Netherlands
The Journal of Medicine

SPECIAL ARTICLE

Clinical pathological conference

A non-Hodgkin's lymphoma patient with persistent anaemia after chemotherapy

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INTRODUCTION

A clinical pathology conference is held each trimester at the Department of Internal Medicine of the Academic Medical Centre Amsterdam. Some weeks before the conference, a senior resident is presented with a 'paper' case to be solved. The resident is provided with some but not all details on the case, including clinical, laboratory and radiological data, and in the current case (presented by a resident in Internal Medicine and Haematology) bone marrow cytology slides, but no reports. Based on this information, the resident puts a case together with a focus on clinical reasoning, leading to a provisional diagnosis. Afterwards, the clinician who provided the case reveals the actual diagnosis and clinical course. Below, a recent case on persistent anaemia following chemotherapy in a non-Hodgkin's lymphoma patient is presented.

KEYWORDS

Anaemia, non-Hodgkin's lymphoma, reticulocytes, parvovirus B19

THE CASE

A 75-year-old man presented at the outpatient haematology clinic for a routine visit. Two months earlier, he had finished six cycles of chemotherapy for a non-Hodgkin's lymphoma. At diagnosis, six months earlier, he had presented with fatigue, night sweats and lymphadenopathy at both sides of the diaphragm. Histological examination of a lymph node biopsy showed large CD20-positive lymphocytes with prominent nucleoli and abundant cytoplasm together with smaller lymphocytes and histiocytes. The bone marrow was not involved. At that time (2002), these pathological findings fitted with the World Heath Organisation (WHO) classification of diffuse large B cell lymphoma (DLBCL) stage IIIb. Interestingly, lymphoma cells were EBER- (EBV) positive and a subpopulation of the malignant cells showed plasma cell differentiation with intracytoplasmic expression of IgA kappa. In the serum an IgA kappa paraprotein was found. According to the revised WHO classification of Tumours and Haematopoietic and Lymphoid Tissues in 2008, this lymphoma would now be classified as an EBV-positive diffuse large B cell lymphoma of the elderly. It occurs in patients >50 years with no known history of immunodeficiency, in contrast to other EBV-driven lymphoproliferative diseases such as post-transplantation lymphoproliferative disorders. Its occurrence may be associated with the physiological age-related immunological deterioration characterised by a decline in T lymphocyte repertoire, numbers and function. Median age at diagnosis is 71 years, and the prognosis of EBV+ DLBCL is generally poor, with a median survival of two years.[1]

The medical history of the patient revealed paroxysmal atrial fibrillation for which he was taking flecainide and acetylsalicylic acid. Two years earlier he had undergone a cholecystectomy because of cholecystolithiasis and at the age of 55, he had been diagnosed with Sjögren's syndrome. He was married and had two sons. He had been sailing the world as a chief engineer officer until his retirement ten

years ago. He had never smoked or used illicit drugs and reported drinking one or two beers a day.

After the diagnosis of DLBCL, the patient was included in the HOVON (Haemato-Oncology Foundation for Adults in the Netherlands) 46 trial, which was recruiting at that time. In this protocol, the added value of the anti-CD20 monoclonal antibody rituximab (Mabthera) to CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) chemotherapy was compared with CHOP chemotherapy alone in elderly patients (>65 years of age) with CD20-positive DLBCL. The patient was randomised to receive standard therapy (CHOP without rituximab). Treatment was complicated by cystitis due to temporary urinary retention, urticaria following adriamycin infusion, axonal sensomotoric polyneuropathy, and recurrent anaemia for which the patient received red cell transfusions. After the third cycle, complete metabolic remission, assessed by gallium imaging, was obtained. Computed tomography (CT) scanning of neck, thorax and abdomen after the sixth cycle confirmed complete remission.

At the current visit, the patient reported no specific complaints, except for fatigue. At physical examination an irregular pulse was noted. No lymphadenopathy or hepatosplenomegaly were found, and the remainder of the examination was also normal. Routine laboratory tests showed a haemoglobin level of 5.1 mmol/l (8.2 g/dl) with a mean corpuscular volume (MCV) of 89 fl, but normal platelet count (342 x 10^9/l) and leucocyte count (5.0 x 10^9/l). Creatinine and lactate dehydrogenase (LDH) were normal at 56 µmol/l and 165 U/l, respectively. The differential leucocyte count showed 44% neutrophils, 7% eosinophils, 0% basophils, 25% lymphocytes, 8.4% monocytes and no reticulocytes.

CLINICAL REASONING

This patient presented with mild fatigue and a persistent normocytic anaemia two months after finishing six courses of CHOP chemotherapy for non-Hodgkin's lymphoma.

Classification of anaemia: mean corpuscular volume and reticulocytes

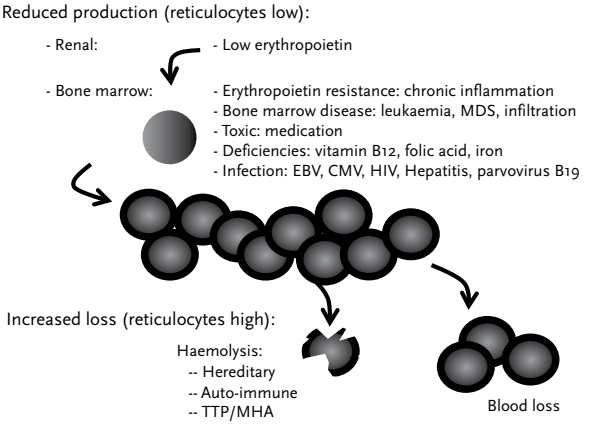
The work-up of anaemia is often guided by additional parameters such as the mean red blood cell volume or corpuscular volume (MCV) of red cells. Typically, this allows classification of anaemia into microcytic (low MCV, the result of insufficient haemoglobin-production); normocytic (normal MCV, reflecting bone marrow production failure); or macrocytic (high MCV, caused by defective DNA synthesis) (*table 1*). Another helpful and perhaps more simple parameter in the differential diagnosis of anaemia is the reticulocyte count. Reticulocytes are immature red blood cells with ribosomal RNA remnants in the cytoplasm that usually compose about 1 to 2% of circulating red blood cells. The reticulocyte count reflects the activity of bone marrow erythrocyte production: it is elevated in case of increased red cell production (to compensate for blood loss or haemolysis) and reduced in case of bone marrow disease (*figure 1*).

Table 1. *Classification of anaemia based on mean corpuscular volume (MCV)*

Low MCV (microcytic)	Normal MCV (normocytic)	High MCV (macrocytic)
Iron deficiency	Bone marrow disease[2]	Vitamin B12 deficiency
Hereditary haemoglobinopathies[1]	Combined nutrient deficiency[3]	Folate deficiency
	Chronic renal insufficiency[4]	Myelodysplastic syndrome
	Erythropoietin resistance[5]	Alcohol
	Haemolysis	Medication[6]

[1]Haemoglobin C, D, E, sickle cell anaemia, and alpha/beta thalassaemia; [2]haematological malignancy or solid tumour metastases; [3]iron and folate and/or vitamin B12 deficiency; [4]reduced renal erythropoietin production; [5]anaemia of chronic disease; [6]e.g. methotrexate (anti-folate), hydroxycarbamide.

Figure 1. *Classification of anaemia based on reticulocyte counts*



Red cell loss leads to compensatory increased red cell production reflected by high reticulocyte counts; suppressed red cell production is associated with low or absent reticulocytes. MDS = myelodysplastic syndrome; EBV = Epstein-Barr virus; CMV = cytomegalovirus; HIV = human immunodeficiency virus; TTP/MHA = thrombocytopenic thrombotic purpura/microangiopathic haemolytic anaemia.

Impaired production versus increased destruction or loss

The disadvantage of using the MCV in the work-up of anaemia is that one has to be familiar with the characteristics of the different forms of anaemia. The reticulocyte count provides a more mechanistic approach, based on the pathophysiology of anaemia.

Our patient presented with a normocytic anaemia and absent reticulocytes in the peripheral blood. This suggests impaired red blood cell production by the bone marrow, the causes of which have been outlined above and in *table 1*. Deficient erythropoietin production seems unlikely in our patient as his renal function is normal. There are quite a few medications that have been described to have myelosuppressive side effects, but flecainide, the antiarrhythmic that he had been taking for years, is not one of them. Infiltration of bone marrow with relapsed lymphoma at this early time point, only affecting red blood cell production but not platelet or leucocyte production, seems improbable. In fact, only two months earlier, CT scanning of neck, thorax and abdomen had shown complete remission. As for nutritional deficiencies, chemotherapy could theoretically lead to a suboptimal nutritional state. In practice, however, chemotherapy treatment in the outpatient setting rarely results in clinically significant nutritional deficiencies. Finally, when a lymphoma patient presents with anaemia, autoimmune haemolysis should be excluded, even when there is no overt paraproteinaemia present. In fact, in about 20% of initially unexplained (idiopathic or primary) autoimmune haemolytic anaemia's a non-Hodgkin's lymphoma turns out to be the underlying cause.[2] Usually haemolytic anaemia is accompanied by a compensatory increase in reticulocyte numbers. The absence of reticulocytes makes haemolysis less likely, but this should be confirmed by a normal LDH and haptoglobin, because in rare cases, haemolytic autoantibodies can be directed against erythrocyte progenitors.[3] In the present case autoimmune haemolytic anaemia seems unlikely given the normal LDH and low reticulocyte number. A normal LDH makes less frequent causes of bone marrow failure, such as EBV-related haemophagocytosis, also unlikely.[4]

Interestingly, when reviewing the medical records of the patient, it was noted that during the six cycles of CHOP chemotherapy, the patient had received over 15 units of red blood cells. At initial presentation, his red blood cell count had been normal. CHOP chemotherapy does have myelosuppressive side effects, with a temporal reduction in haemoglobin, leucocyte and platelet counts as a result. Whereas in the majority of patients this requires support at some point, it is highly unusual to have such a high red cell transfusion dependency. This is related to the fact that CHOP-related myelosuppression is only transient and limited in time, while the median survival of red blood cells is 100 to 120 days. Normally, temporal reductions in red blood cell production are masked by the longevity of previously produced red blood cells, and bone marrow haematopoiesis usually recovers before significant anaemia requiring repeated blood transfusions develops.

In addition, leucocyte and platelet counts were not affected in this patient, an observation that warrants further attention. There are only a few possible explanations for an isolated deficiency of red blood cell production in the bone marrow. First, primary bone marrow disease such as myelodysplastic syndrome (MDS) that predominantly affects erythrocyte production has to be excluded, since MDS may be induced by chemotherapy, even though the time between chemotherapy and the development of anaemia in this case was very short and MDS is typically characterised by a high MCV. Secondly, viral infection of bone marrow, in particular with human parvovirus B19, can lead to 'pure red cell aplasia', bone marrow failure to produce red blood cells. Occasionally, other viral infections of bone marrow such as infection with cytomegalovirus (CMV) or human immunodeficiency virus (HIV) can lead to isolated anaemia. More often, however, CMV and HIV infection result in mild pancytopenia.

DIFFERENTIAL DIAGNOSIS

At this point, myelodysplastic syndrome or viral infection of the bone marrow seem the most likely explanation for this patient's anaemia. Nutrient deficiencies and lymphoma relapse should be excluded.

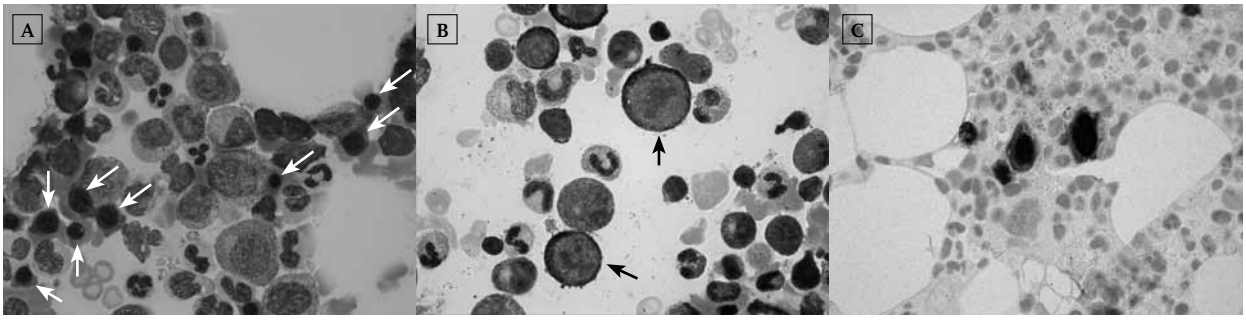
ADDITIONAL TESTING

Guided by the clinical reasoning outlined above, additional laboratory testing and a bone marrow biopsy and aspirate were performed. Laboratory results showed normal iron, folate and vitamin B12 levels. In addition to the normal LDH, also bilirubin and haptoglobin were found to be normal, excluding haemolysis as the cause for his anaemia. Serology of hepatitis B, C, HIV-1, HIV-2 and human parvovirus B19 performed eight months earlier, at the time of lymphoma diagnosis, was negative, while EBV and CMV serology (IgG but not IgM) was positive.

BONE MARROW ASPIRATE AND BIOPSY

Bone marrow aspirate and biopsy are shown in *figure 2*. The bone marrow showed normal cellularity, and normal maturation of the myeloid and megakaryocyte lineages was

Figure 2. *Bone marrow aspirate*



A) Bone marrow aspirate of an healthy individual. Arrows indicate erythroid progenitors. B) Bone marrow aspirate of the patient. Arrows indicate large pro-erythroblasts, the most immature red cell progenitors that can be detected in bone marrow. More mature red cell progenitors (as indicated by arrows in panel A) are lacking. C) Bone marrow biopsy of the patient; anti-parvo B19 immunohistochemical staining shows positive erythroblasts.

confirmed. In the erythroid lineage, however, a complete maturation stop was seen at the pro-erythroblast stadium, and pro-erythroblasts were unusually large (*figure 2B*). This finding is pathognomonic of human parvovirus B19 infection of the bone marrow, and infection was confirmed by anti-parvovirus B19 immunohistochemistry (*figure 2C*). In addition, no clusters of lymphocytes that would suggest lymphoma relapse were seen, nor signs of myelodysplastic syndrome. In fact, apart from giant pro-erythroblasts and absence of normal red cell maturation, the bone marrow showed an entirely normal morphology.

CLINICAL DIAGNOSIS

Aplastic anaemia caused by chronic human parvovirus B19 infection of the bone marrow.

DISCUSSION OF DIAGNOSIS AND PATHOPHYSIOLOGY

Parvovirus B19 infection is common with a seroprevalence rate exceeding 80% among the elderly.[5] The clinical spectrum of infection is broad. In a large proportion of healthy individuals B19 infection is asymptomatic, and if symptomatic, it most commonly presents as erythema infectiosum or 'fifth disease', in particular in young children. In a minority of cases it may cause arthropathy, hydrops foetalis and possibly myocarditis and autoimmune diseases, which are mostly observed in adults. Acute B19 infection temporally hampers erythropoiesis, which does not normally lead to significant anaemia, as the infection is cleared within 14 days in the immunocompetent host. Normal erythropoiesis then resumes long before circulating mature red cells have reached the end of their life spans (100 to 120 days). However, in individuals who depend on high levels of red cell production, for example patients with haemoglobinopathies (i.e. sickle cell disease) or other forms of chronic haemolysis, parvovirus B19 infection can induce transient, but sometimes lethal, aplastic crises. In these patients, the life span of red cells is too short to overcome a temporal reduction in erythropoiesis.

In hindsight, human parvovirus B19 serology was negative in our patient at the time of the diagnosis of lymphoma. It is therefore assumed that he became infected through transfusion of a high-level B19 viraemic blood product (although a primo infection through normal transmission in the community cannot be excluded). About 0.006% of Dutch blood bank donors have such high-level (DNA $>10^6$ IU/ml) parvovirus B19 viraemia.[6] In immunocompetent individuals, transmission of B19 virus through highly viraemic blood products is clinically irrelevant, as the virus is rapidly cleared through pre-existing immunity. Neonates, pregnant women, allogeneic stem cell transplantation recipients in the first year after transplantation and patients with acquired or congenital haemolytic anaemia routinely receive parvovirus B19 negative blood products, as parvo B19 infection in these patients can cause severe complications.

Our patient had no previously acquired immunity against parvovirus B19, putting him at risk for primary infection. In addition, he had been diagnosed with EBV-positive lymphoma which is known to be associated with immune deficiency in general and of the elderly in particular.[1] Chemotherapy, even without rituximab or another form of immunotherapy, may have compromised his immunity even further, and this combination might have been the reason for his inability to clear the virus. In a very similar case an elderly patient with a Hodgkin's lymphoma developed anaemia due to chronic parvovirus infection after polychemotherapy.[7]

Today, it is still under debate whether B19 seronegative patients receiving chemotherapy should receive

B19-negative blood products only. With the high B19 seroprevalence rate in patients and the low incidence of high-level viraemia in blood bank donors, cost-effectiveness studies in this group of patients are not feasible. This case demonstrates, however, how parvovirus B19 infection in a parvovirus B19 seronegative elderly patient on chemotherapy may lead to chronic parvovirus B19 infection, resulting in prolonged anaemia with high transfusion dependency.

EPILOGUE

Active human parvovirus B19 infection in this patient was confirmed by PCR on bone marrow and peripheral blood. Indeed, parvovirus B19 selectively infects erythroid progenitor cells in the bone marrow (*figure 2C*). These progenitor cells express globoside (also known as the blood group P antigen), the receptor for B19, as well as $\alpha5\beta2$ integrin, the co-receptor for B19. Infection of erythroid progenitor cells induces cell-cycle arrest and apoptosis (rather than red cell lysis which would increase LDH levels, such as in vitamin B12 deficiency-induced anaemia),[8] typically leaving the bone marrow with pro-erythroblasts, the earliest progenitors of the red cell line, without mature erythroid cells (*figure 2B*). In addition, proteins that are produced by the virus (such as NS1) can lead to thrombocytopenia and neutropenia, although much less frequently.

Treatment of transiently anaemic patients due to parvovirus B19 infection is primarily supportive, when necessary with red cell transfusions.[5] In immunocompromised patients with chronic infection and persistent anaemia, clearance of the virus can be supported by intravenous immunoglobulin (IVIG). Data on the efficacy of IVIG are based on case reports and small series of patients with a variety of underlying conditions such as solid organ transplantation, advanced HIV infection and indeed treatment with (immuno)chemotherapy. Large clinical trials are lacking. However, from the data available it seems that IVIG treatment of persistent parvovirus B19-induced anaemia is beneficial in the vast majority of cases (reviewed in Mouthon *et al.*[9]). The patient was treated with IVIG after which parvovirus B19 in the serum became undetectable and the anaemia resolved. Three years later, however, the lymphoma relapsed. He declined further chemotherapy and died of pneumonia shortly thereafter.

REFERENCES

1. Dojcinov SD, Venkataraman G, Pittaluga S, et al. Age-related EBV-associated lymphoprolipherative disorders in the Western population: a spectrum of reactive lymphoid hyperplasia and lymphoma. Blood. 2011;117:4726-35.
2. Sallah S, Wan J, Hanrahan L. Future development of lymphoproliferative disorders in patients with autoimmune hemolytic anemia. Clin Cancer Res. 2001;7:791-4.
3. Mangan KF, Besa EC, Shadduck RK, et al. Demonstration of two distinct antibodies in autoimmune haemolytic anemia with reticulocytopenia and red cell aplasia. Exp Hematol. 1984;12:788-93.
4. Bosman G, Langemeijer SMC, Hebeda KM, et al. The role of rituximab in a case of EBV-related lymphoproliferative disease presenting with haemophagocytosis. Neth J Med. 2009;67:364-5.
5. Young NS and Brown KE: Mechanisms of disease: Parvovirus B19. N Engl J Med. 2004;350:586-97.
6. Kooistra K, Mesman HJ, De Waal M, Koppelman MHGM, Zaaijer HL. Epidemiology of high-level parvovirus B19 viremia among Dutch blood donors, 2003-2009. Vox Sanguinis. 2010;100:261-6.
7. Poplar S, Allford S, Rooney N. Chronic parvovirus B19 infection leading to red cell aplasia following treatment of Hodgkin lymphoma. Br J Haematol. 2010;148:671.
8. Morita E, Sugamura K. Human parvovirus B19-induced cell cycle arrest and apoptosis. Semin Immunopathol. 2002;24:187-99.
9. Mouthon L, Guillevin L, Tellier Z. Intravenous immunoglobulins in autoimmune- or parvovirus B19- mediated pure red-cell aplasia. Autoimmun Rev. 2005;4:264-9.