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Macro-B_{12} masking B_{12} deficiency

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SUMMARY

In clinical practice, the finding of an elevated serum B_{12} concentration is often the consequence of supplementation with B_{12} in either oral form or injections. Also, elevated serum B_{12} may be associated with underlying disorders, like liver diseases or a (haematologic) malignancy. Only a few studies have shown that it may also be the consequence of complex formation of B_{12}-vitamin binding proteins with immunoglobulins, the so-called macro-B_{12}. We describe a young woman who previously was diagnosed with B_{12} deficiency, and in whom, after cessation of B_{12} injection treatment, neurologic symptoms re-appeared, and despite this, repeatedly elevated serum B_{12} concentrations above the upper limit of the assay were found. We demonstrated that this was caused by the presence of macro-B_{12}, which not only resulted in erroneous and longstanding elevated serum B_{12}, but also masked her underlying B_{12} deficiency.

BACKGROUND

Vitamin B_{12} (cobalamin, abbreviated B_{12}) deficiency is common. Symptoms develop insidiously, sometimes over the course of several years, and overlap with many other common disorders.[1] The measurement of serum B_{12} concentration as a diagnostic test for cellular B_{12} deficiency is unreliable, as many people with clinical signs of B_{12} deficiency have serum concentrations above the lower population reference limit (140 pmol/L, depending on the assay). In such situations the measurement of the biomarkers methylmalonic acid and homocysteine has been used, but their sensitivity and specificity for demonstrating B_{12} deficiency is also limited.[2 3] This may cause individuals with relevant and readily reversible deficiency to be missed.

Elevated serum B_{12} concentrations most commonly reflect recent supplementation with high dose oral vitamin-B_{12}-containing preparations or injections. Serum B_{12} may also be elevated as a consequence of an underlying disorder such as liver diseases or a (haematologic) malignancy. It has also been demonstrated that immune complexes between serum immunoglobulins and B_{12}-vitamin binding protein (macro-B_{12}) may develop in some individuals, leading to highly elevated serum B_{12} concentrations.[4]

We describe a young woman aged 18, who developed macro-B_{12}, leading to difficulties and delay in making a correct diagnosis of B_{12} deficiency.

CASE PRESENTATION

At the age of 13 years, the patient presented with tremors of the hands, burning sensation in her tongue, painful muscles throughout the body, paraesthesia in hands and feet, muscle cramps, fatigue, as well as diminished concentration and memory, and brain fog. On investigation by her general practitioner (GP), she was found to have B_{12} deficiency. The serum concentration of B_{12} was 150 pmol/L (normal for this age >252 pmol/L[5]), of methylmalonic acid was 357 nmol/L (normal <220 nmol/L[6]). Her nutritional intake of B_{12} was deemed sufficient, so it was presumed that she had impaired intestinal B_{12} absorption, but no further investigations were performed. Treatment was started with hydroxocobalamin injections 1000 mcg twice weekly, with the frequency of injections gradually reduced to once every 6 weeks. Her symptoms resolved. After 2 years of treatment, the GP advised her to stop the injections for an undisclosed reason.

In the months thereafter, the patient started to experience neurologic symptoms again, consisting of fatigue, tremor of the hands and slight paraesthesia of the feet. This hindered this active young woman from sports activities. She consulted a neurologist, who could not find a specific explanation for her symptoms. Based on a serum B_{12} concentration >1476 pmol/L, the possibility of B_{12} deficiency as a cause of her neurological complaints was dismissed despite her previous history. Six months later she was referred by her GP to our outpatient clinic for further evaluation as she still had serum B_{12} concentrations >1476 pmol/L, the upper limit of the assay, although she did not have any cobalamin injections nor had been using any oral vitamin-B_{12}-containing supplements for 2 years. Her weight was completely stable, food intake normal, including products of animal origin like meat, eggs and milk, and she had no specific cardiovascular or gastrointestinal complaints, or abnormalities in stool frequency. Besides a delicate but clear tremor of both hands, physical examination was normal.

INVESTIGATIONS

Routine haematologic laboratory evaluation showed no specific abnormalities, liver enzymes were normal, as was her renal function and C reactive protein. Serum B_{12} concentration was >1476 pmol/L, methylmalonic acid was 222 nmol/L and total homocysteine was 7.8 µmol/L (normal <10 µmol/L[5]). Thyroid function was normal. Tests for the presence of antibodies against parietal cells, intrinsic factor and thyroid peroxidase were negative.

To evaluate the presence of macro-B_{12}, that is, complexes of B_{12}-vitamin binding proteins with immunoglobulins, we performed a series of studies

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using the protocol described by Remacha *et al*,[4] in which macroproteins are precipitated during incubation at 37°C for 30 min with 40% w/v polyethylene glycol (PEG) 6000, centrifuged at 1100 g, after which B_{12} concentration is measured in the supernatant. Total serum B_{12} was estimated to be 4400 pmol/L after correcting for dilution. Concentration of B_{12} dropped to 136 pmol/L after PEG precipitation. This is consistent with the existence of >95% macro-B_{12}, and was considered to be a serum B_{12} concentration consistent with her earlier diagnosis of B_{12} deficiency.

DIFFERENTIAL DIAGNOSIS

In many instances, high concentrations of serum B_{12} are the consequence of the use of supplements containing a pharmacological concentration of B_{12}, or recent injections of hydroxocobalamin or cyanocobalamin. Elevated serum B_{12} can also be found in patients with hepatic disease, myeloproliferative disorders, as well as metastasised cancers.[3] Macro-B_{12} is also a cause of high serum B_{12} concentrations. Age of the patient, history, physical examination and additional laboratory investigations ruled out the major reasons or disorders associated with high serum B_{12} concentrations in this patient. Therefore, we specifically focused on demonstrating macro-B_{12} as a cause for the laboratory findings. With this, we specifically wondered whether the earlier diagnosis of B_{12} deficiency could be demonstrated, now that supplementation with B_{12} had been withheld for more than 2 years.

TREATMENT, OUTCOME AND FOLLOW-UP

Treatment with intramuscular hydroxocobalamin injections, 1000 mcg intramuscular twice weekly, was reinstituted. Importantly, based on the earlier findings and diagnosis, this treatment has to be considered a lifelong treatment for this patient. After restarting the injections, symptoms gradually resolved over a period of 2–3 months, and injection frequency was gradually decreased to 1000 mcg intramuscularly every month. Only a slight tremor of the hands remained, which was much less than before B_{12} treatment.

DISCUSSION

Once a diagnosis of B_{12} deficiency due to poor absorption of B_{12} has been made, treatment consists of intramuscular hydroxocobalamin injections, which should be maintained lifelong.[3] Initial loading takes place with 1000 mcg hydroxocobalamin injections twice weekly or on alternating days, until all symptoms have resolved or do not improve further. Frequency of injections is then gradually reduced, and most patients will maintain clinical improvement and absence of complaints with one 1000 mcg injection every 2 weeks to 2 months.

Cessation of B_{12} replacement therapy was the cause of a recurrence of symptoms in this patient which went undiagnosed for a period of almost 2 years, because the erroneous serum B_{12} concentration was above the upper limit of the laboratory assay. It has been known that immune complexes may form between B_{12}-vitamin binding proteins and immunoglobulins in the blood, similar to the situation with prolactin,[7] creatine phosphokinase[8] and several liver enzymes. Bowen *et al*[9] reported a patient with markedly increased serum B_{12} concentrations attributed to immune complexes of B_{12} with IgG and IgM. This formation of macro-B_{12} will lead to spuriously elevated serum B_{12} concentrations, often highly above the upper limit of reference of the assay. Due to the binding to immunoglobulins, this B_{12} is not metabolically active. Initially, the prevalence of macro-B_{12} as a cause of elevated serum B_{12} was suggested to be 8%,[10] but in a more recent series, macro-B_{12} was demonstrated in 25%[4] and 30% of samples of patients with serum B_{12} >1476 pmol/L.[11] In addition, Soleimani *et al* report that 6 out of 15 patients had an associated autoimmune disease (rheumatoid arthritis or Hashimoto's thyroiditis), while 3 had a haematologic disorder (hemochromatosis and aplastic anaemia), 2 had liver steatosis, 2 suffered from kidney and heart failure and 2 were classified as having unknown aetiology.[11] Interestingly, one patient appeared to be B_{12}-deficient according to the measurement of serum B_{12} after PEG precipitation. Similarly, Remacha *et al* mention rheumatoid arthritis, myeloma and lymphoma, but also lung cancer in some of their patients with macro-B_{12}.[4] In their series, three individuals had relatively low B_{12} concentrations, between 160 and 170 pmol/L, after PEG precipitation. Until now it is unknown why macro-B_{12} develops.

The cause of B_{12} deficiency in this patient was not established. It has been long known that only half of people of western European descent with demonstrated pernicious anaemia have anti-IF antibodies.[12] In such situations, upper gastrointestinal endoscopy may be helpful in demonstrating a possible diagnosis of autoimmune gastritis, and measuring faecal calprotectin may be helpful in excluding inflammatory bowel disease.[13]

Patient's perspective

After re-starting vitamin B_{12} injections, almost all my complaints have disappeared, I only have a slight tremor, which is much less than before.

Learning points

- Elevated serum B_{12} is often a result of supplementation with B_{12} in oral form or injections.
- In addition, elevated serum B_{12} concentrations can also be associated with underlying disorders, like liver diseases or a (haematologic) malignancy, but it can also be the consequence of complex formation of B_{12}-vitamin binding proteins with immunoglobulins, the so-called macro-B_{12}.
- In this specific patient, the development of macro-B_{12} resulted in erroneous and longstanding elevated serum B_{12}, which masked her underlying B_{12} deficiency as evident by low serum B_{12} postpolyethylene glycol precipitation and resolution of symptoms related to B_{12} deficiency post-treatment.

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Case reports provide a valuable learning resource for the scientific community and can indicate areas of interest for future research. They should not be used in isolation to guide treatment choices or public health policy.

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