liver in the bile. Because only cobalamin binds selectively to IF that is present in the duodenal lumen, approximately 90% of the secreted cobalamin is reabsorbed [2]. The total amount of cobalamin participating in this enterohepatic circulation is about 2 to 5 times the normal daily intake of cobalamin. The cobalamin analogues on the other hand, leave the body with the faeces (see Fig. 2).

Although the majority of circulating cobalamin is bound to HC, this glycoprotein does not play a role in the uptake of cobalamin by the tissues. Removal from the circulation is achieved by deglycosylation followed by nonspecific protein uptake by the liver. The half life of HC in the circulation is 6 to 9 days.

5. Hematologic disorders with elevated levels of plasma cobalamin

In patients with chronic myelogeneous leukemia (CML), plasma levels of cobalamin are often significantly elevated, sometimes up to 10 times the upper margin of the reference values (see Fig. 3) [4,10]. This phenomenon is probably related to an elevated production of HC by an increased number of leukocytes. When HC is released from these cells it becomes saturated with cobalamin liberated from various tissues and the expanded granulocyte pool. Both the plasma concentrations of apo-HC and cobalamin follow the changes in the leukocyte count during the course of the disease. It has been suggested that the concentration of apo-HC has prognostic value [11]. Whether or not it is an independent prognostic parameter for the course of CML requires further investigation.

In 30 to 50% of the patients with polycythemia vera (PV) an elevated level of plasma cobalamin is found [12-14]. Compared to CML these increases are less dramatic, but the elevated plasma concentration of cobalamin is also caused by an increase of HC. It is however striking that the sialic acid-poor form of HC also shows a strong increase. This results in a strongly elevated unsaturated binding capacity for cobalamin. It is assumed that the enlarged pool of mature leukocytes is responsible for this phenomenon. Currently, an elevated level of plasma cobalamin is considered as a minor criterion for the diagnosis PV, but it may very

well be of diagnostic value in discriminating primary and secundary polycythemia.

Hypereosinophilic syndrome is characterized by a spectacular increase of plasma cobalamin. In a study that reports an eosinophilic granulocyte count between 2 and 26 $\times$ 10^9 per liter, cobalamin values of over 30 times the reference values were found [15]. This considerable increase is probably caused by the increased production of HC by an enlarged pool of both eosinophilic and neutrophilic granulocytes and their precursors. Secondary eosinophilia, as for example based on parasitary infections, is not associated with a comparable increase.

In acute myelogenous leukemia (AML) an increase in apo-HC and/or apo-TC II is often seen. In only 30% of the cases this is associated with an elevation of the concentration of cobalamin in plasma [11,14,16]. An exception to this is acute promyelocytic leukemia, which almost always shows strong increases of circulating cobalamin [17]. When elevated in AML, plasma cobalamin follows the extent of the tumor load during the course of the disease. Unfortunately the number of relevant studies on this topic is very small.

Also in about half of the cases of myelofibrosis an increase in the levels of apo-HC and/or apo TC II is seen. The few publications dealing with this subject demonstrate that in approximately one third of the patients an increase in plasma cobalamin is found [14,18].

Chronic myelomonocytic leukemia, which can be regarded as a myeloproliferative disease according to the new WHO classification, can also cause increases in plasma cobalamin levels [19].

Lymphoproliferative diseases rarely or never show an increase in plasma cobalamin. An elevated apo-TC II level is sometimes found in patients with a malignant lymphoma and in patients with hairy cell leukemia [20].

6. Liver diseases with elevated levels of plasma cobalamin

Since the liver plays an important role in the storage and transport of cobalamin, it is not surprising that liver pathology is associated with major changes in plasma cobalamin concentrations. For example in acute hepatitis, elevated levels in plasma have been found in 25 to 40% of the patients [21,22]. Inflammation-induced cell degradation hereby causes the release of stored cobalamin, which in the circulation predominantly binds to HC. This latter process becomes reinforced by a diminished concentration of TC II, which is the result of an impaired synthesizing capacity of the liver.

In liver cirrhosis the increase of plasma cobalamin is also associated with tissue depletion. Several studies show a significant decrease of intracellular cobalamin in liver biopsies [23–25]. The increase of plasma cobalamin is related to the severity of the cirrhosis, and can reach 4 to 5 times the upper limit of the reference values. However, hepatocytes are degraded to a lesser degree than is the case in acute hepatitis. It is therefore assumed that a diminished uptake of HC-bound cobalamin by the affected liver also contributes to the elevated levels of cobalamin in plasma.

In most children with cystic fibrosis (CF) cobalamin concentrations in blood are increased. Analogous to the situation in liver cirrhosis, the concentrations of apo-HC and apo-TC II are not elevated in CF [26]. CF-mediated liver disease may therefore be responsible for the increase of cobalamin in the circulation.

In over 50% of the patients with hepatocellular carcinoma increased values of mainly HC-bound plasma cobalamin are found, [27,28] which is most likely the result of a diminished clearance of HC by the liver. Possible causes for this phenomenon include poor vascularization of the liver tumor and/or a reduction in the amount of asialoglycoprotein receptors on the malignant hepatocytes. In several cases a correlation between tumor size and the plasma cobalamin concentration was observed [29,30].

In other malignancies such as cancers of the breast, colon, pancreas, and stomach, strong increases of apo-HC and/or apo-TC II are occasionally seen. In patients with metastases of the liver, 30 to 40% show elevated plasma levels of cobalamin, [31-33] in some cases even up to 30 times the upper limit of the reference values (*see* Fig. 1). These high concentrations are most likely caused by the degradation of hepatocytes. However, there is no clear explanation for the finding that, in some cases, the two unsaturated binding proteins are present in increased amounts as well. Increase of apo-HC might be caused by tumor-associated stimulation of the granulocytes. In addition, it has been suggested that some tumors produce HC themselves [34]. Increase of apo-TC II (seen in renal carcinoma) is regarded as an immunologic response to tumor progression [35].

In addition to the just mentioned clinical pictures causing an elevation of cobalamin in the circulation, several particular cases have been described in the literature.

Carmel, *et al.* described a patient with a strongly elevated level of plasma cobalamin based on a circulating antibody against TC II [36]. The formation of the TC II-antibody complex induced a marked prolongation of the halflife of TC II in the circulation. Although uptake of cobalamin by the tissues was disturbed, no obvious signs of cobalamin deficiency were observed.

Reynolds, *et al.* described a patient with a strongly elevated level of plasma cobalamin which was caused by an unknown circulating binding protein [37]. The patient presented with macrocytosis and a subacute combined degeneration matching a severe cobalamin deficiency. Intensive treatment with hydrocobalamin resulted in an improvement of the neurologic symptoms and normalization of the mean corpuscular volume (MCV).

7. Effect of pathology-associated increase of plasma cobalamin

The functional cobalamin status of a patient can be read from the activities of the cobalamin dependent enzymes. In principle, this status is above all dependent on intracellular cobalamin, and not on circulating cobalamin. Therefore the plasma concentrations of homocysteine and methylmalonic acid, which are the substrates of methionine synthase and methylmalonyl CoA mutase respectively, have a diagnostic value in tracing a functional deficiency of cobalamin [38]. Several studies have already shown that, also in the case of slightly reduced or even normal plasma cobalamin levels, a functional cobalamin deficiency can exist presenting with hematologic and/or neurologic symptoms [38,39].

Theoretically a significantly elevated level of plasma cobalamin can be associated with a functional cobalamin deficiency. Cell damage can directly lead to a reduced concentration of intracellular cobalamin [23,24]. This condition can also result from the increased binding of cobalamin to HC, which has an inhibiting effect on cobalamin binding to the physiologic transport protein required for intracellular uptake (TC II). The paradox of high plasma cobalamin and elevated homocysteine and/or methylmalonic acid has actually been described in patients with cirrhosis and CML [22,40,41]. Apart from these reports, the knowledge on this subject is restricted to several casuistic announcements in the literature, in which highly elevated plasma cobalamin levels were associated with neurologic and/or hematologic complications [31,32,42]. Research on this phenomenon however is hampered mostly by the underlying, complicated course of the disease.

8. Conclusion

The overview outlined above demonstrates that elevated plasma levels of cobalamin have been found in several disease states. The potential relevance of this finding predominantly lies in the diagnostic phase. First, it is possible to discriminate between primary and secondary forms of PV and hypereosinophilic syndrome based on the cobalamin concentration in blood. Furthermore follow-up examinations are indicated upon the coincidental identification of (highly) increased plasma levels of cobalamin. A thorough patient history is essential, because cobalamin injections can cause elevated levels in blood as well. Additionally, the use of vitamin preparations and dietary supplementation may lead to elevations of plasma cobalamin.

Finally, the treating physician of a patient with one of the above mentioned pathologies has to be aware that the result of a cobalamin test may not be informative. The usefulness of holo TC II determination (now available for routine clinical use) in these situations is still not clear. However, the determination of plasma homocysteine and/or plasma methylmalonic acid might under these conditions be more suited for a conclusive picture of the functional cobalamin status [25].

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