



Fatal Epstein-Barr Virus Primo Infection in a 25-Year-Old Man Treated with Azathioprine for Crohn's Disease

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Y N'Guyen, L Andreoletti, M Patey, C Lecoq-Lafon, P Cornillet, et al.. Fatal Epstein-Barr Virus Primo Infection in a 25-Year-Old Man Treated with Azathioprine for Crohn's Disease. *Journal of Clinical Microbiology*, 2009, 47 (4), pp.1252 - 1254. <10.1128/jcm.02052-08>. <hal-03267106>

HAL Id: hal-03267106

<https://hal.science/hal-03267106v1>

Submitted on 29 Jun 2021

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HAL Authorization

Case report (1383 words)

Full title

**Fatal Epstein Barr-Virus Primo Infection in a 25 year-old man
Treated by Azathioprine for Crohn 's Disease**

Running title

Fatal EBV infection and Crohn's Disease

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Abstract (55 words):

We report a case of Epstein Barr-Virus primo infection with the development of successive infectious mononucleosis, hemophagocytic lymphohistiocytosis and B lymphoproliferative disorder in a patient treated by azathioprine for Crohn 's disease. This case-report suggests that specific EBV-related clinical and virological managements should be considered when treating a patient with inflammatory bowel disease with azathioprine.

Key-words: Epstein-Barr Virus, Primoinfection, Lymphoproliferative disorder, Azathioprine, Crohn, hemophagocytic lymphohistiocytosis.

Case report:

A 25 year-old man was admitted in the infectious diseases department of the university medical centre of Reims (France) hospital in April 2007 (day 1 of hospitalisation) for a chronic fatigue syndrome going on for 5 weeks. This patient was known to suffer from an uncomplicated Crohn's disease diagnosed 6-year ago and treated for 3 years by azathioprine. Five weeks before the time of hospitalisation, the patient had suffered from a clinical syndrome consisting of asthenia, weight loss, nocturnal sweats and sore throat. His General Practitioner (GP) established the diagnosis of infectious mononucleosis syndrome in regard of Epstein Barr virus (EBV) serological data: EBV Viral Capsid Antigen IgM and IgG: 31 and 35 IU/l respectively (N<15 IU/l) and 37 and 21 UI/l respectively one week later. Anti-Epstein Barr Nuclear Antigens (EBNA) IgG were only detected at 15UI/l (N<9 IU/l) on the second serum sample. On clinical examination, a 10 percent weight loss and a continuous fever up to 40°C were notified. An eyelid oedema, a voluminous pharyngitis, mouth mucous membrane ulcerations, a generalized lymphadenopathy and a splenomegaly were observed. The azathioprine treatment was immediately stopped. The classical laboratory findings evidenced the presence of a pancytopenia (haemoglobin 81 g/l, thrombocytes 40000/mm³ and 800 leukocytes/mm³ without atypical leukocytes or blasts. Exploration of liver functions showed the following results: Aspartate aminotransferase 294 IU/l (N<40 IU/l), Alanine aminotransferase 119 IU/l (N<40 IU/l), Lactico deshydrogenase 1111 IU/l (N: 200-450 IU/l). C reactive proteine was 216 mg/l (N<10 mg/l). Because of the presence of a pancytopenia, a bone marrow aspiration was performed on day 1 and classical cytological analysis evidenced normal cellular counts associated with pronounced macrophage hyperplasia and signs of hemophagocytosis (principally phagocytosis of platelets and erythrocytes) with the absence of abnormal cells (Figure 1). Taken together these data allowed us to establish a diagnosis of EBV-related

hemophagocytic lymphohistiocytosis (HLH). A treatment by intravenous (IV) methylprednisolone (2 mg/kg per day) in association with total human immunoglobulins (1g/kg per day during 2 days) was immediately started (day 1). Because of an apparently non-clinical response to this therapeutic management, immunoglobulins associated with acyclovir and methylprednisolone IV bolus injection (1g per day every two days during 5 days and usual 2mg/kg/day the other day) were administered on day 9. Moreover, EBV genome quantification by real time polymerase chain reaction (EBV-PCR, Argene Bosoft, Varhiles, France) displayed high levels of viral load in the peripheral blood (130,000 copies /ml of serum). On day 11, cervical lymph node biopsy was performed. Histological analysis of surgical biopsy of cervical lymph node revealed a polymorphic B cell lymphoid proliferation with extensive ischemic necrosis. The normal architecture of lymph node was effaced by an infiltration of lymphoid cells with variable size, immunoblastic and plasmocytoid features (Figure 2A) with some sternbergoid cells. The erythrophagocytosis was prominent in macrophages. The B cell origin was demonstrated by positive immunohistochemical staining for CD20 and CD79a (Figure 2B). The majority of cells were positive for EBV LMP1 antigen immunostaining and for EBV latency associated RNA (EBER) by in situ hybridization (Figure 2C). Moreover, we evidenced the presence of monoclonal IgG Kappa production in tumoral cells (Figure 2D) associated with high EBV viral load in lymph node tissues (230,000 copies/ μ g of total extracted DNA). The diagnosis of EBV- related B cell lymphoproliferative disorder was confirmed on day 14 by the demonstration of a monoclonal B cell population evidenced by PCR amplification assay of the heavy chain variable region gene CDRII and CDRIII using home-made PCR with known primers (1,17). Because of a respiratory distress resulting from upper way obstruction by giant tonsilla and oedema of the tongue basis, our patient was admitted in intensive care unit where he benefited from emergency tracheotomy and invasive mechanical ventilation assistance. A

chest X ray showed multiple bilateral alveolar opacities. Under wide spectrum antibiotherapy, polychemotherapy consisting of cyclophosphamide, doxorubicin, vincristine and prednisone associated with rituximab (R-CHOP) was administered on day 15. EBV genome quantitative detection showed a significant increase of the peripheral blood EBV DNA load levels on day 17: (253,000 copies/ml). The clinical course went on worsening with a massive upper digestive tract bleeding on day 21. Emergency gastroscopy found a pale ischemic gastric mucous membrane with numerous blood clots. Our patient died the same day of multiple organ failure. Because X-linked lymphoproliferative disorder (XLP) was suspected, DNA was retrospectively extracted from frozen lymph node tissues. Exons and intronic flanking regions of *SH2D1A*, were directly amplified using pairs of primers (primers and PCR conditions available upon request). PCR products were sequenced by dideoxynucleotide termination and the BigDye terminator kit, on an ABIPrism3130 apparatus (Applied biosystems, cCoutaboeuf, France). Sequences analysis of the PCR products was wild type for the genes predisposing to XLP. Moreover, HIV serology testing was negative. No autopsy was performed.

EBV infection is a common human gamma *herpesviridae* usually responsible for infectious mononucleosis in the general population. Moreover, EBV can induce HLH, post-tranplant lymphoproliferative disorder (PTLD) and lymphoma in immunocompromised hosts (7, 10, 14). Case reports describing HLH and/or lymphoma in patients suffering for Crohn's disease have also been published (5, 8, 13). Azathioprine and 6-mercaptopurine are the first choice therapeutic strategy for the treatment of steroid-refractory inflammatory bowel disease. These immunomodulating drugs can be associated with the development of EBV-positive HLH or lymphoma (5, 11, 15). This is the first report of a fatal EBV primo infection case associated with the development of successive hemophagocytic lymphohistiocytosis and B lymphoproliferative disorder

in an adult patient treated by Azathioprine for Crohn's Disease. In the present case, the histopathological pictures were morphologically compatible both with an EBV-associated fulminant mononucleosis and a B cell lymphoma. Despite the tumoral morphological aspect, our case should be classified as an “early“ lesion: infectious mononucleosis–like PTLN according to the WHO 2001 classification (2).

Hügler and al had already described a similar clinical history with lymphoma in a patient with an evidenced X-linked lymphoproliferative disorder (6). However in the present case, there was no familial or molecular evidence for XLP disorder. In the present report, the only explanation for this fatal EBV induced acute infection was the immunosuppressive treatment by azathioprine initiated 3 years ago. The development of lymphoproliferative disorder up to lymphoma (8, 13) and HLH (5) syndromes have been already associated with azathioprine treatment in patients with Crohn's disease but this is the first reported case of a simultaneous EBV related HLH and B lymphoproliferative disorder.

The risk of lymphoma is more elevated in patients treated by azathioprine for Crohn's disease (4, 9). However the relationship between developments of lymphoma in Crohn's disease without azathioprine remain unclear and controversial (12). Dayharsh and al (3) showed the etiological role of EBV in lymphoma in patients suffering from Crohn's disease specifically those treated by azathioprine. Azathioprine decreases significantly the T cell lymphocytes proliferation therefore preventing the immunological clearance of EBV immortalized B cell. The absence of an efficient control of EBV infection can lead to the successive or concomitant development of HLH and lymphoproliferative disorder up to lymphoma (6).

In the present report, late onset salvage cytostatic polychemotherapy did not allow saving our patient, and acyclovir regimen was useless in accordance to rare therapeutic success in previous published articles (7). Early treatments, specifically etoposide among HLH patients and

rituximab among PTLN patients, seem to improve mortality (7, 14). We can notify that increased EBV viral loads despite chemotherapy are related with a poor clinical outcome (16). Our patient died because of multiple organ failure related to an upper digestive tract haemorrhage. The explanation of this haemorrhage and the fundus aspect in gastroscopy remains unclear.

In conclusion, we report a fatal case of Epstein Barr-Virus (EBV) primo infection with the development of successive infectious mononucleosis, hemophagocytic lymphohistiocytosis and B lymphoproliferative disorder in a 25 year-old man treated by azathioprine for Crohn 's disease. This case-report suggests that specific EBV-related clinical and virological managements should be considered when treating a patient with inflammatory bowel disease with azathioprine. EBV serological assays have to be performed to obtain the EBV immunological status of steroid-refractory Crohn's disease patients before the beginning of an azathioprine treatment. Moreover in order to stop azathioprine and to quickly begin aggressive cytostatic chemotherapy and rituximab, peripheral blood viral load monitoring must be performed to diagnosed EBV induced HLH or lymphoproliferative disorder in patients treated by azathioprine for Crohn 's disease.

175 **Acknowledgments:**

176 **The authors thank Dr Vince Nicolas, MD, (EA-3963) for his help in sequencing the**
177 ***SH2D1A gene region.***

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Legends of Figures:

Figure 1: High phagocytic activity among macrophages: arrow indicate marked erythrophagocytosis.

Figure 2: Histopathological and immunohistological findings

- 2a- Infiltration of lymphoid cells with variable size, immunoblastic and plasmocytoid features
- 2b- Lymphoid proliferation (positive CD20 + staining)
- 2c- Positive EBER stained cells in the lymph node tissue by in situ hybridization
- 2d- Kappa positively stained tumoral cells (immunohistochemistry assay)