CASE REPORT

Hypersegmented neutrophils and oval macrocytes in the setting of B₁₂ deficiency and pancytopenia

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SUMMARY

Vitamin B₁₂ deficiency is a recognised pathology in several populations, with a particular prevalence in an older adult population. We present two cases whereby vitamin B₁₂ deficiency is the causative factor in marked pancytopenia. Oval macrocytosis and hypersegmented neutrophils were noted on both peripheral blood samples, which are a characteristic finding in macrocytic anaemia due to B₁₂ deficiency. Distinct underlying pathologies were identified in both cases; food-cobalamin malabsorption and pernicious anaemia. Parenteral vitamin B₁₂ supplementation resulted in a marked reticulocytosis and rapid improvement of haematological indices in both cases. We present this series to serve as a reminder that B₁₂ deficiency can present as life-threatening pancytopenia. It has multiple underlying pathologies, defined risk populations and has characteristic blood film findings which can guide investigations, diagnosis and treatment.

BACKGROUND

Pancytopenia is characterised by low levels of haemoglobin (Hb), white blood cells (WBC) and platelets. The list of differentials which can account for this abnormality is extensive, as are the potential investigations and associated treatments.¹ We describe two separate cases of pancytopenia due to vitamin B₁₂ deficiency, outlining our investigative approach, the two different underlying pathologies resulting in B₁₂ deficiency and subsequent management of same. The case series is useful in that it illustrates the insidious and polysymptomatic nature of B₁₂ deficiency and the potentially life-threatening haematological consequences. In addition, we aim to highlight the importance of screening high risk populations for B₁₂ deficiency and create an awareness for gastric malignancy in the setting of pernicious anaemia.

CASE PRESENTATION**Case 1**

A 70-year-old man had been referred to the emergency department in light of reported pancytopenia while under investigation for recent weight loss. He had been unwell for a period of 8 weeks, reporting an altered bowel habit, anorexia and worsening dyspnoea. The altered bowel habit was characterised by constipation and diarrhoea, although he denied melaena and frank blood per rectum (PR). There were no upper GI symptoms reported. He denied any night sweats or haemoptysis. There

were no reported sick contacts or recent travel. He was a smoker and while he had an unremarkable medical history, there was family history notable for colon cancer in a sibling. On examination, he was haemodynamically stable and inspection revealed notable scleral icterus with some bruising. His cardiac examination showed no evidence of an elevated JVP or peripheral oedema. Auscultation of the precordium was unremarkable while there was a noted wheeze on auscultation of the respiratory system. His abdomen was soft and non-tender, with no organomegaly. No masses were found on PR examination. Examination of both the neurological system and the lymphatic system were unremarkable.

Case 2

A 76-year-old previously healthy woman was referred to the emergency department with severe fatigue and Hb of 5.5 g/dL. She had been unwell for 4 weeks with progressive lethargy. Of note, she had a recent elective hospital admission for a total knee replacement and had been prescribed a course of aspirin and ibuprofen on discharge. Her sole complaint was of debilitating fatigue and lethargy. She denied any altered bowel habit, melaena or blood PR. Her appetite had remained stable and there were no complaints of dysphagia, nausea, early satiety or vomiting. There was no weight loss, no reported night sweats or lymphadenopathy. She was a lifelong non-smoker. On presentation to the emergency department, she did not have any cardiac symptoms. On examination, the patient was haemodynamically stable. She was notably pale with no observed bruising or vitiligo. Cardiovascular examination was normal. There was no noted lymphadenopathy or organomegaly. Her neurological examination was unremarkable.

INVESTIGATIONS**Case 1**

The initial haematological investigations revealed a marked pancytopenia, Hb 4.8 g/dL with a mean cell volume of 121, WBC $2.9 \times 10^9/L$ and platelets of $26 \times 10^9/L$. Of further note were reticulocytes $16 \times 10^9/L$ (0.9%), folate of 2.9, B₁₂ <125, ferritin 330, lactate dehydrogenase (LDH) 8500 IU/L, fibrinogen of 1.6, bilirubin 53 with unremarkable renal and liver function profiles. An autoimmune antibody screen, including but not limited to anti-neutrophil cytoplasmic antibodies (ANCA), anti-transglutaminase and anti-parietal cell antibodies, was negative.

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Figure 1 Hypersegmented neutrophil and oval macrocytes.

Stool culture was unremarkable with serial negative faecal occult blood tests. A CT thorax-abdomen-pelvis performed did not reveal any pathologically enlarged lymph nodes or organs, nor did it demonstrate any focal masses. A peripheral blood film was notable for oval macrocytosis and hypersegmented neutrophils (figure 1), highly suggestive of vitamin B₁₂ deficiency. Gastroscopy performed revealed a hiatus hernia but did not demonstrate any gross lesion. Histology demonstrated chronic gastritis in the antrum, incisura and corpus with mild activity throughout and no noted metaplasia. There were normal duodenal biopsies. There was no evidence of *Helicobacter pylori* (*H.pylori*) infection. A diagnosis of food-cobalamin malabsorption was made.

Case 2

Routine blood tests on admission were notable for a Hb 5.6 g/dL with a mean cell volume of 111, WBC $3.4 \times 10^9/L$, platelets $129 \times 10^9/L$, reticulocytes of $10.5 \times 10^9/L$ (0.7%), B₁₂ <125, folate 3.3, LDH 3518 IU/L, iron 36.1 with normal renal, liver and thyroid profiles. Autoimmune serology was positive for intrinsic-factor antibodies, but negative for ANCA, anti-transglutaminase and anti-parietal cell antibodies. Faecal occult blood tests were negative. An ultrasound of the abdomen was unremarkable. A blood film performed was reported as notable for oval macrocytosis and hypersegmented neutrophils, confirming B₁₂ as a likely aetiology of the haematological findings. A gastroscopy performed showed no gross abnormality but histology revealed moderate chronic gastritis with extensive intestinal metaplasia in the body and fundus of stomach. A diagnosis of pernicious anaemia was made.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis is broad but not limited to haemolytic anaemia, haematological malignancy, nutritional deficiency, pharmacological agent, lymphoma and multiple myeloma.¹

TREATMENT

Case 1

The patient was initially transfused 2 units of red cell concentrate (RCC) on initial presentation by the emergency department. After preliminary investigations and consultation with haematology the patient was given 1000 µg B₁₂ intramuscularly, initially once a day for 1 week, reducing to three times a week for 1 week, once a month for 3 months and 3 monthly thereafter. The patient was also given folic acid 5 mg once a day and oral

Figure 2 Response of haemoglobin and reticulocyte levels to B₁₂ supplementation.

iron supplementation. While platelet levels were low initially, there was no indication for a platelet transfusion at any time during the admission.

Case 2

Unlike Case 1, there was no initial RCC transfusion. Rather, after haematology consult, the patient was treated with intramuscular B₁₂, oral iron and folic acid replacement therapy, on the regimen as that outlined in Case 1 above. Similarly, despite the evidence of a thrombocytopaenia, there was no indication for a platelet transfusion.

OUTCOME AND FOLLOW-UP

The response to treatment was reassuring in both cases despite slightly different approaches.

In Case 1, there was an initial lag period, followed by an exponential rise in the reticulocyte count and subsequent steady increase in Hb levels (figure 2). As evidenced by figure 3, there was an increase in all haematological markers in response to treatment. Follow-up tests at 2 and 4 months in Case 1 revealed Hb 15.8 g/dL, WBC $4.9 \times 10^9/L$, platelets $136 \times 10^9/L$ and Hb 15.2 g/dL, WBC $6.0 \times 10^9/L$, platelets $135 \times 10^9/L$, respectively. The patient was asymptomatic and was discharged with 3 monthly B₁₂ injections.

In the context of Case 2, similar findings were noted, with a brisk reticulocytosis and improvement in all haematological indices. Repeat testing at 1 and 4 months revealed Hb 7.0 g/dL, WBC $4.3 \times 10^9/L$, platelets $431 \times 10^9/L$ and Hb 12.6 g/dL, WBC $5.8 \times 10^9/L$, platelets $257 \times 10^9/L$, respectively. The patient was in

Figure 3 Response of haemoglobin, WBC and platelet levels to B₁₂ supplementation. WBC, white blood cell.

good health and was continued on her B₁₂ considering her newly diagnosed pernicious anaemia.

DISCUSSION

In this case series, we describe two cases of pancytopenia secondary to vitamin B₁₂ deficiency. What is of interest in these cases is the rare severity of the haematological deficit, the characteristic blood film and both the different clinical presentation and underlying aetiologies of vitamin B₁₂ deficiency.

The importance of B₁₂ as a cofactor in the body for varying reactions has been well studied. It plays an essential role in DNA synthesis, haematopoiesis and myelination. Given the haematological picture presented in both cases, the list of differentials was extensive. However, the characteristic finding of oval macrocytosis and hypersegmented neutrophils was key in both cases, ultimately resulting in the diagnosis of B₁₂ deficiency.² A normal neutrophil has up to three to four segments in its nucleus, and a hypersegmented neutrophil has six or greater. Microscopic criteria cite 1% neutrophils with six lobes or 5% with five lobes as a relevant finding. There were several other abnormalities in the blood profile, namely the elevated LDH and elevated bilirubin. The latter findings create a picture which is similar to a haemolytic anaemia; however in this case, the aetiology is lysis of immature cells due to ineffective erythropoiesis and associated release of LDH.^{3,4}

In Case 1 the initial presentation was with altered bowel habit and malaise. Based on the aforementioned peripheral blood sample and listed investigations, a diagnosis of food-cobalamin malabsorption was made. Food-cobalamin malabsorption was first described by Carmel in 1995, originally described in the setting of a negative Schilling test but more recently characterised as a diagnosis of exclusion in the presence of vitamin B₁₂ deficiency. The diagnosis requires the absence of a nutritional deficiency and the presence of one of the following: atrophic gastritis with or without *H pylori* infection, bacterial overgrowth, chronic alcohol abuse, long-term treatment with acid suppressive drugs or biguanides, history of gastric surgery, pancreatic insufficiency or age (idiopathic).^{5,6} It is characterised by the inability to release cobalamin from food or a deficiency of intestinal cobalamin transport proteins or both. Patients can absorb 'unbound' cobalamin through intrinsic factor or passive diffusion mechanisms.⁷ In this case, the likely aetiology relates to a state of chronic active gastritis as is evidenced from the histology report. Previous studies have revealed atrophic gastritis on biopsy with or without *H pylori* infection in food-cobalamin malabsorption. Atrophic gastritis relating to *H pylori* infection is a multifocal pangastritis, involving independent foci in the corpus and antrum of the stomach and as such biopsies at these sites are appropriate. While many studies linked chronic *H pylori* infection in the progression from chronic to atrophic gastritis, there is a recognition that it is a multifactorial process.^{8,9} There is often heterogeneity between the severity of atrophy on histological review and the degree of malabsorption documented.¹⁰ Importantly, the recognition of the syndrome permits new developments of oral cobalamin therapy.⁵

In contrast, the profound symptom in Case 2 was that of fatigue. Following investigations, the underlying pathology in Case 2 was identified as pernicious anaemia. Pernicious anaemia can be defined as a macrocytic anaemia that is caused by a lack of vitamin B₁₂, secondary to a lack of intrinsic factor. It is considered an autoimmune process. Classically, the Schilling test was used in diagnosis but this test is no longer favoured and now surrogate markers, including anti-intrinsic factor, anti-parietal

cell antibodies and characteristic histological findings can be considered diagnostic.¹¹ The diagnosis in Case 2 was based on positive intrinsic-factor antibodies and characteristic histology on gastric biopsy.

Vitamin B₁₂ deficiency is prevalent among the older population. The Framingham study demonstrated a prevalence of 12% among older people living in the community while some estimates suggest as many as 30%–40% in institutions, with the predominant pathology food-cobalamin malabsorption and pernicious anaemia.⁷ The presentation is usually insidious due to the gradual reduction in the stores of B₁₂ rather than an acute decline. Symptoms are polymorphic and include haematological, neuropsychiatric, digestive including altered bowel habit and possibly gynaecological.^{2,7}

The treatment of both conditions is replacement of vitamin B₁₂ and the parenteral route is normally used, with serial blood monitoring thereafter. The regimen used in these severe cases is outlined in the treatment section above. There is a body of evidence that oral supplementation can also be sufficient to replenish stores in food-cobalamin malabsorption.⁵ There is ongoing research into the role of regular endoscopy/histopathology monitoring in patients with pernicious anaemia due to an increased risk of gastric neoplasms.¹² A consensus has yet to be reached, however, a low threshold should be held in performing endoscopy in these patients if there are any symptoms suggestive of malignancy. There are no formal guidelines in place as regarding screening for B₁₂ deficiency in the adult population, although there have been multiple recommendations for screening in the older adult population.¹³

Learning points

- ▶ Vitamin B₁₂ deficiency can present as marked pancytopenia.
- ▶ The aetiology of vitamin B₁₂ deficiency is diverse, with food-cobalamin malabsorption of relevance in the older adult population.
- ▶ There is an increased risk of gastric malignancy in pernicious anaemia and vigilance for alarm symptoms is important.
- ▶ Oval macrocytes and hypersegmented neutrophils are characteristic of macrocytic anaemia due to B₁₂ deficiency.

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Reminder of important clinical lesson

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