Basophil Activation Test: An Additional Diagnostic Tool in Vitamin B12 Allergy

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Vitamin B12 (Vit B12), also known as cobalamin, is a complex molecule with a central cobalt atom. It is essential for various biological processes in the body [1]. Two predominant etiologies have been proposed for Vit B12 deficiency. The first involves deficiency arising from inadequate intake of nutrients, which is addressed through oral supplementation; the second variant is associated with an absence of intrinsic factor, often due to pernicious anemia or the aftermath of gastric bypass, which must be replaced parenterally. Vit B12 can be delivered either intramuscularly (IM) or subcutaneously (SC) in the form of hydroxocobalamin or cyanocobalamin. Of these, cyanocobalamin is frequently the formulation chosen for oral Vit B12 fortification [2].

We present the case of an 83-year-old woman with a history of autoimmune atrophic gastritis and current Vit B12 deficiency. When she was first diagnosed in 2019, IM cyanocobalamin was administered, with good tolerance. In 2023, the patient was advised to increase supplementary Vit B12 because of poor levels. On this occasion, she started with oral cyanocobalamin and developed lingual edema and pharyngeal pruritus that resolved within an hour without the need for treatment. She has not taken cyanocobalamin since. Although the patient's most recent blood analysis showed Vit B12 levels within the normal range, she was referred to the allergy department for further evaluation. This involved skin prick tests (SPTs) with injectable cyanocobalamin, which is the formulation of Vit B12 used at our hospital (hydroxocobalamin is not available). The result of the SPT with cyanocobalamin (prepared at 1 mg/mL) was negative. An intradermal test (IDT) was not conducted, as our experience indicates that most cases are attributable to irritant reactions. The patient underwent an oral challenge test with cyanocobalamin starting at 0.25 mg (a quarter of the total dose) and developed odynophagia,

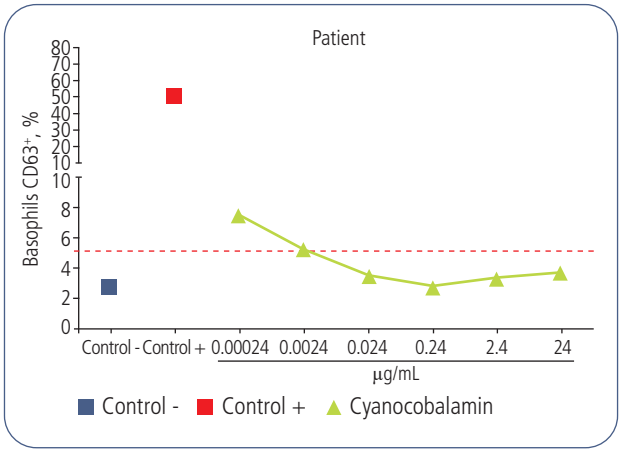

Figure. Basophil activation test with cyanocobalamin. The dose-response curves of both the healthy control and the patient were generated using 6 × 10-fold concentrations of the drug. The negative control consisted of unstimulated cells, while in the positive control, cells were stimulated with anti-IgE antibody and N-formyl-Met-Leu-Phe. Activated basophils were identified as $SSC^{low}/CD203c^{+}/CD63^{+}$ using flow cytometry. The results are expressed as the percentage of $CD63^{+}$ cells. The patient exhibited values above the dashed cut-off at 0.00024 µg/mL (≥5% and double the negative value), indicating a positive result.

lingual edema, nausea, and abdominal pain 30 minutes after administration of the first dose. Nasal fibroendoscopy revealed mild edema of the epiglottis and the pharyngeal walls. No skin lesions were observed. Adrenaline 0.3 mL IM was administered, and a peripheral venous line was inserted for the administration of 40 mg of methylprednisolone intravenously (IV), dexchlorpheniramine 5 mg IV, and hydrocortisone 200 mg IV. The symptoms resolved in less than 1 hour.

A basophil activation test (BAT [BasoFlowEx Kit, EXBIO]) was performed with cyanocobalamin according to the manufacturer's instructions and using both the patient's blood sample and blood obtained from a nonatopic healthy control. The population of basophils was defined as $SSC^{low}/CD203c^{+}$ by flow cytometry, with at least 500 basophils acquired. The results are expressed as the percentage of $CD63^{+}$ basophils. Additionally, the stimulation index (SI) was calculated as the ratio between the percentage of activated basophils ($SSC^{low}/CD203c^{+}/CD63^{+}$ cells) with cyanocobalamin and the negative control (unstimulated basophils). Previous studies have established that the threshold for activated basophils following interaction with the drug must be ≥5% [3,4]. Dose-response curves were generated using 6 × 10-fold dilutions (ranging from 1:1 to 1:100 000), with an initial concentration of 24 µg/mL in a phosphate-buffered saline 1X solution (Figure). A cross-linking anti-IgE antibody combined with a stimulating peptide, N-formyl-Met-Leu-Phe, served as the positive control. A pronounced dose-response relationship was observed with cyanocobalamin, reaching a peak of 7.48% of activated basophils (SI of 2.87) (Figure). The healthy control percentage of unstimulated cells was about 1.65% (Supplementary Figure 2). Despite the negative SPT result, both the oral challenge with cyanocobalamin and the BAT with cyanocobalamin were positive, confirming allergy to Vit B12. This finding reinforces the utility of this test. At present, the patient does not require urgent treatment since her Vit B12 levels remain within the normal range. We intend to conduct skin tests with hydroxocobalamin and a new BAT. The patient consented to the publication of her case.

Allergic hypersensitivity reactions associated with the administration of Vit B12 (cyanocobalamin or hydroxocobalamin) have been reported, although they are uncommon [5]. In 2023, El Rhermoul et al [2] conducted a multicenter retrospective analysis of data from 29 patients with Vit B12 hypersensitivity from 3 centers in the United Kingdom gathered over an 8-year period [2]. Eighteen patients (62%) had had immediate hypersensitivity reactions. Of these, 8 (44%) displayed symptoms consistent with anaphylaxis, affecting at least 2 organ systems. While Vit B12 deficiency is a well-recognized and extensively researched topic, there is limited information on the prevalence and severity of allergy to the drug [2,6]. Consequently, the present case offers valuable insights to fellow medical professionals and aids in the management of similar cases in the future.

The typical symptoms of allergic hypersensitivity reactions to Vit B12 can range from mild to severe and may include the following: localized skin reactions such as itching, hives (urticaria), redness, and rash; angioedema, typically around the eyes and lips, and sometimes affecting the hands, feet, and throat; and anaphylaxis, a severe and potentially life-threatening allergic reaction with a rapid onset. The symptoms of anaphylaxis include dyspnea, hypotension, tachycardia, dizziness, syncope, and gastrointestinal symptoms such as vomiting and diarrhea [2,7]. Furthermore, we should note that patients with only a positive cobalt patch test result do not require skin testing with Vit B12 and can receive the drug. This is especially important, since patch test results for cobalt are very frequently positive [2].

In conclusion, confirmed allergy to Vit B12 is rare. We recommend enhancing the evaluation of Vit B12 allergy with a BAT, as this provides additional diagnostic value.

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Conflicts of Interest

JMRM reports receiving payments for lectures and educational events from AstraZeneca and GSK. The remaining authors declare that they have no conflicts of interest.

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