Microangiopathic haemolytic anaemia and thrombocytopenia due to combined vitamin B12 and folate deficiency masquerading as thrombotic thrombocytopenic purpura

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Abstract

Vitamin B12 deficiency and folate deficiency are common causes of macrocytic anaemia and both are important for many cellular processes. These deficiencies could be due to inadequate dietary intake, impaired absorption or drug ingestion. We present a case of a 47-year-old male with a history of diffuse large B-cell lymphoma (DLBCL) who was admitted for fatigue, persistent frontal headache and left upper-quadrant abdominal pain. Further investigation showed that he had pancytopenia with microangiopathic haemolytic anaemia (MAHA) and intracranial bleeding (ICB). Serum vitamin B12 and folate were later found to be low and a diagnosis of combined vitamin B12 and folate deficiency mimicking thrombotic thrombocytopenic purpura (TTP) was made. The patient responded well to vitamin B12 and folate replacement.

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introduction

Vitamin B12 deficiency and folate deficiency are common causes of macrocytic anaemia and both are important for many cellular processes. These deficiencies could be due to inadequate dietary intake, impaired absorption or drug ingestion. We present an interesting case of pancytopenia with microangiopathic haemolytic anaemia (MAHA) and intracranial bleeding (ICB) due to combined vitamin B12 and folate deficiency, mimicking thrombotic thrombocytopenic purpura (TTP).

Case presentation

A 47-year-old male was admitted to the haematology ward for fatigue, persistent frontal headache and left upper-quadrant abdominal pain that had lasted one week. He was diagnosed with diffuse large B-cell lymphoma (DLBCL) which was treated with six cycles of dose-adjusted EPOCH-rituximab regimen. An end of treatment positron emission tomography (PET) scan reported no active fluorodeoxyglucose (FDG) uptake, consistent with disease in remission. He had a history of jejunal resection at the age of 42 for bowel ischaemia with unknown aetiology and defaulted surgical follow-up. He was on treatment for type 2 diabetes mellitus and hypertension.

Physical examination revealed scleral icterus, pallor and excess weight. There was an old midline laparotomy scar on the abdomen. Other physical and neurological examination findings were unremarkable. The initial laboratory study (Table 1) showed white blood cell (WBC) count of 3.4 x 109/l, haemoglobin (Hb) of 76g/l platelet count of 67 x 109/l, mean corpuscular volume (MCV) of 91.2fl and reticulocyte count of 10.4%. He had indirect hyperbilirubinemia 62µmol/l and markedly raised lactate dehydrogenase (LDH) 9894U/l. Peripheral blood film (PBF) reported anisopoikilocytosis, polychromasia, ovalocytes, spherocytes, numerous teardrop and fragmented red cells, thrombocytopenia (Figure 1) and hypersegmented neutrophils. His Coomb's test was negative, consistent with non-immune haemolytic anaemia. Coagulation profile, renal, liver and thyroid function tests

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Figure 1 Peripheral blood film showing anisopoikilocytosis, spherocytes, polychromasia (arrows), teardrop and fragmented RBCs (circles) and thrombocytopenia.

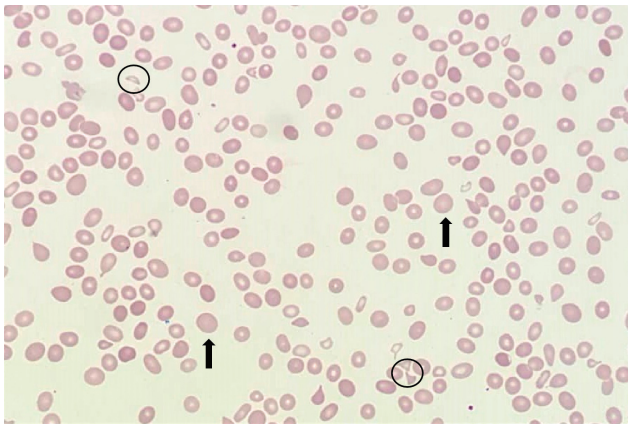
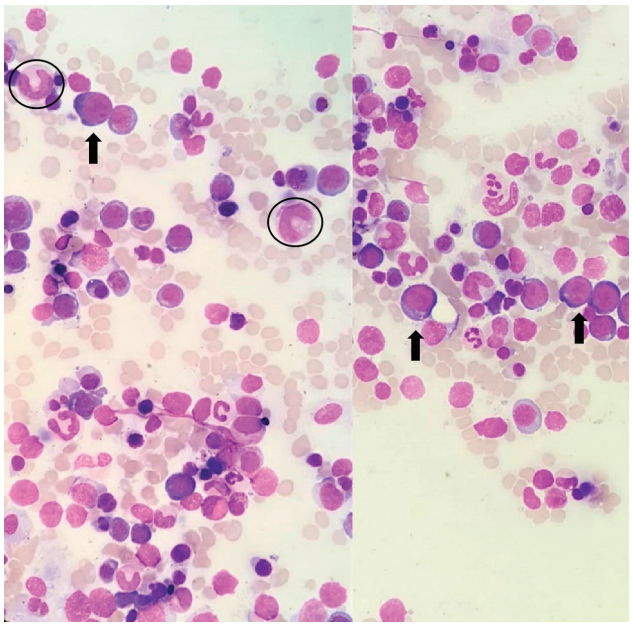


Figure 2 Bone marrow aspirate showing megaloblastic changes: marked erythroid hyperplasia (arrows) and giant metamyelocytes (circles).



were normal. Computed tomography (CT) of the brain was performed for persistent headache and it showed a left fronto-temporo-parietal subdural haematoma that measured 0.8cm in its maximal thickness. The clinical diagnosis of TTP was made based on neurological symptoms, MAHA and thrombocytopenia. Corticosteroid was started immediately and plasmapheresis was arranged.

Serum vitamin B12 and folate levels were also required due to the presence of hypersegmented neutrophils in the PBF. Surprisingly, he was deficient in vitamin B12 and folate, which were 70pg/ml and 3ng/ml respectively (Table 1). He received oral folic acid 5mg daily and parenteral vitamin B12 1000mcg daily for one week followed by weekly doses for one month. His headache, abdominal pain, full blood count and haemolytic markers improved within a few days of therapy.

Plasma exchange was therefore not started. Bone marrow aspiration and trephine biopsy revealed megaloblastic changes (Figure 2) without evidence of lymphomatous infiltration. A negative PET scan ruled out relapse of lymphoma. A normal level of ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) was against TTP. Based on these findings, the final diagnosis was pseudothrombotic microangiopathy due to combined vitamin B12 and folate deficiency.

Table 1 Laboratory study at presentation and after vitamin B12/folate administration

Blood parameters	Admission	D5	D9	D20	Normal range
White blood cells	3.4	3.6	4.1	5.7	4–10 x109 /L
Haemoglobin	7.6	8.8	8.5	11.2	13–17 g/dL
Mean corpuscular volume	91.2	85.6	87.0	83.0	83–101 fL
Mean corpuscular haemoglobin	31.8	27.6	26.9	27.2	27–32 pg
Reticulocyte count	10.4	–	1.77	–	0.5–2.5 %
Platelet count	67	88	112	124	150–400 x109 /L
Coomb's test	Negative	–	–	–	–
Total bilirubin	75	58	40	16	< 21 umol/L
Indirect bilirubin	62	28	17	–	0–16 umol/L
Lactate dehydrogenase	9894	1726	1380	290	< 250 U/L
Serum vitamin B12	70	–	–	–	145–569 pg/mL
Serum folate	3	–	–	–	10–70.2 ng/mL
Serum anti-parietal cell antibody	Positive	–	–	–	–
Serum intrinsic factor antibody	Negative	–	–	–	–
Prothrombin time	12.5	–	–	–	9.4–12.5 sec
International normalization ratio	1.1	–	–	–	1–1.2
Activated partial thromboplastin time	24.8	–	–	–	25.1–36.5 sec

D5, D9, D20: day 5, day 9, day 20 of parenteral vitamin B12 and oral folic acid.

To further investigate the cause of vitamin B12 and folate deficiency, he underwent upper endoscopy, which revealed antral gastritis and a positive urease test. He was given a course of eradication therapy for *Helicobacter pylori* infection. Antral biopsy showed no evidence of atrophic gastritis. Serum for intrinsic factor antibody (IFA) was negative but anti-parietal cell antibody (APCA) was positive. He was discharged with oral folic acid and received 3-monthly intramuscular vitamin B12 injections.

Discussion

This case demonstrates the importance of combined vitamin B12 and folate deficiency as the rare cause of MAHA and thrombocytopenia. Vitamin B12 and folate are essential co-factors in DNA synthesis and cell metabolism. They are involved in remethylation of homocysteine to methionine and in recycling of folic acid. An interruption in this metabolism leads to ineffective haematopoiesis and accumulation of homocysteine.[1,2] Excessive homocysteine causes inflammation of blood vessels and oxidative stress.[3] Oxidative damage to the blood vessels may predispose to bleeding.[4] Vitamin B12 is also required for normal neurological functioning. It is absorbed in the terminal ileum after binding to intrinsic factor produced by gastric parietal cells while the proximal jejunum is the main site of folate absorption.

Deficiency of vitamin B12 or folate contributes to megaloblastic anaemia. Ineffective erythropoiesis due to impaired DNA synthesis and cell maturation gives rise to haemolytic anaemia and macrocytosis. Leucopenia and thrombocytopenia are not uncommon. In extreme cases, red cell anisopoikilocytosis can result in MCV values within the normal range. Markedly elevated LDH reflects ongoing destruction of nucleated erythrocytes in the bone marrow. This causes a reduced lifespan in red blood cells. PBF often shows oval macrocytosis, poikilocytosis, basophilic stippling, Howell-Jolly bodies, circulating megaloblasts, hypersegmented neutrophils and thrombocytopenia.[5,6] Fragmented red blood cells or schistocytes are seen occasionally in cases with very severe vitamin B12 or folate deficiency.[7] Bone marrow aspirate usually reveals marked erythroid hyperplasia, predominance of early erythroid precursors, asynchrony with nuclear maturation lagging behind cytoplasmic maturation and giant metamyelocytes.[6] Haematological findings in folate deficiency are identical to those seen in vitamin B12 deficiency. However, red cell folate level gives a more reliable indication of folate status than serum folate. Serum folate rises rapidly with folate supplementation and thus it reflects recent folate intake. Conversely, folate accumulates in red cells during erythropoiesis and red cell folate is a useful tool to indicate long term folate status in the body.[8]

It was important to identify the cause of combined vitamin B12 and folate deficiency in our patient. Gastric parietal cells secrete intrinsic factor which helps in vitamin B12 absorption. The absence of atrophic gastritis and IFA does not fully exclude pernicious anaemia. APCA is positive in this case but it is not a diagnostic test as it is also found in healthy individuals. IFA is a more reliable test but only positive in about 50–70% of patients with pernicious anaemia.[9,10] There are two possible mechanisms to explain combined vitamin B12 and folate deficiency. Impaired absorption of folic acid due to jejunal resection causes folate deficiency. Besides, there is a strong association between *Helicobacter pylori* infection and formation of autoantibodies to gastric parietal cell and intrinsic factor.[10,11] A significant number of patients with vitamin B12 deficiency were successfully treated with eradication therapy. This finding suggests that *Helicobacter pylori* infection plays a role in the pathogenesis of vitamin B12 deficiency, perhaps via food-cobalamin malabsorption.[11,12] However, it must be remembered that eradication may correct long-term malabsorption, but is not a treatment for megaloblastic anaemia which requires urgent B12/folate replacement.

Distinguishing between TTP and combined vitamin B12 and folate deficiency is difficult due to shared clinical features such as MAHA, thrombocytopenia and neurological symptoms. In a case series of 201 patients with vitamin B12 deficiency, only 2.5% of patients exhibited MAHA symptoms.[7] In a resource-limited settings such as ours, it would be difficult to receive ADMATS-13 level and inhibitor results within a week. Nevertheless, TTP is a clinical diagnosis. Without timely recognition and intervention such as plasmapheresis, the mortality is high. On the other hand, an erroneous diagnosis of TTP may lead to unnecessary and expensive treatment, such as plasma exchange, intensive care admission and risk of infections. It is noteworthy that reticulocyte in megaloblastic anaemia is usually low, but it was elevated in our patient. Hence, PBF is crucial as hypersegmented neutrophil is the only clue which points toward possible megaloblastic anaemia due to vitamin B12 or folate deficiency.

Conclusion

Our case highlights combined vitamin B12 and folate deficiency in a patient presented with neurological symptoms, MAHA, thrombocytopenia, normochromic normocytic RBC and reticulocytosis, mimicking TTP.

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