Macro-\(B_{12}\) masking \(B_{12}\) deficiency

Bruce H R Wolffenbuttel,1 Anneke C Muller Kobold,2 Agata Sobczyńska-Malefora,3,4 Dominic J Harrington3,4

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 reappeared, 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), of methylmalonic acid was 357 nmol/L (normal <220 nmol/L). 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 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). 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...
Case report

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 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 pharmaco logical 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 re instituted. 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 the recurrence of symptoms in this patient who 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, creatine phosphokinase and several liver enzymes. Bowen et al5,6 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% 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-B12.3 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.

Acknowledgements The authors thank Thijs Heerink, Dept. of Laboratory Medicine, University of Groningen, University Medical Center Groningen, NL, and Ella Freeke, The Nutristasis Unit, Viapath, St. Thomas’ Hospital, London, UK, for their expertise.

Contributors BHRW is the treating physician, ACMK, ASM and DIH oversaw and performed all laboratory measurements. All authors participated in the case discussion and description. All authors approved the final version of the manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Consent obtained directly from patient(s).

Provenance and peer review Not commissioned; externally peer reviewed.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

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.

REFERENCES