CASE REPORT

What should I know before ordering a bone marrow aspiration/biopsy in patients with vitamin B₁₂ deficiency?

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SUMMARY

Vitamin B₁₂ deficiency is a well recognised cause of macrocytic anaemia and bone marrow failure. Bone marrow aspiration/biopsy is infrequently indicated for the diagnosis in this setting. However, if a bone marrow aspiration/biopsy is performed, it is important to recognise that it may show dysplastic changes mimicking myelodysplastic syndrome (MDS) or acute leukaemia. We report a case of a 66-year-old non-vegetarian man presenting with generalised weakness for 1 month and misdiagnosed on bone marrow biopsy as MDS. However, laboratory investigations revealed severe deficiency of vitamin B₁₂. Four weeks after starting vitamin B₁₂ replacement the patient's complete blood counts reverted to normal.

BACKGROUND

Vitamin B₁₂ deficiency is a well recognised and reversible cause of bone marrow failure.¹ Macrocytic anaemias secondary to folate and/or vitamin B₁₂ deficiency are characterised by typical morphological abnormalities in the bone marrow, attributable to flaws in both the synthesis and repair of DNA. Nonetheless, bone marrow aspiration/biopsy is infrequently indicated for the diagnosis of macrocytic anaemia and its causes since it may be misleading, especially in cases of severe pancytopenia with marrow hypercellularity.¹ The cause of the vast majority of macrocytic anaemias (alcoholism, liver disease, hypothyroidism, a variety of drugs, vitamin B₁₂ or folate deficiency) can ordinarily be determined by history and/or conventional blood testing. Most importantly, the dysplastic bone marrow in vitamin B₁₂ and folate deficiency can be mistaken for myelodysplastic syndrome (MDS) or even acute leukaemia.^{1–3} In turn this may result in unnecessary and inordinately expensive additional testing to rule out these possibilities. We present an example of a mistaken morphological diagnosis of MDS, which could have been avoided had the recommended diagnostic criteria for MDS been adhered to.⁴

CASE PRESENTATION

A 66-year-old non-vegetarian man presented with increasing generalised weakness for 1 month. He also reported numbness and tingling in his extremities for the same time period. The physical examination was significant for pale appearance and generalised weakness. Laboratory results revealed pancytopenia, with white blood cell (WBC) count of 1310/μL (normal range (NR), 4–11 k/μL), haemoglobin 5.5 g/dL (NR,

13–17 g/dL), haematocrit 16.3% (NR, 39–51%), platelets 55 000/μL (NR, 150 000–400 000/μL) and mean cell volume (MCV) 113.2 fL (NR, 80–100 fL) with red blood cell distribution width of 24.5% (NR 11.5–15%). Peripheral blood WBC differential counts were: 53% neutrophils, 36% lymphocytes, 4% monocytes, 5% eosinophils, 1% basophils and 1% myelocytes. The red blood cells exhibited macrocytosis, anisocytosis, moderate ovalocytosis and polychromasia, while platelets were diminished. Total serum bilirubin was 1.6 mg/dL (NR, 0–1.5 mg/dL), aspartate aminotransferase 106 U/L (NR, 7–40 U/L), alanine transaminase 52 U/L (NR, 5–50 U/L), serum lactate dehydrogenase >2500 U/L (NR, 100–220 U/L), haptoglobin <20 mg/dL (NR, 37–246 mg/dL), folate >20 ng/mL (NR, 2.8–18 ng/mL), reticulocytes 1.1% (NR, 0.4–2.0%) and vitamin B₁₂ level <63 pg/mL (NR, 221–700 pg/mL). A hepatitis panel, stool for occult blood and HIV screen were negative. Esophagogastroduodenoscopy showed severe atrophic gastritis. The patient was transfused four units of red cells for symptomatic anaemia and was started on vitamin B₁₂ supplementation. Despite an evident diagnosis of vitamin B₁₂ deficiency anaemia, a bone marrow aspiration/biopsy was performed.

The reported differential count on 200 cells was: 8% neutrophil myelocytes and metamyelocytes (NR, 10–40%), 21% neutrophils (which included band forms and segmented cells) (NR, 20–60%), 5% eosinophil myelocytes, metamyelocytes and segmented (NR, 0–6%), 3% monocytes (NR 0–2%) and 10% lymphocytes (NR, 3–24%). There were 55% erythroid precursors with a marked left-shift in maturation; notably 30.5% of these were early erythroblasts (NR, 1–4%). The myeloid/erythroid ratio was decreased at 0.7 (NR, 3:1–4:1). A bone marrow flow cytometry was performed, which revealed no immunophenotypic evidence of acute leukaemia or involvement by a lymphoproliferative disorder. The pathological diagnosis was reported as MDS with erythroid hyperplasia. In turn, conventional cytogenetic analysis without mitogens was performed by the Mayo Clinic laboratories and demonstrated a 46XY (30) complement without clonal abnormality. To further seek confirmation that this was MDS, a bone marrow interphase fluorescence in situ hybridisation was performed. It was negative for monosomy 5/del (5q), monosomy 7/del (7q), del (20q) and trisomy 8.

OUTCOME AND FOLLOW-UP

Following 1 week of vitamin B₁₂ supplementation, WBC was 2160/μL, haemoglobin 9.6 g/dL,

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haematocrit 29%, platelets 118 000/ μ L and MCV 97 fL. A follow-up complete blood count (CBC) at 1 month showed WBC 4040/ μ L, haemoglobin 13.2 g/dL, haematocrit 39.2%, platelets 141 000/ μ L and MCV 92.9 fL. A positive anti-intrinsic factor and antiparietal cell antibody determination were subsequently reported, suggesting the diagnosis of pernicious anaemia.

DISCUSSION

Diagnosis of vitamin B₁₂ deficiency does not ordinarily require bone marrow aspiration/biopsy. However, many patients still undergo this procedure, especially when presenting with severe pancytopenia.² Unfortunately this may complicate the initial assessment and result in patient misdiagnosis, leading to further unnecessary and costly testing. For example, severe vitamin B₁₂ deficiency if accompanied by folic acid deficiency can present with transient chromosomal abnormalities. In one case report, a 7 ½-year-old boy with combined vitamin B₁₂ and folic acid deficiency presented with megaloblastic anaemia and underwent bone marrow examination that showed dysplastic changes and asynchronous maturation. Cytogenetics revealed a non-random del(7q) and complex karyotype in three cells, which indicates a clonal origin according to the International System of Human Cytogenetic Nomenclature. Furthermore, complex karyotypes and non-random abnormalities including del(7q) can be associated with MDS or acute myeloid leukaemia. However, both the marrow morphological and cytogenetic abnormalities were resolved after replacement with the deficient vitamins.³ While it is currently not known whether these patients are at risk of developing haematological malignancies later in life, these findings of chromosomal abnormalities although temporary, may lead to unnecessary physician patient follow-ups. In another case series of 12 patients with pancytopenia misdiagnosed as MDS on bone marrow biopsies, the patients responded fairly well to vitamin B₁₂ replacement with normalisation of their CBC in as few as 7 days.⁵ The morphological changes in the bone marrow may sometimes take weeks to months to revert to normal, as seen in a 53-year-old woman with pancytopenia whose bone marrow aspirate showed typical features of megaloblastic anaemia. Her serum vitamin B₁₂ level was 53 pg/mL (normal 211–911 pg/mL).⁶ Bone marrow cytogenetics demonstrated a del(3p) in three metaphases, suggesting a clonal process. Flow cytometry results showed an aberrant antigen maturational pattern (eg, CD13/CD11b) in granulocytes. Interestingly, a repeat bone marrow examination 2 weeks after complete haematological recovery still showed dysplastic changes in the absence of increased blasts. However, the cytogenetic and flow cytometric aberrations disappeared.⁶ The ineffective haematopoiesis caused by vitamin B₁₂ deficiency with impaired DNA synthesis is thought to cause genomic instability. The disappearance of the cytogenetic and flow cytometric aberrations after cobalamin substitution provides evidence that these chromosomal breaks, rearrangements and deletions indicative of chromosomal fragility are transient and may occur in vitamin B₁₂ deficiency.⁷

In order to better understand the apparent misdiagnosis of our case, a second review of the bone marrow aspirate/biopsy was undertaken by a haematopathologist. A 500 cell differential count revealed the following: 1% blasts (NR 0–2%), 1% promyelocytes (NR 1–5%), 27% myelocytes/metamyelocytes/bands and segmented neutrophils (NR 32–72%), 5% eosinophils and precursors (NR 1–6%), 0% basophils (NR 0–1%), 54% erythroid precursors (NR 13–37%), 10% lymphocytes (NR 7–23%) and 1% plasma cells (NR 0–2%). There was erythroid hyperplasia with left shift in maturation and marked megaloblastic

Figure 1 (A) Peripheral blood smear with macrocytic anaemia (Wright stain, $\times 1000$ oil). (B,C) Bone marrow aspirate smears with (B) numerous megaloblastic erythroid precursors and (C) trilineage megaloblastic haematopoiesis including nuclear to cytoplasmic maturational dysynchrony in a megakaryocyte, giant neutrophilic band forms, and megaloblastic pronormoblasts and normoblasts (Wright stain, $\times 1000$ oil). (D) Bone marrow trephine biopsy that is hypercellular for age due to erythroid hyperplasia (H&E, $\times 400$).

changes, most notably in the erythroid precursors but also in the granulocytic and megakaryocytic lineages (figure 1). Dysplastic changes in the erythroid cells included nuclear abnormalities such as budding, multilobation and basophilic stippling. There were numerous giant bands and megakaryocytes with asynchronous maturation. The bone marrow trephine biopsy was hypercellular (80%) due to erythroid hyperplasia (figure 1D). On a morphological basis, the presence of dysplasia and maturational abnormalities were not enough for definitive evidence of a clonal disorder such as MDS. The differential diagnosis was considered to be either a low-grade MDS such as refractory anaemia with multilineage dysplasia, or a megaloblastic anaemia due to vitamin B₁₂ or folate deficiency, other essential element abnormalities (such as copper deficiency or zinc toxicity), exposure to heavy metals such as arsenic and several commonly used drugs (such as methotrexate). Review of 'The minimal diagnostic criteria for MDS proposed by the 2006 Vienna Workshop' specified two prerequisite requirements in order to make this diagnosis.⁴

1. Marked cytopenia (ie, haemoglobin < 11 g/dL, absolute neutrophil count $< 1.5 \times 10^9$ /L or platelets $< 100 \times 10^9$ /L in at least one lineage lasting for ≥ 6 months, unless cytogenetic studies reveal MDS).
2. Exclusion of another clonal or non-clonal haematopoietic disease or non-haematopoietic disease.

In addition, the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (4th edition) specifies that many other factors (nutritional, toxic, drugs, biological agents) can cause myelodysplastic changes and that one must consider these other non-clonal disorders before rendering a diagnosis of MDS.⁸ While our patient only partially met the first criterion in that the cytopenia did not meet the 6 month duration stipulation, he failed to meet the second since the vitamin B₁₂ level was extremely low. Absent the recognition of this criterion and a trial of vitamin B₁₂ therapy, unnecessary and expensive additional testing was undertaken to rule out a plausible, morphologic only, consideration of MDS.

CONCLUSION

In a patient with vitamin B₁₂ deficiency and pancytopenia, the morphological bone marrow picture may mimic that of MDS. For this reason, and in light of the aforementioned Vienna criteria, bone marrow aspiration/biopsy before a therapeutic trial with vitamin B₁₂ is not indicated. In the event it is performed, it is imperative to recognise that it may show dysplastic changes mimicking MDS or acute leukaemia,¹ in turn leading to potentially unnecessary and expensive flow cytometry and cytogenetic testing whose results may not be definitive.

Learning points

- ▶ The diagnostic work-up for macrocytic anaemia when diminished serum levels of vitamin B₁₂ or folate are present infrequently requires bone marrow biopsy. The diagnosis can be quickly confirmed by a rapid response to treatment with vitamin B₁₂ and/or folate. Signs of recovery can be seen within 2 weeks although complete resolution to normal of the complete blood count can take up to 2 months after starting replacement.
- ▶ However, if a bone marrow aspiration/biopsy is performed, physicians must appreciate that it may show dysplastic changes mimicking myelodysplastic syndrome or acute leukaemia.

Competing interests None.

Patient consent Obtained.

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