Int J Clin Exp Pathol 2014;7(4):1742-1747
www.ijcep.com /ISSN:1936-2625/IJCEP1401105

Case Report

Diffuse alveolar damage in a patient with Epstein Barr virus (EBV)-positive diffuse large B-cell lymphoma, severely low serum folate, and megaloblastic anemia: a case report of autopsy

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Received January 28, 2014; Accepted February 26, 2014; Epub March 15, 2014; Published April 1, 2014

Abstract: We report a case of a 49-year-old Japanese man, who was admitted to our hospital because of severe dyspnea. He was found to have severely low serum folate and megaloblastic anemia, which was at first suspected to cause his dyspnea. It was assumed that severely low serum folate might be related to his malnutrition, probably caused by habitual alcohol consumption. He died in several days because of acute respiratory distress syndrome. Autopsy revealed diffuse alveolar damage (DAD) in the lungs and systemic lymph node swelling by EBV-positive diffuse large B-cell lymphoma, in addition to megaloblastic anemia in the bone marrow. Together with histological hemophagocytosis and high level of serum iron and ferritin, DAD was considered to be caused by hypercytokinemia triggered by the presence of EBV-positive diffuse large B-cell lymphoma. On the other hand, pathological findings suggestive of habitual alcohol consumption were not apparent. We considered that low serum folate in this case was not by the low intake but by increased consumption of folate by rapid progression of the lymphoma.

Keywords: Diffuse alveolar damage, lymphoma, folate, megaloblastic anemia

Introduction

Diffuse alveolar damage (DAD) is a histopathological correlate of acute respiratory distress syndrome characterized by rapidly progressing dyspnea. One of the histopathological hallmarks for DAD is a deposition of fibrin-like hyalinized material which diffusely covers alveolar surfaces. Pathophysiological backgrounds for DAD include high level of inflammatory cytokines in the serum (hypercytokinemia), which is triggered by the presence of various infectious diseases and malignancies. In many cases, hypercytokinemia is associated with hemophagocytosis clinically and histopathologically.

Epstein Barr virus (EBV)-positive lymphomas are malignancies caused by infection and/or reactivation of EBV in lymphoid cells [1]. Majority of them are of B-cell type. There has been recent rise in occurrences of EBV-positive lymphomas, presumably due to decrease in host immunity by ageing and/or iatrogenic causes [2, 3]. The latter include transplantation and use of immunosuppressive drugs, such as methotrexate (MTX).

MTX is frequently used not only for immunosuppressants for autoimmune diseases but also for anti-cancer agents. MTX is an antagonist for folate metabolism and decreases folate level in the serum if not appropriately supplemented. Low serum folate is caused not only by drugs including MTX, but also by its low intake or its increased consumption. Examples for low folate intake include malnutrition caused by chronic alcohol consumption and various absorption disorders in digestive tracts. Megaloblastic anemia is a classic example associated with low serum folate.

In this paper, we report a case of a 49-year-old Japanese man, who was admitted to our hospital because of severe dyspnea. He was found to

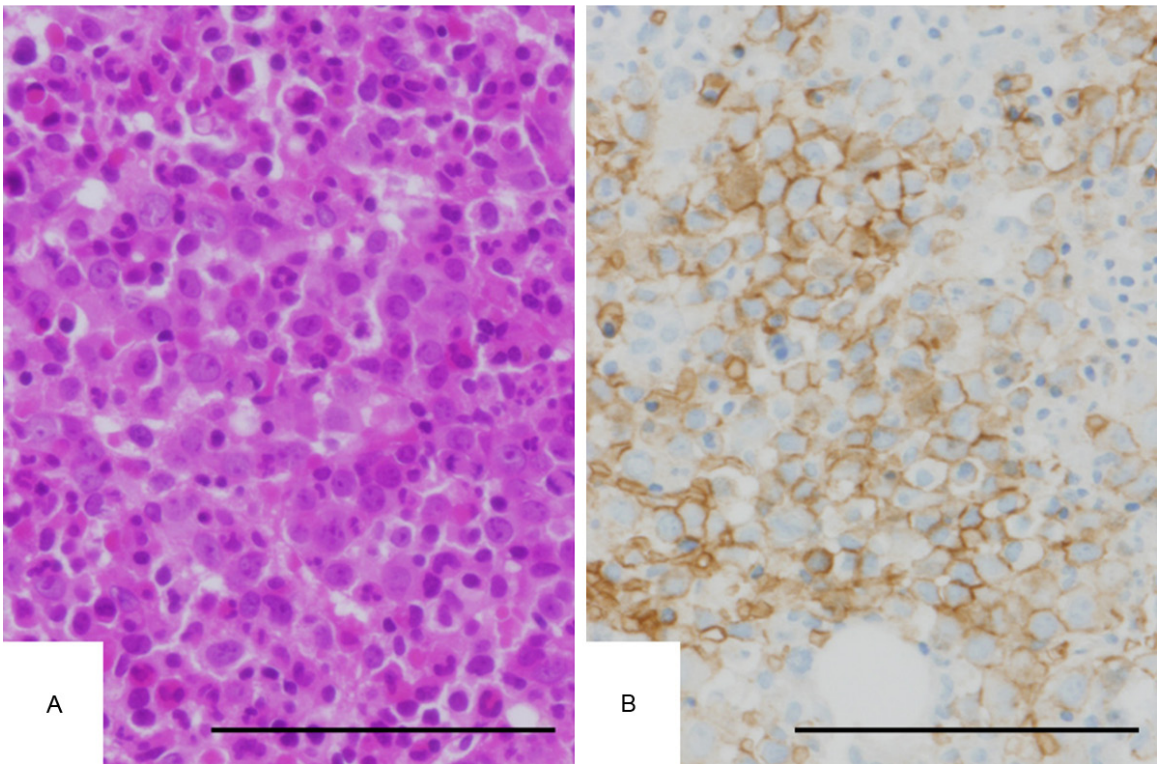

Figure 1. Histopathology of the bone marrow. A. Hematoxylin and eosin (HE) image showed diffuse proliferation of megaloblastic erythroblasts. B. Glycophorin C immunohistochemistry showed that the large cells in **Figure 1A** were stained positive, confirming that they were erythroid. Original magnification: x400. Bar: 100 μm.

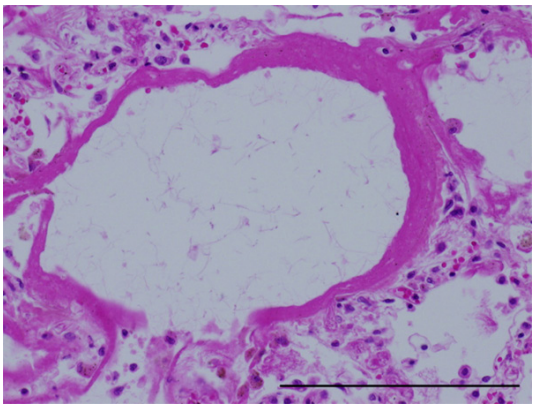

Figure 2. HE image of the lung. Alveolar surface was covered with eosinophilic, hyalinized membranous material. Original magnification: x200. Bar: 200 μm.

have severely low serum folate and megaloblastic anemia, which was at first suspected to cause his dyspnea. It was assumed that severely low serum folate might be related to his malnutrition, probably caused by chronic alcohol consumption. He died in several days because of acute respiratory distress syndrome. Autopsy revealed DAD in the lungs and systemic lymph node swelling by EBV-positive diffuse large B-cell lymphoma, in addition to megaloblastic anemia in the bone marrow.

Case report

A 49-year-old Japanese man was admitted to the emergency department of the hospital because of rapidly progressing respiratory distress. His dyspnea had started several days before the admission. He had had a habit of drinking around 60 to 80 g of pure alcohol per day for at least several years. He had taken non-steroidal anti-inflammatory drugs for cervical spondylosis for six years.

On admission, examination of his peripheral blood showed severe anemia with 4.7 g/dL (normal range: 13.5-17.5) of hemoglobin. Mean corpuscular volume was 126.0 fl (normal range: 89.0-99.0), indicating macrocytic anemia. Dyspnea due to severe anemia was suspected at first. In order to explore the cause of the anemia, bone marrow aspiration was performed. It revealed hypercellular bone marrow with diffuse proliferation of megaloblastic erythro-

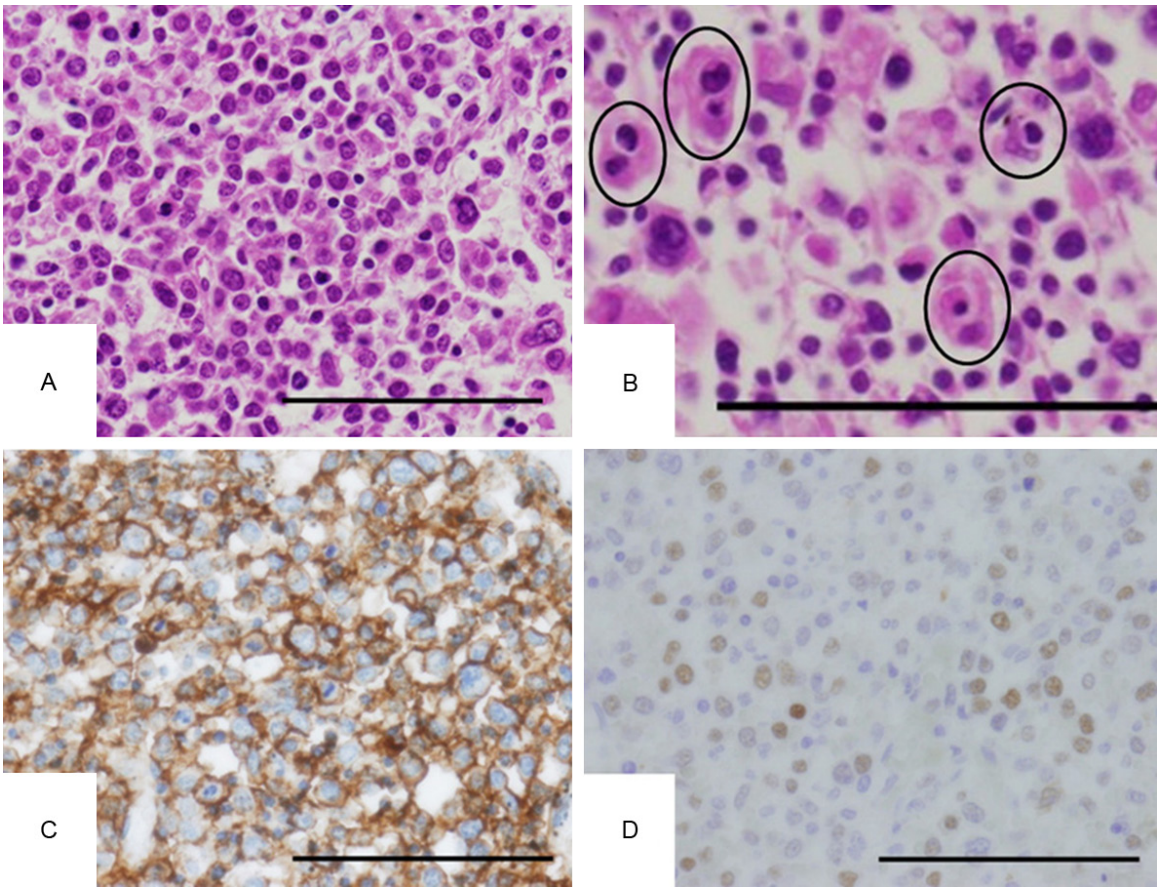

Figure 3. Histopathology of lymphoma. A. HE image of the tumor cells. B. HE image of hemophagocytosis, highlighted with open circles. C. CD20 immunohistochemistry. The tumor cells were stained positive. D. *In situ* hybridization of EBER. The tumor cells were stained positive. Original magnification: x400. Bar: 100 μm.

blasts (**Figure 1A**), which was positive for Glycophorin C by immunohistochemistry (**Figure 1B**). Together with severely low serum folate less than 0.6 ng/mL (normal range: 3.6-12.9), the diagnosis of megaloblastic anemia was made, presumably related to malnutrition due to habitual drinking. His medication record was not remarkable to suggest its involvement in low serum folate. He died of rapidly progressing dyspnea in a few days, which was clinically diagnosed as respiratory distress syndrome.

In autopsy, most part of alveolar surface in his lungs was covered with hyalinized membranous material, compatible with a diagnosis of DAD in acute phase (**Figure 2**). Neither involvement of infectious pneumonia nor infiltration of malignant cells was apparent. Systemic swelling of lymph nodes, mainly 30-40 mm in diameter, was observed in cervical regions as well as in the vicinity of the aortic arch, fundus and antrum of the stomach, and abdominal aorta. These lymph nodes were filled with atypical large lymphoid cells (**Figure 3A**). Geographical necrosis (data not shown) and hemophagocytosis by macrophages (**Figure 3B**) were observed. Immunohistochemical studies revealed that these large atypical cells were stained positive for CD20 (**Figure 3C**), CD79a (data not shown), and negative for CD3 (data not shown). Furthermore, *in situ* hybridization showed that these large atypical cells were positive for EBV-encoded RNA (EBER) (**Figure 3D**). The diagnosis of EBV-positive diffuse large B-cell lymphoma was made for the lymph node lesions. The lymphoma cells infiltrated the stomach to make multiple deep ulcers (data not shown). However, no macroscopic bleeding from the ulcers was observed. We excluded it from a major cause for his severe anemia. Except the stomach, no remarkable findings were observed in upper and lower digestive tract (data not shown).

Together with histological hemophagocytosis, blood test revealed high level of serum ferritin (816.5 ng/mL; normal range: 50.0-200), low

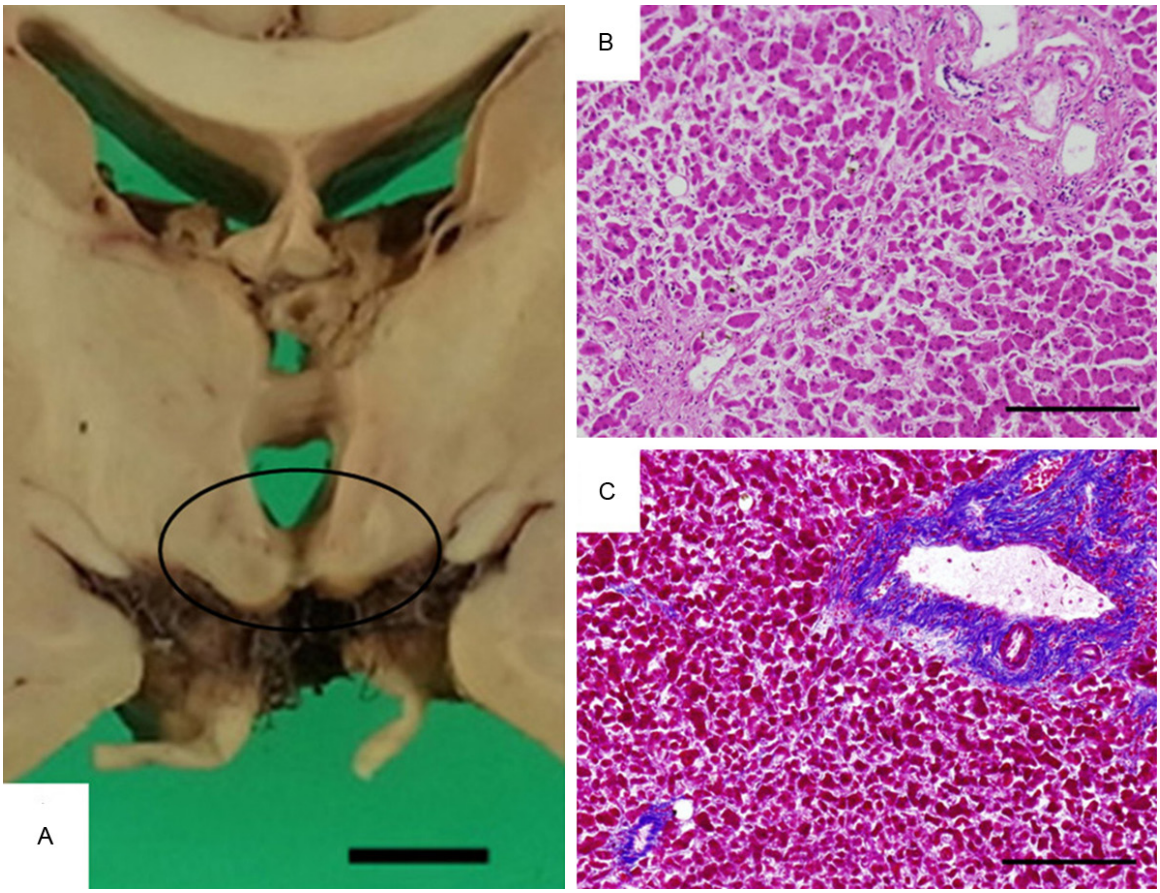

Figure 4. Macroscopic finding of the mammillary body and microscopic findings of the liver. A. Macroscopic view of the mammillary body. The bilateral mammillary bodies are highlighted by an open oval. Their atrophy was not apparent. Bar: 1 cm. B. HE image of the liver. C. Azan Mallory staining of the liver. Neither fat deposition nor pericellular fibrosis was evident. Original magnification: x100. Bar: 200 μm.

platelet count (8 x 10^9; normal range: 140-379 x 10^9), high serum D-dimer (19.3 μg/mL; normal range: less than 1.0), high serum LDH (1552 U/L; normal range 100-210), and elevated level of soluble interleukin-2 receptor (1740 U/mL; normal range: 145-519). Thus, we concluded that the main cause of his death was DAD due to hypercytokinemia triggered by the rapid progression of EBV-positive diffuse large B-cell lymphoma.

Despite the presence of severely low folate in the serum and megaloblastic anemia in the bone marrow biopsy, pathological findings suggestive of chronic alcohol consumption were not apparent. Atrophy of mammillary bodies in the brain was not evident (**Figure 4A**), nor was fat deposition in the liver (**Figure 4B**). Mild centrilobular degeneration was observed (**Figure 4B**) but fibrosis was negligible (**Figure 4C**), which was not typical for alcoholic liver disease. Liver dysfunction was not apparent either in the serum biochemistry, as aspartate aminotransferase was 37 U/L (normal range: 8-40), alanine aminotransferase was 16 U/L (normal range: 4-45), and alkaline phosphatase was 180 U/L (normal range: 100-340). Mild diffuse fibrosis was observed in the heart (data not shown), which was consistent with but not specific for habitual alcohol consumption.

Discussion

Here, we report a case of DAD in a patient with EBV-positive diffuse large B-cell lymphoma, severely low serum folate, and megaloblastic anemia. His anemia progressed rapidly within 1 month: the level of hemoglobin in his peripheral blood had been 14.9 g/dL (normal range: 13.5-17.5) seven months before his death, 11.1 g/dL one month before his death, and it was 4.7 g/dL at death. In parallel, his platelet count in

the peripheral blood was 134 x 10^9/L (normal range: 140-379 x 10^9) seven months before his death, 84 x 10^9/L one month before his death, and it was 8 x 10^9/L at death. His serological tests did not show apparent dysfunction of the liver, which was excluded as a cause of rapid decrease in his platelet count. It is considered that the rapid progression of anemia was not solely due to bleeding from the gastric ulcer caused by infiltration of the lymphoma cells.

The possible causes of low serum folate include its low intake or absorption from alimentary tract, side effects of drugs, and endogenous consumption by tumors or during pregnancy. It was reported that the primary cause of folate deficiency is low intake of sources rich in the vitamin, such as legumes and green leafy vegetables [4]. Other situations in which the risk of folate deficiency increases include lactation and alcoholism [4].

There were multiple ulcers in the stomach formed by infiltration of lymphoma cells. However, there were no remarkable macroscopic abnormalities in the other parts of the alimentary tract. Since folate is mainly absorbed from the jejunum, severely low serum folate cannot be explained solely by its malabsorption from the alimentary tract. Despite the presence of severely low folate in the serum and megaloblastic anemia in the bone marrow biopsy, the pathological findings suggestive of chronic alcohol consumption were not apparent as described above. Therefore, low serum folate in this case is not completely explained by malnutrition due to chronic alcohol consumption. Furthermore, the patient did not take any drugs which may affect level of serum folate, including MTX and antiepileptics.

Consumption of folate by pregnancy, malignancy, and so on, is the third possible cause of low serum folate. We consider that low serum folate in this case was caused by its consumption by the rapidly progressing B-cell lymphoma through our autopsy findings. We also consider that megaloblastic anemia was caused by the folate deficiency subsequently.

From the literature, we found four case reports on concurrent lymphoma/leukemia and megaloblastic anemia. Compared with these cases, our case appears to be unique in that lymphoma is considered to cause anemia, and that the progression of the anemia was relatively rapid. Jafroodi *et al*. reported a case of Hodgkin's lymphoma during follow-up of megaloblastic anemia caused by celiac disease [5]. In this case, megaloblastic anemia was presumably due to low intake of folate caused by celiac disease. Sagristani *et al*. reported a case of malignant lymphoma in a patient who had been followed for megaloblastic anemia several years [6]. On the other hand, Saitoh *et al*. reported a case of megaloblastic anemia occurring a few years after diagnosis of malignant lymphoma [7]. Finally, Sallah *et al*. reported cases of intrathecal MTX-induced megaloblastic anemia in acute leukemia patients [8]. In this report, megaloblastic anemia was observed within a few weeks after intrathecal administration of MTX. Interestingly, in our case, anemia progressed at a similar time course (1 month), as described above.

Lowering intracellular folate by MTX is a pharmacological and biochemical basis of MTX's anti-tumor activity [9]. Therefore, low serum folate in this case might not provide an appropriate microenvironment for proliferation of the lymphoma cells. It is controversial and inconclusive whether low dietary folate or chronic alcohol consumption can be a risk factor for lymphomagenesis [10, 11]. On the other hand, lowering intracellular folate by MTX may be a basis of MTX's immunosuppressive activity as well. It has been recently more and more recognized that during treatment of rheumatoid arthritis with MTX, EBV-positive lymphoproliferative disorder can occur due to reactivation of latently-infected EBV. In this regard, it is probable that low serum folate in the present case might be involved in progression of the lymphoma through suppression of the host immunity against the lymphoma.

We conclude that in the present case, rapid progression of the lymphoma led to consumption of folate and severely low serum folate, followed by megaloblastic anemia. Rapid progression of the lymphoma also led to DAD through hypercytokinemia. Concurrence of DAD with severe anemia resulted in lethal dyspnea in the present case. A lesson from this case is that megaloblastic anemia may be caused not only by exogenous factors (for example, malnutrition) but also by endogenous factors (for example, consumption of folate by internal malignancy) subacutely within 1 month.

Acknowledgements

We thank Dr. Kayo Ueda (Yodogawa Christian Hospital) for discussion in brain pathology in the autopsy. We also thank all the colleagues in the Department of Surgical Pathology, Hyogo College of Medicine, and those in the Pathology Department, Takarazuka Municipal Hospital, for preparation of pathological specimen.

Disclosure of conflict of interest

All authors have no conflict of interest in association with this work.

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