Review

QJM

The pathophysiology of elevated vitamin B12 in clinical practice

E. ANDRÈS[1], K. SERRAJ[1], J. ZHU[2] and A.J.M. VERMORKEN[2]

From the [1]Department of Internal Medicine, Diabetes and Metabolic Disorders, University Hospital of Strasbourg, 1 Place de l'Hôpital, 67091 Strasbourg cedex, France and [2]Laboratory for Molecular Oncology, Department of Human Genetics, Herestraat 49 - Box 602, KU Leuven, B-3000 Leuven, Belgium

Address correspondence to Prof. E. Andrès, Service de Médecine Interne, Diabète et Maladies Métaboliques, Clinique Médicale B, Hôpital Civil, Hôpitaux Universitaires de Strasbourg, 1 porte de l'Hôpital, 67091 Strasbourg Cedex, France. email: emmanuel.andres@chru-strasbourg.fr

Summary

Hypercobalaminemia (high serum vitamin B12 levels) is a frequent and underestimated anomaly. Clinically, it can be paradoxically accompanied by signs of deficiency, reflecting a functional deficiency linked to qualitative abnormalities, which are related to defects in tissue uptake and action of vitamin B12. The aetiological profile of high serum cobalamin predominantly encompasses severe disease entities for which early diagnosis is critical for prognosis. These entities are essentially comprised of solid neoplasms, haematological malignancies and liver and kidney diseases. This review reflects the potential importance of the vitamin B12 assay as an early diagnostic marker of these diseases. A codified approach is needed to determine the potential indications of a search for high serum cobalamin and the practical clinical strategy to adopt upon discovery of elevated cobalamin levels. While low serum cobalamin levels do not necessarily imply deficiency, an abnormally high serum cobalamin level forms a warning sign requiring exclusion of a number of serious underlying pathologies. Functional cobalamin deficiency can thus occur at any serum level.

Strengths

- High serum cobalamin (vitamin B12) is a frequent and underestimated anomaly.
- Hypercobalaminemia can yield clinical signs indicating functional and qualitative vitamin B12 deficiency.
- Methylmalonic acid and homocysteinemia are key biomarkers for the diagnosis of functional deficits in vitamin B12. However, as there is still no 'gold standard' for diagnosis of cobalamin deficiency, therapeutic trials are warranted when clinical symptoms are consistent with deficiency.
- The majority of causes of hypercobalaminemia is related to quantitative anomalies pertaining to transcobalamins.

- Solid neoplasms, myeloproliferative blood disorders, liver metastases, liver diseases and kidney failure are the main aetiologies to look for when dealing with high serum cobalamin levels.

Introduction

High serum cobalamin (vitamin B12) has long been a misunderstood and underestimated anomaly, being considered as irrelevant from a diagnostic and clinical standpoint. Both fundamental and clinical research efforts were indeed concentrated, for a long time, on cobalamin deficiency. However, the growing awareness by clinicians of the frequency and clinical impact of this deficiency resulted in

an increasing demand for a biological assay of vitamin B12. This enabled to highlight firsthand the non-negligible frequency of patients presenting high cobalamin blood levels.[1] Furthermore, the aetiological profile of high serum cobalamin was found to be primarily comprised of potentially serious diseases, where early diagnosis is often a determinant prognostic factor.[1–3] Despite this knowledge, there is to date no codified approach enabling to determine clinical situations indicating the search for excess vitamin B12, let alone a diagnostic process to be followed upon discovery of high serum cobalamin.

In this article, we review the current concepts of the metabolism and physiology of vitamin B12 to address, in a second instance, the diagnostic implications in clinical practice of the discovery of excess vitamin B12.

Biological definition and current epidemiological data

High serum vitamin B12 is defined by a rate above 950 pg/ml (701 pmol/l), which corresponds by biological standards, to the upper limit of biological normality, in the absence of any sign and/or clinical anomaly.[1] It should be noted that older studies (before the 1990s) are not to be considered, given that the assay of vitamin B12 has only been standardized in recent years.

The high frequency of high serum cobalamin was recently exemplified in a retrospective study by Deneuville *et al.*, which included 3702 hospitalized patients in whom high levels of vitamin B12 were found in 12% of cases, whereas a deficiency was only observed in 10% of cases.[3] The study from the Carmel's group found a prevalence of 14% of high coabalaminemia (>664 pmol/l) in an hospital laboratory.[4] The recent study from Arendt documented a prevalence of 'high' (600–1000 pmol/l corresponding to 813–1355 pg/ml) and 'very high' (>1000 pmol/l) cobalamin levels in, respectively, 13 and 7% of hospital-treated patients ($n = 12\,070$).[2] In the multicentre 'BDOSE' study, the frequency of high serum cobalamin was 18%.[5] To our knowledge, these are the only currently available studies. Large-scale studies are thus needed to assess the actual incidence and prevalence of this anomaly in the general population.

Pathophysiological basis

Vitamin B12 and its absorption factors

Vitamin B12 was discovered as the antipernicious anaemia factor that was found in the liver.[6–8] The discovery, by two independent teams, of liver as a dietary factor that could reverse anaemia in pernicious anaemia patients led to the Nobel prize in 1934. In the 1930s, researchers started to attempt isolating the 'active principle' in liver that contained the antipernicious anaemia factor. In the mean time, the importance of gastric juice for inducing reticulocytosis in pernicious anaemia patients was identified.[9–12] Only in 1947, two groups independently managed crystallizing the active principle.[13,14] No other micronutrient than vitamin B12 is known to require a specific factor for its absorption. Without this absorption factor vitamin B12 deficiency follows. Only during the 1950s, this vitamin B12 absorption factor, called intrinsic factor (IF) as opposed to the extrisic factor (vitamin B12 itself) had been to some extent purified.[15,16] An excellent historical review of the discovery of vitamin B12 and its absorption factor has been written by Dr Kunia Okuda.[17]

Vitamin B12 is absorbed in the small intestine where the vitamin B12–IF complex binds to a receptor.[18] It was called cubilin and found to function in the endocytosis of several ligands including the vitamin B12–IF complex and also apolipoprotein A-I and high-density lipoprotein, for example.[19] Later the vitamin B12–IF receptor was shown to consist of two subunits coded by different genes. The second gene appeared to be coding for the protein amnionless, which is essential for gastrulation in mice.[20] The vitamin B12–IF receptor has been called cubam.[21] Mutations in the genes coding for either of the subunits cubilin or amnionless lead to a vitamin B12 malabsorption syndrome. This is accompanied by proteinuria, because cubam is also mediating the tubular reabsorption of protein from the primary urine.[22]

Achlorydria, lack of IF and dysfunction of the cubam receptor all predispose to vitamin B12 deficiency. It has long time been thought that without the functional sophisticated mechanism for vitamin B12 absorption, such as, for example, in patients with pernicious anaemia or in patients after a total gastrectomy, parenteral treatment with vitamin B12 was indispensable. It is today, however, clear that 1% of free vitamin B12 is absorbed passively, independently of the IF and of its cubam receptor.[23] Therapeutic schemes have not been definitely validated,[24,25] but everything seems to indicate that oral treatment is both effective and sufficient, including in the case of pernicious anaemia.[22,23]

Metabolism of vitamin B12

The source of vitamin B12 in men is thus exogenous, predominantly of animal origin. However,

neither fungi and plants nor animals are able to synthesize vitamin B12. Only bacteria and archaea, also single-celled microorganisms but with an evolutionary history different from that of bacteria, have the enzymes required for its synthesis. Many foods are, however, a natural source of vitamin B12 because of bacterial symbiosis. Daily requirements were originally estimated at 2–3 μg quantities largely provided by a balanced diet.[27] Later studies show that vitamin B12 levels above 400 pg/ml (295 pmol/l, i.e. double the accepted lower limit of normal) do reduce micronucleus formation in peripheral blood lymphocytes[27,28] and uracil misincorporation into leukocyte DNA.[29] It has therefore been suggested that the current recommended daily intake for vitamin B12 may be inadequate to ensure genomic stability and that a vitamin B12 intake of 7 μg/day, needed for a plasma level of 400 pg/ml would be more appropriate.[30]

After ingestion of vitamin B12, its dissociation from its carrier proteins by gastric acid and pancreatic secretions is an essential prerequisite for the binding of vitamin B12 to the IF secreted by gastric parietal cells.[31–35] The vitamin B12–IF complex then reaches the terminal ileum where, as discussed earlier, absorption of vitamin B12 occurs according to an active and saturable mechanism, involving the specific receptor cubam.[1,36,37]

The transport of vitamin B12 in the blood as well as its tissue and hepatic uptake require the presence of transcobalamins (TCBs).[37,38] TCB types I (TCB I) and III (TCB III) ensure the binding of ~80% of circulating vitamin B12; however, TCB type II (TCB II) plays the predominant role in the key processes of tissue and hepatic uptake of vitamin B12. Clinically, measuring this active fraction of vitamin B12, bound to TCB II is ensured by the determination of holotranscobalamin.[39] Holotranscobalamin II is composed of vitamin B12 attached to TCB II, and it represents the biologically active fraction that can be delivered into all DNA synthesizing cells.[40] Liver storage of vitamin B12 is mediated by endothelial cells, hepatocytes being naturally devoid of TCB II receptors.[37] The enterohepatic cycle (5–7 μg daily) and proximal tubular reabsorption of vitamin B12 help maintain physiological reserves of cobalamin at significant levels (up to 5-year reserves).[31,37]

The majority of unduly high serum cobalamin cases pathophysiologically involve a qualitative and/or quantitative disorder of TCBs. Hence, a better knowledge of these proteins, their sites of production, their distribution and their physiological functions is vital to understand the pathophysiological mechanisms and aetiological implications of high serum cobalamin.

Transcobalamins

TCB I and III are 60–70 kDa molecular weight proteins. They belong to the haptocorrin (HC) superfamily in which they represent the serum forms.[41,42] The HCs, also called R-binders or cobalophilins, are found in various tissues and biological fluids.[43,44] TCB I and III are derived from the granulocyte line and are markers of neutrophilic secondary granules, which explains their increase in myeloproliferative disorders (MPDs).[1,45]

Aside from a suggested antibacterial role, the function of TCB I and III remains scarcely elucidated and appears, in all instances, incidental to the role of TCB II.[37]

TCB II is a 42–47 kDa protein, which specifically transports cobalamins in serum. It is an essential protein in the delivery of vitamin B12 to cells and tissues (active fraction of serum vitamin B12 assayed) although it only transports 20% of circulating cobalamins.[45,46] The production site of TCB II is primarily hepatocytic, and secondarily endothelial, monocytic and intestinal.[1,45,47] Severe disorders are observed in congenital deficiencies in TCB II, illustrating the vital role played by this protein, including developmental neuropsychiatric disorders as well as pancytopenia-type haematological disorders and aregenerative megaloblastic anaemia.[1,37]

Determination of vitamin B12 and diagnosis of deficiency

As mentioned, about one quarter of vitamin B12 in serum is bound to TCB while the remainder is bound to other proteins. Before vitamin B12 can be measured it has to be freed of protein and converted to cyanocobalamin. Different methods exist for the measurement of the free cyanocobalamin. Older assays, still in use in some laboratories, depended on the growth of microorganisms. For obvious reasons the presence of antibiotics in serum risks to lead to falsely low results with these assays. Most vitamin B12 determinations today are performed using automated equipment. These assays are mostly based on competition for IF between radioactive vitamin B12 and the cyanocobalamin in the serum sample. Discrepancies between results of vitamin B12 levels determined by different methods are not uncommon. It is therefore necessary to use the reference values of the laboratory that performed the measurement (for reviews, see Vogeser and Lorenzl[48] and Karmi *et al.*[49]).

Regrettably neither specific clinical symptoms nor precise serum vitamin B12 levels allow the straightforward diagnosis of a case of deficiency. Originally

megaloblastic anaemia was believed to be a specific symptom for vitamin B12 deficiency. After eight decades of reluctance it has been widely accepted only since 1988 that neurologic dysfunction can be the only clinical symptom of vitamin B12 deficiency.[50]

The lack of a clear association between the serum level of vitamin B12 and deficiency of the vitamin led to the introduction of the measurement of metabolites as indicators of functional deficiency. Methylmalonic acid and homocysteine are used. Functional deficiency of vitamin B12 leads to increased levels of methylmalonic acid because the vitamin plays a role in the conversion of methyl-malonyl coenzyme A to succinyl coenzyme A by the enzyme methylmalonyl coenzyme A mutase. Homocysteine levels are raised because methionine synthase requires methylcobalamin for the conversion of homocysteine to methionine. Only in the 1980s, the techniques for measuring these metabolites became sufficiently sensitive.[50] Today these metabolites do still play a crucial role in the diagnosis of deficiency although even the use of these metabolic indicators of vitamin B12 deficiency does neither predict nor preclude with absolute certainty the presence of vitamin B12—responsive haematologic or neurologic disorders.[51] In this context, medullary aspiration has a place of choice, despite some difficulties in interpretation (differential diagnostic with myelodysplastic syndromes).[31,32] Meanwhile, the lack of reliability of the homocysteine concentration as a metabolic indicator of a reduced conversion towards methionine has been shown in situations with oxidative stress.[52] It is thus no surprise that in patients with diabetes, a disease known to cause oxidative stress, clinical response of neuropathy was noted at cobalamin levels above 400 pg/ml (as seen above, double the accepted lower limit of normal).[53] As a consequence one must agree that there is still no 'gold standard' for the diagnosis of cobalamin deficiency, and therapeutic trials are warranted when the clinical picture is consistent with this disorder.[54]

As we will see later in this article even high serum cobalamin levels can be accompanied by functional deficiency.

Mechanisms related to high serum cobalamin levels

High serum levels of cobalamin involve three essential pathophysiological mechanisms, which meet virtually all aetiologies to search for and that will be detailed later on. These mechanisms are[1,37]:

● a direct increase in plasma vitamin B12 by excess intake or administration,

● a direct increase in plasma vitamin B12 by liberation from an internal reservoir,
● an increase in TCB via excess production or lack of clearance and
● a quantitative deficiency or lack of affinity of TCB for vitamin B12.

Figure 1 outlines the metabolism of vitamin B12 and the various aetiological mechanisms of high serum cobalamin.

Pathological consequences of high serum cobalamin

Vitamin B12 is a ubiquitous coenzyme mainly involved in reactions leading to the synthesis of DNA and of that of methionine from homocysteine.[55,56] This explains, on the one hand, the haematological, neurological and epithelial clinical manifestations observed during vitamin B12 deficiency, and on the other hand, the plasma accumulation of substrates of enzymatic reactions involving vitamin B12, namely methylmalonic acid and homocysteine.

Contrary to vitamin B12 deficiency, the pathophysiology and clinical consequences of high serum cobalamin have, until now, been very little studied. It is however currently considered that an increase in plasma levels of vitamin B12 may be an indicator of a functional deficit with clinical consequences paradoxically similar to those of vitamin B12 deficiency. Indeed, an increase in the binding of vitamin B12 to HCs, secondary to an elevation in their plasma levels (especially for TCB I and III which are by far the majority), leads to a potential decline in its attachment to TCB II and therefore alters its delivery to the cells. Thus, a functional deficit in vitamin B12 with an increase in homocysteine
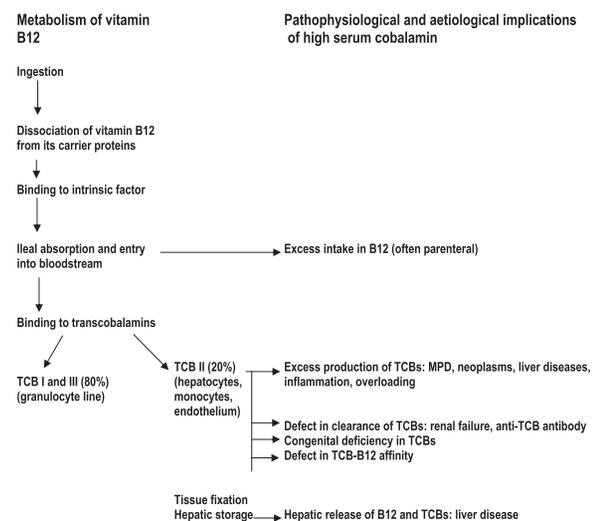

Figure 1. Metabolism of vitamin B12 and pathophysiological correlations with high serum cobalamin.

and/or methylmalonic acid levels can occur, even though the initial anomaly in this instance is not a deficiency in vitamin B12.[1,57,58] Beside by this mechanism, functional deficiency may also be caused by the failure of damaged liver to take up cobalamin from the serum and/or by leakage of total vitamin B12 from the liver tissue into the plasma.[56–59] Cases of liver disease and MPDs reproducing these anomalies have been described in the literature.[56–62]

Aetiological profile of high serum cobalamin

The simple increase in plasma vitamin B12 being most often clinically asymptomatic (aside from potential functional deficits), it is much more accurate to speak of hypervitaminemia B12 rather than of hypervitaminosis B12.

From a practical standpoint, the finding of high serum cobalamin should first and foremost be followed by the search for the cause of this condition. Specific correction of the hypervitaminemia B12 can frequently be obtained by treatment of the underlying disorder. Moreover, although high cobalamin blood levels are not unusual they are significantly associated to both malignant hemopathies and solid tumours. It is noteworthy that most of diagnosed neoplastic diseases were previously unknown and at a non-metastatic stage.[1,37] In the aformentionned study from Arendt, high serum cobalamin levels were significantly associated with alcoholism (odds ratio (OR) = 5.74 [2.76–11.96], liver disease (OR = 8.53 [3.59–20.23]) and cancer (OR = 5.48 [2.85–10.55]).[2] In this study, elevated vitamin B12 levels were also seen in patients with renal-, autoimmune- and bronchopulmonary disease.

As described earlier, it is necessary in clinical practice to consider the three aforementioned basic pathophysiological mechanisms, which meet virtually all aetiologies to search for.

Excess vitamin B12 intake

Excessive oral intake of vitamin B12 is usually relatively easy to identify in the course of the anamnesis.[1] In addition, long-term parenteral administration of vitamin B12 can lead to the development of anti-TCB II autoantibodies, which result in a reduction in TCB II clearance.[1,63,64] This induced auto-immunization was observed in 30% of cases in a series of Danish patients treated for pernicious anaemia.[65]

However, in clinical routine, elevated serum cobalamin levels of exogenous origin are encountered primarily in two major situations:

1. The ingestion by the patient of multivitamin complex tablets containing vitamin B12. This self-medication, sometimes overlooked by the patient, should be systematically investigated at examination, as it is often not spontaneously reported.
2. Parenteral administration of vitamin B12. This was previously most often the result of exogenous intake during the course of a Schilling test (not currently used because no longer available), which includes by definition an intramuscular loading dose of vitamin B12.[66] Currently, it is mostly administered in the treatment of a documented vitamin deficiency. In this regard, it should be reminded that the parenteral route is decreasingly justified in the majority of aetiologies of vitamin B12 deficiency and that, as mentioned earlier, oral treatment has been found to be both effective and sufficient, including in the case of pernicious anaemia.[33,67] It is also important to underline that in case high serum cobalamin is observed during follow-up of pernicious anaemia a gastric neoplasm, more frequent in this type of patients (relative risk multiplied by three) must be excluded. A simple cobalamin excess should remain a diagnosis of exclusion.[1]

High serum cobalamin and solid neoplasms

The association of excess serum cobalamin and solid neoplasms was first described and documented by Carmel *et al.* in 1975 and in 1977.[61,68] Several studies have since supported this observation. These latter studies have also attempted to identify the practical diagnostic and prognostic implications of this association.

The carcinomas most frequently involved are hepatocellular carcinoma (HCC) and secondary liver tumours, breast cancer, colon cancer, cancer of the stomach and pancreatic tumours. In the series of Fremont *et al.*, approximately half of the patients with HCC presented with high serum cobalamin.[69] In patients with liver metastases, the frequency of high serum cobalamin is estimated at 30–40%, with vitamin B12 levels at times reaching extreme thresholds.[2,37,70] In the series of Chiche *et al.*, 23% of patients with high serum cobalamin had a previously unknown solid cancer in 73% of cases, which was still at a non-metastatic stage in 80% of cases.[1] The association between high serum cobalamin and neoplastic disease has also been demonstrated by Deneuville *et al.* with an OR of 1.8 for all cancers combined, 2.9 for metastatic tumours, 3.3 for HCC, 4.7 for other primary hepatic tumours and 6.2 for neoplasms with liver metastases.[3]

From a prognostic point of view, the correlation observed in some cases between the size of certain tumours, particularly of the liver, and the degree of elevation of vitamin B12 has suggested serum cobalamin levels as a possible tumour marker for poor prognosis.[71,72] In this context, it should be emphasized that a strong predictive value of high serum cobalamin for mortality was found in a group of cancer patients at the palliative stage and in another group of geriatric patients.[1,73]

In liver tumours, the primary mechanisms implicated in the genesis of elevated serum cobalamin are the decrease in hepatic clearance of the HC–cobalamin complex and increased plasma levels of TCB due to excess degradation of hepatocytes.[1] The decrease in hepatic clearance is thought to be due to poor hepatic vascularization and to the reduction in the number of HC receptors on the surface of tumour hepatocytes.

In other solid tumours, high serum cobalamin is thought to be mainly related to an excess synthesis of TCB by the tumour or to an increase in HCs due to induction of hyperleukocytosis.[1,37]

High serum cobalamin and blood disorders

High serum cobalamin is an anomaly frequently observed in malignant blood diseases and these essentially involve MPDs, including chronic myelomonocytic leukemia and primary hypereosinophilic syndrome (HES), myelodysplastic syndromes and acute leukemias (ALs), notably promyelocytic leukemia (AML3).[1,65] In ancient literature, high serum cobalamin is included in the biological parameters strongly pointing to a myeloproliferative syndrome including chronic myeloid leukemia, primary polycethemia (Vasquez disease), thrombocythemia and other myelofibroses. Table 1 summarizes the key data in the literature regarding high serum cobalamin observed in haematological disorders. It should be noted that lymphoproliferative disorders are rarely providers of high serum cobalamin except for multiple myeloma, where both hypervitaminemia and hypovitaminemia B12 can be observed (personal observation[1]).

In their work, Chiche et al. found a statistically significant association between vitamin B12 levels >1275 pg/ml and the existence of a malignant blood disease, hence suggesting an in-depth aetiological search for a possible blood disease when plasma levels of vitamin B12 are particularly elevated.[1]

An elevated serum cobalamin level in myeloid proliferations is primarily linked to the release of HCs by tumour granulocytes and their precursors.[74–80]

High serum cobalamin and liver diseases

The role played by the liver in the metabolism of cobalamin increases the likelihood that acute and chronic liver diseases, regardless of their aetiology, are purveyor situations of high serum cobalamin.[58–60] Schematically, the three major conditions to be distinguished are acute liver diseases, chronic liver diseases and HCC already discussed earlier. In the series of Chiche et al., 20 of 65 patients (31%) had a non-neoplastic liver disease in which 80% were chronic and 25% at the cirrhosis stage.[1] In

Table 1 High serum cobalamin related to haematological disorders and their clinical characteristics

|	Extent of high serum cobalamin	Mechanism of high serum cobalamin	Potential clinical implications
Chronic myeloid leukemia	- Very frequent - Up to 10 times normal value	Production of granulocyte HCs	- Probable prognostic value of apo-HCs
Polycythemia vera (PV)	- 30–50% of cases - Up to three times normal value	Release of granulocyte HCs	- Minor diagnostic criterion of PV - Differential diagnosis with secondary polycythemia
Primary myelofibrosis	- one-third of cases	Elevated apo-HC and apo-TCB II levels	
Primary HES	- Up to 30 times normal value	Production of HCs (eosinophils and neutrophils)	- Diagnostic argument for HES - Differential diagnosis with secondary eosinophilia
ALs	- 30% of cases - More frequent in promyelocytic AL (AML3)		- Potential indicator of tumour mass
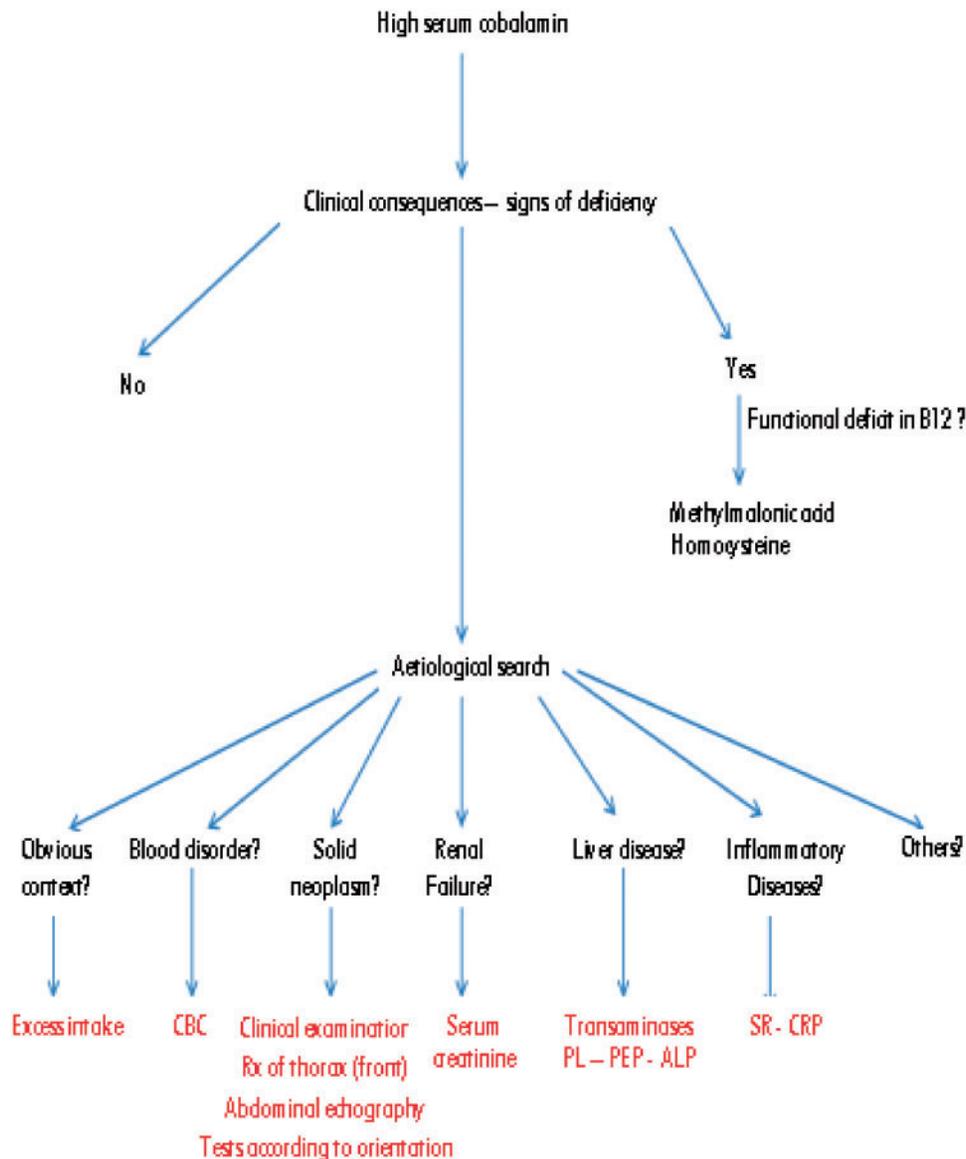
Figure 2. Conduct to follow and first line examinations when confronted with high serum cobalamin. PEP, protein electrophoresis; ALP, alkaline phosphatases; PL, prothrombin level; SR, sedimentation rate; CRP, C-reactive protein; CBC, complete blood count.

the same manner, Deneuville *et al.* demonstrated a significant association between high serum cobalamin and liver diseases with an OR of 4.3.[3]

Acute hepatitis can hence be accompanied by high serum cobalamin in 25–40% of cases. This elevation of vitamin B12 has been ascribed to an excess release of cobalamin by the liver and decreased hepatic synthesis of TCB II, an essential element for tissue binding of vitamin B12.[37,81]

In liver cirrhosis, high serum cobalamin can be found five times above the upper limit. In this context, the degree of elevated cobalamin is thought to be correlated with the severity of cirrhosis.[57,59] In cirrhoses, the decrease in tissue and cellular liver uptake of vitamin B12 and of the HC–cobalamin

complex are the main mechanisms involved and have been typified by biopsy studies performed in cirrhotic patients.[37,58]

Finally, it should be emphasized that alcoholic liver diseases occupy an important place amongst high serum cobalamin cases stemming from liver disease.[82] Indeed, an ethylic origin was found by Chiche *et al.* in 80% of patients with liver disease.[1] In addition, Baker *et al.* provided an objective biological assessment in a population of patients with severe alcoholic liver disease.[58] They were able to demonstrate an increase in plasma levels of TCB I and III which, by binding to vitamin B12, would prevent plasma vitamin B12 from eventually being excreted. In the same study, a decrease in TCB II
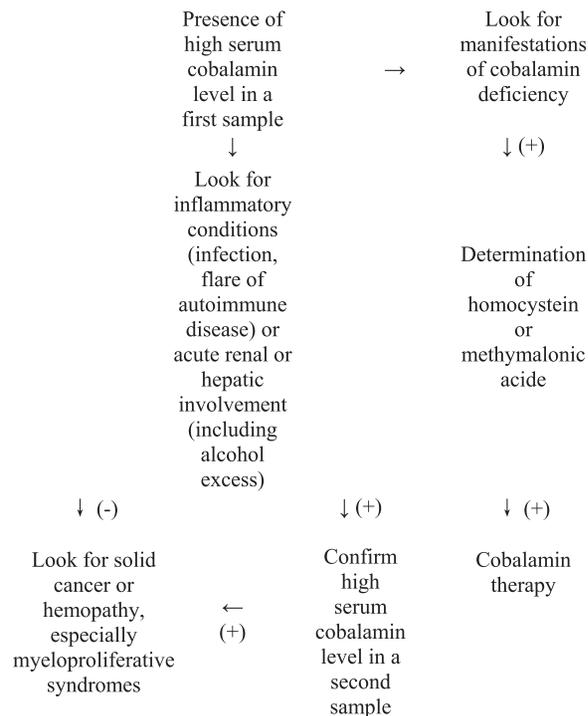
Presence of
high serum
cobalamin   →   Look for
level in a   manifestations
first sample   of cobalamin
  ↓   deficiency
Look for   ↓ (+)
inflammatory
conditions
(infection,
flare of   Determination
autoimmune   of
disease) or   homocystein
acute renal or   or
hepatic   methymalonic
involvement   acide
(including
alcohol
excess)
  ↓ (-)   ↓ (+)   ↓ (+)
Look for solid   Confirm   Cobalamin
cancer or   high   therapy
hemopathy,   ← serum
especially   (+) cobalamin
myeloproliferative level in a
syndromes   second
                 sample

Figure 3. Guidelines for clinician in case of high serum cobalamin level.

was found to cause impaired tissue entry of vitamin B12. This again draws attention to the fact that increased plasma vitamin B12 may well be associated with a concomitant functional decline reproducing the same clinical consequences as a genuine vitamin deficiency.[58]

Other causes of high serum cobalamin

The role of the kidney in the metabolism of vitamin B12 is currently well accepted, albeit not completely understood. Kidney failure is among the causes to look for when confronted with high serum cobalamin. The suggested mechanism is serum accumulation of TCBs.[83] In the series of Deneuville et al., a significant association between high serum cobalamin and interstitial nephropathy has been reported with an OR of 2.7.[3] This fact was also documented by Carmel.[4] He hypothesized that cellular uptake of cobalamin by the abundant TC II receptors in the kidney may be impaired.

Anecdotal cases of high serum cobalamin of various aetiologies have equally been reported in the literature. These cases generally involve the presence of anti-TCB II antibodies resulting in a decrease in their clearance from the antibody–TCB II complex thus formed,[84] or to high serum cobalamin due to the presence of an abnormal plasma vitamin B12-binding protein.[85]

Cases of Gaucher disease, systemic lupus, rheumatoid arthritis and Still's disease with high serum cobalamin have also been reported. High serum cobalamin in dysimmune and inflammatory diseases may be linked to an increase in TCB II during the acute phase of inflammation.[1,86–88]

Finally, contrary to secondary neutrophilic polynucleoses which are common contributors of high serum cobalamin, cases of secondary hypereosinophilia with high serum cobalamin remain isolated and anecdotal, and may thus provide high serum cobalamin a potential discriminatory value between primary HES of myeloproliferative origin and other causes of eosinophilia.[89]

In Figure 2, we propose a practical course of action when confronted with the finding of high serum cobalamin, taking into account its potential clinical consequences and its most common aetiologies.[90] In Figure 3, we propose guidelines for clinicians.

Conclusions

High serum cobalamin is a frequent and underestimated anomaly. Clinically, it can sometimes be paradoxically accompanied by signs of deficiency resulting in a functional deficit linked to qualitative anomalies, which are related to defects in tissue uptake and action of vitamin B12. The aetiological profile of high serum cobalamin mostly encompasses severe disease entities for which early diagnosis is crucial to prognosis. These entities are essentially comprised of solid neoplasms, haematologic malignancies and liver diseases. This reflects the potential importance of a vitamin B12 assay as a possible early marker in the working diagnosis of these diseases. A codified approach is needed to determine the potential indications of the search for high serum cobalamin and the approach to adopt upon discovery of elevated cobalamin levels. As in many fields of medicine, further studies are needed more than ever to better understand the clinical data related to high serum cobalamin.

Acknowledgements

deficiency at the University Hospital of Strasbourg (CARE B12) and is a member of GRAMI: Groupe de Recherche sur les Anémies en Médecine Interne (Research Group on Anemia in Internal Medicine). He is an expert consultant to several laboratories involved in haematology (AMGEN, ROCHE, CHUGAI, GSK, VIFOR Pharma, FERRING, BMS, SHERRING, GENZYME, ACTELION) and has participated in numerous international and national studies sponsored by these laboratories or academic works.

Conflict of interest: None declared.

References

1. Chiche L, Jean R, Romain F, Roux F, Thomas G, Canavese S, et al. Implications cliniques de la découverte d'une hypervitaminémie B12 en médecine interne. *Rev Med Interne* 2008; **29**:187–94.

2. Arendt JFB, Nexo E. Cobalamin related parameters and disease patterns in patients with increased serum cobalamin levels. *PLoS ONE* 2012; **9**:e45979.

3. Deneuville T, Mario N, Tiev KP, Tolédano C, Josselin-Mahr L, Gain M, et al. Concentration plasmatique élevée de la vitamine B12: un indicateur des maladies hépatiques ou tumorales? *Rev Med Interne* 2009; **30**(**Suppl. 2**):S73.

4. Carmel R, Vasireddy H, Aurangzeb I, George K. High serum cobalamin levels in the clinical setting—clinical associations and holo-transcobalamin changes. *Clin Lab Haematol* 2001; **23**:365–71.

5. Chiche L, Mancini J, Arlet JB, investigateurs. de l'étude BDOSE. Hypervitaminémies B12 en médecine interne: résultats de l'étude BDOSE. *Rev Med Interne* 2009; **30**(**Suppl. 4**):S377.

6. Whipple GH, Hooper CW, Robscheit FS. Blood regeneration following anemia. IV. Influence of meat, liver, and various extractives, alone or combined with standard diets. *Am J Physiol* 1920; **53**:236–62.

7. Minot GR, Murphy WP. Treatment of pernicious anemia by special diet. *JAMA* 1926; **87**:470–6.

8. Minot GR, Murphy WP, Stetson RP. Response of reticulocytes to liver therapy: particularly in pernicious anemia. *Am J Med Sci* 1928; **175**:581–99.

9. Castle WB. Observations on etiologic relationship of achylia gastrica to pernicious anemia. I. Effect of administration to patients with pernicious anemia of contents of normal human stomach recovered after ingestion of beef muscle. *Am J Med Sci* 1929; **178**:748–64.

10. Castle WB, Townsend WC, Heath CW. Observations on etiologic relationship of achylia gastrica to pernicious anemia. II. Effect of administration to patients with pernicious anemia of beef muscle after incubation with normal human gastric juice. *Am J Med Sci* 1929; **178**:764–77.

11. Castle WB, Townsend WC, Heath CW. Observations on etiologic relationship of achylia gastrica to pernicious anemia. III. Nature of reaction between normal human gastric juice and beef muscle leading to clinical improvement and increased blood formation similar to effect of liver feeding. *Am J Med Sci* 1930; **180**:305–35.

12. Castle WB, Heath CW, Strauss MB, Heinle RW. Observations on etiologic relationship of achylia gastrica to pernicious anemia.VI. Site of interaction of food (extrinsic) and gastric (intrinsic) factors: failure of in vitro incubation to produce thermostable hematopoietic principle. *Am J Med Sci* 1937; **294**:618–25.

13. Smith EL. Purification of anti-pernicious anemia factors from liver. *Nature* 1948; **161**:638–9.

14. Rickes EL, Brink NG, Koniuszy FR, Wood TR, Folkers K. Crystalline vitamin B12. *Science* 1948; **107**:396–7.

15. Gärsbeck R. Studies on the vitamin B12-binding principle and other biocolloids of human gastric juice. *Acta Med Scand* 1956; **314**(**Suppl.**):1–87.

16. Gärsbeck R. Fractionation of human gastric juice and saliva employing starch electrophoresis. *Gastroenterologia* 1955; **84**:99–102.

17. Okuda K. Discovery of vitamin B12 in the liver and its absorption factor in the stomach: a historical review. *J Gastroenterol Hepatol* 1999; **14**:301–8.

18. Glass GBJ. Gastric intrinsic factor and its function in the metabolism of vitamin B12. *Physiol Rev* 1963; **43**:529–849.

19. Kozyraki R, Fyfe J, Kristiansen M, Gerdes C, Jacobsen C, Cui S, et al. The intrinsic factor-vitamin B12 receptor, cubilin, is a high-affinity apolipoprotein A-I receptor facilitating endocytosis of high-density lipoprotein. *Nat Med* 1999; **5**:656–61.

20. Tanner SM, Aminoff M, Wright FA, Liyanarachchi S, Kuronen M, Saarinen A, et al. Amnionless, essential for mouse gastrulation, is mutated in recessive hereditary megaloblastic anemia. *Nat Genet* 2003; **33**:426–9.

21. Fyfe FC, Madsen M, Hojrup P, Christensen EI, Tanner SM, de la Chapelle A, et al. The functional cobalamin (vitamin B12)-intrinsic factor receptor is a novel complex of cubilin and amnionless. *Blood* 2004; **103**:1573–9.

22. Gräsbeck R. Imerslund-Gräsbeck syndrome (selective vitamin B(12) malabsorption with proteinuria). *Orphanet J Rare Dis* 2006; **1**:17.

23. Andrès E, Fothergill H, Mecili M. Efficacy of oral cobalamin (vitamin B12) therapy. *Expert Opin Pharmacother* 2010; **11**:249–56.

24. Andrès E, Henoun Loukili N, Noel E, Maloisel F, Vinzio S, Kaltenbach G, et al. Oral cobalamin (daily dose of 1000 μg) therapy for the treatment of patients with pernicious anemia. An open label study of 10 patients. *Curr Ther Res* 2005; **66**:13–22.

25. Solomon LR. Oral pharmacologic doses of cobalamin may not be as effective as parenteral cobalamin therapy in reversing hyperhomocystinemia and methylmalonic acidemia in apparently normal subjects. *Clin Lab Haematol* 2006; **28**:275–8.

26. Herbert V. Recommended dietary intakes (RDI) of vitamin B-12 in humans. *Am J Clin Nutr* 1987; **45**:671–8.

27. Fenech MF, Dresoti IE, Rinaldi JR. Folate, vitamin B12, homocysteine status and chromosome damage rate in lymphocytes of older men. *Carcinogenesis* 1997; **18**:1329–36.

28. Fenech M, Aitken C, Rinaldi J. Folate, vitamin B12, homocysteine status and DNA damage in young Australian adults. *Carcinogenesis* 1998; **19**:1163–71.

29. Kapiszewska M, Kalemba M, Wojciech U, Milewicz T. Uracil misincorporation into DNA of leukocytes of young women with positive folate balance depends on plasma vitamin B12 concentrations and methylenetetrahydrofolate reductase polymorphisms. A pilot study. *J Nutr Biochem* 2005; **16**:467–78.

30. Solomon LR. Disorders of cobalamin (vitamin B12) metabolism: emerging concepts in pathophysiology, diagnosis and treatment. *Blood Rev* 2007; **21**:113–30.

31. Andrès E, Vidal-Alaball J, Federici L, Loukili NH, Zimmer J, Kaltenbach G. Clinical aspects of cobalamin deficiency in elderly patients. Epidemiology, causes, clinical manifestations, and treatment with special focus on oral cobalamin therapy. *Eur J Intern Med* 2007; **18**:456–62.

32. Andrès E, Affenberger S, Vinzio S, Noel E, Kaltenbach G, Schlienger JL. Carences en vitamine B12 chez l'adulte: étiologies, manifestations cliniques et traitement. *Rev Med Interne* 2005; **26**:938–46.

33. Serraj K, Mecili M, Andres E. Signes et symptômes de la carence en vitamine B12: revue critique de la littérature. *Méd Thér* 2010; **16**:13–20.

34. Andrès E, Loukili NH, Noel E, Kaltenbach G, Ben Abdelgheni M, Perrin AE, *et al.* Vitamin B12 (cobalamin) deficiency in elderly patients. *CAMJ* 2004; **171**:251–60.

35. Russel RM. Vitamin requirements in old age. *Age Nutr* 1992; **3**:20–3.

36. Federici L, Henoun Loukili N, Zimmer J, Affenberger S, Maloisel F, Andrès E. Manifestations hématologiques de la carence en vitamine B12: données personnelles et revue de la littérature. *Rev Med Interne* 2007; **28**:225–31.

37. Ermens AA, Vlasveld LT, Lindemans J. Significance of elevated cobalamin (vitamin B12) levels in blood. *Clin Biochem* 2003; **36**:585–90.

38. Lindemans J, Kroes ACM, van Geel J, Van Kapel J, Schoester M, Abels J. Uptake of transcobalamin II-bound cobalamin by HL-60 cells: effects of differentiation induction. *J Exp Cell Res* 1989; **184**:449–60.

39. Herzlich B, Herbert V. Depletion of serum holotranscobalamin II. An early sign of negative vitamin B12 balance. *Lab Invest* 1988; **58**:332–7.

40. Herrmann W, Schorr H, Obeid R, Geisel J. Vitamin B-12 status, particularly holotranscobalamin II and methylmalonic acid concentrations, and hyperhomocysteinemia in vegetarians. *Am J Clin Nutr* 2003; **78**:131–6.

41. Grasbeck R. Biochemistry and clinical chemistry of vitamin B12 transport and the related diseases. *J Clin Biochem* 1984; **17**:99–107.

42. Nicolas JP, Gueant JL, Frémont S. Cobalamin binding glycoproteins. In: Bhatt HR, James VHT, Besser GM, Bottazzo GF, Keen H, eds, *Advances in Thomas Addison's Diseases.* Bristol: Journal of Endocrinology Ltd., 1994:253–67.

43. Gräsbeck R. Intrinsic factor and the other vitamin B12 transport proteins. *Prog Hematol* 1969; **6**:233–60.

44. Burger RL, Mehlman CS, Allen RH. Human plasma R-type vitamin B12-binding proteins. I. Isolation and characterization of transcobalamin I, transcobalamin III and the normal granulocyte vitamin B12-binding protein. *J Biol Chem* 1975; **250**:7700–6.

45. Zittoun J. Cobalamins: an update of fundamental and clinical data. *Hématologie* 1996; **2**:119–29.

46. Li N, Seetharam S, Lindemans J, Alpers DH, Arwert F, Seetharam B. Isolation and sequence analysis of variant forms of human transcobalamin II. *Biochim Biophys Acta* 1993; **1172**:21–30.

47. Herbert V. Etiology of vitamin B12 (cobalamin) deficiency and staging of B12 status. In: Bhatt HR, James VHT, Besser GM, Bottazzo GF, Keen H, eds, *Advances in Thomas Addison's Diseases.* Bristol: Journal of Endocrinology Ltd, 1994:139–48.

48. Vogeser M, Lorenzl S. Comparison of automated assays for the determination of vitamin B12 in serum. *Clin Biochem* 2007; **40**:1342–5.

49. Karmi O, Zayed A, Baraghethi S, Qadi M, Ghanem R. Measurement of vitamin B12 concentration: a review of available methods. *IIOAB J* 2011; **2**:23–32.

50. Carmel R. Current concepts in cobalamin deficiency. *Annu Rev Med* 2000; **51**:357–75.

51. Solomon LR. Cobalamin-responsive disorders in the ambulatory care setting: unreliability of cobalamin, methylmalonic acid, and homocysteine testing. *Blood* 2005; **105**:978–85.

52. Rogers EJ, Chen S, Chan A. Folate deficiency and plasma homocysteine during increased oxidative stress. *N Engl J Med* 2007; **357**:421–2.

53. Solomon LR. Diabetes as a cause of clinically significant functional cobalamin deficiency. *Diabetes Care* 2011; **34**:1077–80.

54. Solomon LR. More on failures of cobalamin assays in pernicious anemia. *N Engl J Med* 2012; **367**:1570.

55. Andrès E, Serraj K, Mecili M, Ciobanu E, Vogel T, Weitten T. Mise au point sur la vitamine B12 administrée par voie orale. *Ann Endocrinol* 2009; **70**:453–61.

56. Solomon LR. Disorders of cobalamin (vitamin B12) metabolism: emerging concepts in pathophysiology, diagnosis and treatment. *Blood Rev* 2007; **21**:113–30.

57. Kanazawa S, Herbert V. Total corrinoid, cobalamin and cobalamin analogue levels may be normal in serum despite cobalamin in liver depletion in patients with alcoholism. *Lab Invest* 1985; **53**:108–10.

58. Baker H, Leevy CB, DeAngelis B, Frank O, Baker ER. Cobalamin and holotranscobalamin changes in plasma and liver tissue in alcoholics with liver disease. *J Am Coll Nutr* 1998; **17**:235–8.

59. Hagelskjaer L, Rasmussen K. Methylmalonic acid concentration in serum not affected in hepatic disease. *Clin Chem* 1992; **38**:493–5.

60. Lambert D, Benhayoun S, Adjalla C, Gélot MM, Renkes P, Gérard P, *et al.* Alcoholic cirrhosis and cobalamin metabolism. *Digestion* 1997; **58**:64–71.

61. Carmel R, Eisenberg L. Serum vitamin B12 and transcobalamin abnormalities in patients with cancer. *Cancer* 1977; **40**:1348–53.

62. Gauchan D, Joshi N, Gill AS, Patel V, De Bari VA, Guron G, *et al.* Does an elevated serum vitamin B12 level mask actual vitamin B12 deficiency in myeloproliferative disorders? *Clin Lymph Myel Leukemia* 2012; **12**:269–73.

63. Carmel R, Tatsis B, Baril L. Circulating antibody to transcobalamin II causing retention of vitamin B12 in the blood. *Blood* 1977; **49**:987–1000.

64. Bowen RA, Drake SK, Vanjani R, Huey ED, Grafman J, Horne MK 3rd. Markedly increased vitamin B12 concentrations attributable to IgG-IgM-vitamin B12 immune complexes. *Clin Chem* 2006; **52**:2107–14.

65. Schwartz M, Bastrup-Madsen P. A new vitamin B12 binding protein in serum causing excessively high serum vitamin B12 values. *Scand J Haematol* 1968; **5**:35–40.

66. Rain JD. Intérêt en hématologie du dosage des cobalamines. *Immunoanal Biol Spec* 1992; **32**:17–24.

67. Serraj K, Vogel T, Federici L, Ciobanu E, Mecili M, Kaltenbach G, et al. Le syndrome de Non-Dissociation de la Vitamine B12 de ses Protéines Porteuses ou de Mal Digestion des cobalamines alimentaires. *Presse Med* 2009; **38**:55–62.

68. Carmel R. Extreme elevation of serum transcobalamin I in patients with metastatic cancer. *N Engl J Med* 1975; **292**:282–4.

69. Fremont S, Chamigneulle B, Gerard P, Felden P, Lambert D, Gueant JL, et al. Blood transcobalamin levels in malignant hepatoma. *Tumor Biol* 1991; **12**:353–9.

70. Gimsing P, Hippe E. Increased concentration of transcobalamin I in a patient with metastatic carcinoma of the breast. *Scand J Haematol* 1978; **21**:243–9.

71. Kane SP, Murray-Lyon IM, Paradinas FJ, Johnson PJ, Williams R, Orr HA, et al. Vitamin B12 binding protein as a tumour marker for hepatocellular carcinoma. *Gut* 1978; **19**:1105–9.

72. Wheeler K, Pritchard J, Luck W, Rossiter M. Transcobalamin I as a marker for fibrolamellar hepatoma. *Med Ped Oncol* 1986; **14**:227–9.

73. Salles N, Herrmann F, Sakbani K, Rapin CH, Sieber C. High vitamin B12 level: a strong predictor of mortality in elderly inpatients. *J Am Geriatr Soc* 2005; **53**:917–8.

74. Gimsing P. Cobalamin metabolism in chronic myelogenous leukemia. *Dan Med Bull* 1998; **45**:459–79.

75. Arnalich F, Zamorano AF, Martinez-Hernandez P, Pena JM, Barbado FJ, Vazquez JJ. Additional predictive value of serum unsaturated vitamin B12 proteins in multiple myeloma. *J Med* 1990; **21**:277–86.

76. Vlasveld LT. Low cobalamin levels in multiple myeloma: a retrospective study. *Neth J Med* 2003; **61**:249–52.

77. Gimsing P. The cobalamin forms and analogues in plasma and myeloid cells during the course of chronic myelogenous leukemia related to the clinical condition. *Br J Haematol* 1995; **89**:812–9.

78. Rachmilewitz B, Manny N, Rachmilewitz M. The transcobalamins in polycythemia vera. *Scand J Haematol* 1977; **19**:453–62.

79. Zittoun J, Farcet JP, Marquet J, Sultan C, Zittoun R. Cobalamin vitamin B12 (vitamin B12) and B12 binding proteins in hypereosinophilic syndromes and secondary eosinophilia. *Blood* 1984; **63**:779–83.

80. Rachmilewitz D, Rachmilewitz EA, Polliack A, Herchko CA. Acute promyelocytic leukemia. A report of five cases with a comment on the diagnostic significance of serum vitamin B12 determination. *Br J Haematol* 1972; **22**:87–92.

81. Hagelskjaer L, Rasmussen K. Methylmalonic acid concentration in serum not affected in hepatic disease. *Clin Chem* 1992; **38**:493–5.

82. Fragasso A, Mannarella C, Ciancio A, Sacco A. Functional vitamin B12 deficiency in alcoholics: an intriguing finding in a retrospective study of megaloblastic anemic patients. *Eur J Intern Med* 2010; **21**:97–100.

83. Hofer LJ, Elian KM. Parenteral vitamin B12 therapy of hyperhomocysteinemia in end-stage renal disease. *Med Clin Exp* 2004; **27**:10–3.

84. Carmel R, Tatsis B, Baril L. Circulating antibody to transcobalamin II causing retention of vitamin B12 in the blood. *Blood* 1977; **49**:987–1000.

85. Reynolds EH, Bottiglieri T, Laundry M, Stern J, Payan J, Linnell J, et al. Subacute combined degeneration with high serum vitamin B12 level and abnormal vitamin B12 binding protein. *Arch Neurol* 1993; **50**:739–42.

86. Molad Y, Rachmilewitz B, Sidi Y, Pinkhas J, Weinberger A. Serum transcobalamin and transcobalamin levels in systemic lupus erythematosus. *Am J Med* 1989; **88**:141–4.

87. Grindulis KA, Calverley M, Cox C. Rheumatoid arthritis: is serum vitamin B12 high in active disease? *J Rheumatol* 1984; **11**:211–2.

88. Kalyoncu U, Buyukasik Y, Akdogan A, Karadag O, Bilgen SA, Kiraz S, et al. Increased serum vitamin B12 levels are associated with adult-onset Still's disease with reactive macrophage activation syndrome. *Joint Bone Spine* 2010; **77**:131–4.

89. Zittoun J, Farcet JP, Marquet J, Sultan C, Zittoun R. Cobalamin and B12 binding proteins in hypereosinophilic syndromes and hypereosinophilia. *Blood* 1984; **63**:779–83.

90. Serraj K, Mecili M, Housni I, Andrès E. L'hypervitaminémie B12: physiopathologie et intérêt en pratique clinique. *Press Med* 2011; **40**:1120–7.