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Macro-B₁₂ masking B₁₂ deficiency

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SUMMARY

In clinical practice, the finding of an elevated serum B₁₂ concentration is often the consequence of supplementation with B₁₂ in either oral form or injections. Also, elevated serum B₁₂ 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 B₁₂-vitamin binding proteins with immunoglobulins, the so-called macro-B₁₂. We describe a young woman who previously was diagnosed with B₁₂ deficiency, and in whom, after cessation of B₁₂ injection treatment, neurologic symptoms re-appeared, and despite this, repeatedly elevated serum B₁₂ concentrations above the upper limit of the assay were found. We demonstrated that this was caused by the presence of macro-B₁₂, which not only resulted in erroneous and longstanding elevated serum B₁₂, but also masked her underlying B₁₂ deficiency.

BACKGROUND

Vitamin B₁₂ (cobalamin, abbreviated B₁₂) 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 B₁₂ concentration as a diagnostic test for cellular B₁₂ deficiency is unreliable, as many people with clinical signs of B₁₂ 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 B₁₂ deficiency is also limited.^{2,3} This may cause individuals with relevant and readily reversible deficiency to be missed.

Elevated serum B₁₂ concentrations most commonly reflect recent supplementation with high dose oral vitamin-B₁₂-containing preparations or injections. Serum B₁₂ 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 B₁₂-vitamin binding protein (macro-B₁₂) may develop in some individuals, leading to highly elevated serum B₁₂ concentrations.⁴

We describe a young woman aged 18, who developed macro-B₁₂, leading to difficulties and delay in making a correct diagnosis of B₁₂ 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 B₁₂ deficiency. The serum concentration of B₁₂ was 150 pmol/L (normal for this age >252 pmol/L⁵), of methylmalonic acid was 357 nmol/L (normal <220 nmol/L⁶). Her nutritional intake of B₁₂ was deemed sufficient, so it was presumed that she had impaired intestinal B₁₂ 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 B₁₂ concentration >1476 pmol/L, the possibility of B₁₂ 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 B₁₂ 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-B₁₂-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 B₁₂ concentration was >1476 pmol/L, methylmalonic acid was 222 nmol/L and total homocysteine was 7.8 µmol/L (normal <10 µmol/L⁵). 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-B₁₂, that is, complexes of B₁₂-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 macro-proteins are precipitated during incubation at 37°C for 30 min with 40% w/v polyethylene glycol (PEG) 6000, centrifuged at 1100g, after which B₁₂ concentration is measured in the supernatant. Total serum B₁₂ was estimated to be 4400 pmol/L after correcting for dilution. Concentration of B₁₂ dropped to 136 pmol/L after PEG precipitation. This is consistent with the existence of >95% macro-B₁₂, and was considered to be a serum B₁₂ concentration consistent with her earlier diagnosis of B₁₂ deficiency.

DIFFERENTIAL DIAGNOSIS

In many instances, high concentrations of serum B₁₂ are the consequence of the use of supplements containing a pharmacological concentration of B₁₂, or recent injections of hydroxocobalamin or cyanocobalamin. Elevated serum B₁₂ can also be found in patients with hepatic disease, myeloproliferative disorders, as well as metastasised cancers.³ Macro-B₁₂ is also a cause of high serum B₁₂ concentrations. Age of the patient, history, physical examination and additional laboratory investigations ruled out the major reasons or disorders associated with high serum B₁₂ concentrations in this patient. Therefore, we specifically focused on demonstrating macro-B₁₂ as a cause for the laboratory findings. With this, we specifically wondered whether the earlier diagnosis of B₁₂ deficiency could be demonstrated, now that supplementation with B₁₂ had been withheld for more than 2 years.

TREATMENT, OUTCOME AND FOLLOW-UP

Treatment with intramuscular hydroxocobalamin injections, 1000 mcg intramuscular twice weekly, was reinstated. 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 B₁₂ treatment.

DISCUSSION

Once a diagnosis of B₁₂ deficiency due to poor absorption of B₁₂ 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 B₁₂ 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 B₁₂ concentration was above the upper limit of the laboratory assay. It has been known that immune complexes may form between B₁₂-vitamin binding proteins and immunoglobulins in the blood, similar to the situation with prolactin,⁷ creatine phosphokinase⁸ and several liver enzymes. Bowen *et al*⁹ reported a patient with markedly increased serum B₁₂ concentrations attributed to immune complexes of B₁₂ with IgG and IgM. This formation of macro-B₁₂ will lead to spuriously elevated serum B₁₂ concentrations, often highly above the upper limit of reference of the assay. Due to the binding to immunoglobulins, this B₁₂ is not metabolically active. Initially, the prevalence of macro-B₁₂ as a

cause of elevated serum B₁₂ was suggested to be 8%,¹⁰ but in a more recent series, macro-B₁₂ was demonstrated in 25%⁴ and 30% of samples of patients with serum B₁₂ >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 B₁₂-deficient according to the measurement of serum B₁₂ after PEG precipitation. Similarly, Remacha *et al* mention rheumatoid arthritis, myeloma and lymphoma, but also lung cancer in some of their patients with macro-B₁₂.⁴ In their series, three individuals had relatively low B₁₂ concentrations, between 160 and 170 pmol/L, after PEG precipitation. Until now it is unknown why macro-B₁₂ develops.

The cause of B₁₂ 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 B₁₂ injections, almost all my complaints have disappeared, I only have a slight tremor, which is much less than before.

Learning points

- ▶ Elevated serum B₁₂ is often a result of supplementation with B₁₂ in oral form or injections.
- ▶ In addition, elevated serum B₁₂ 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 B₁₂-vitamin binding proteins with immunoglobulins, the so-called macro-B₁₂.
- ▶ In this specific patient, the development of macro-B₁₂ resulted in erroneous and longstanding elevated serum B₁₂, which masked her underlying B₁₂ deficiency as evident by low serum B₁₂ postpolyethylene glycol precipitation and resolution of symptoms related to B₁₂ 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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