



A 20-year-old girl with an unusual febrile illness

Roberto Castoldi¹ · Francesca Bai¹ · Sabrina Caberlon² · Daris Ferrari³ · Alessia Moro⁴ · Gian Marco Podda² · Antonella d'Arminio Monforte¹ · Giulia Carla Marchetti¹

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Case presentation

Dr. Castoldi

In December 2018, more than 1 year before the spread of COVID-19 epidemic in Italy, a 20-year-old Sicilian girl, back from a study period in Spain, presented for persistent fever that lasted for over a week with temperature higher than 39 °C. In the past 3 days, she took Acetaminophen without benefit.

She had moved from Sicily to Milan when she was 10 years old; in the previous 6 months, she had lived near Bilbao where she was attending the University. She did not have pets and she did not recently travel outside Europe. She did not smoke cigarettes, drink alcohol, or use illicit drugs. She reported an unprotected sexual intercourse 1 week before the onset of the fever.

In the past, she had undergone tonsillectomy when she was 8 years old for recurrent pharyngitis; at that time, laboratory data showed Epstein–Barr virus (EBV) IgG antibody positivity, while EBV-IgM antibodies were negative.

At present, she was receiving only oral contraceptives for polycystic ovarian disease. She had no known allergies.

In the last few months, on May 2018, she has been admitted to our Department of Infectious Diseases for similar symptoms: persistent fever associated with pancytopenia,

reactive lymphocytosis, and abnormally elevated results of liver-function tests. At that time, Hepatitis A virus IgM antibodies, Hepatitis B surface antigen, Hepatitis B virus core IgM antibodies, Hepatitis C antibodies, and Human Immunodeficiency virus (HIV) antibodies were negative; the serology for Epstein–Barr virus, Cytomegalovirus, and Parvovirus B19 was compatible with a previous infection (IgG antibodies resulted positive, while IgM antibodies were negative). Blood and urine cultures were negative; since the patient had lived in Sicily and a close relative was previously diagnosed with visceral Leishmaniasis, serology, and PCR on peripheral blood were performed and were negative. The fever resolved spontaneously after 2 weeks and the patient was discharged with a diagnosis of unspecific viral infection.

Prof. Marchetti

During the first physical examination, her vital parameters were normal, except for body temperature close to 39°C and profuse sweating; the physician discovered splenomegaly and latero-cervical, axillary, and inguinal lymphadenopathy, while the remainder of the physical examination was normal. The lymph nodes were smaller than 1 cm, mobile, unpainful, non-tender, with parenchymatous consistency.

Laboratory tests showed a white blood cell count at the lower reference level (3460/uL, with 45% of lymphocytes), thrombocytopenia (platelets: 109000/uL) with a slight rise in inflammation indexes (CRP 27,4 m/L); AST/ALT rate was 118/134 U/L and lactic dehydrogenase (LDH) was 816 U/L; other laboratory test results are shown in Table 1.

The electrocardiogram showed signs suggestive of pericardial effusions and chest radiography showed pleural effusion without lung infiltrates; ultrasonography of the abdomen revealed mild hepatomegaly and an enlargement of the spleen, measuring up to 16 cm in the craniocaudal dimension with a little hyper-echogenic area at the lower pole.

After discussion with the Emergency Medicine specialist, the patient was admitted to the unit of Infectious Diseases.

✉ Giulia Carla Marchetti
giulia.marchetti@unimi.it

¹ Department of Health Sciences, Clinic of Infectious Diseases, ASST Santi Paolo E Carlo, University of Milan, via A Di Rudini, 8 20142, Milan, Italy

² Department of Health Sciences, Clinic of Internal Medicine III, ASST Santi Paolo E Carlo, University of Milan, Milan, Italy

³ Department of Medical Oncology, ASST Santi Paolo E Carlo, Milan, Italy

⁴ Division of Pathology, ASST Santi Paolo E Carlo, Milan, Italy

Table 1 Blood test results

Analytes	Unit	Range	12/28	12/30	12/31	01/02	01/04	01/06	01/09	01/11	01/13	01/15	01/21	02/01	03/18
Hemoglobin	g/dL	12—15.2	13.4	11.9	10.6	10.4	10.3	9.9	10.3	9.9	9.5	11.7	12.5	13.1	12.5
Platelets	10 ³ /uL	160—350	90	83	88	104	127	123	100	109	124	175	271	201	221
Leukocytes	10 ³ /uL	3.6—9.2	3.21	3.41	3.74	4.15	4.88	6.08	5.38	4.85	5.3	7.22	7.67	5.86	5.68
Neutrophils	%	47—68	56.1	46	35.5	30.6	24	20	14.5	18	19.8	25.2	32	45	56.4
Lymphocytes	%	27—37	34.9	44.3	56.7	63.6	71.1	76.2	79.9	76.9	73.6	66.3	55	46	33.1
ALT	U/L	9—52	134	386	425	346	278	222	208	138	117	107	85	43	31
AST	U/L	14—36	118	333	302	225	191	184	228	151	115	85		34	31
ALP	U/L	38—126			147	195	243		530			350		98	
Albumin	g/dL	3.5—5						2.8	2.75	2.9					
LDH	U/L	313—618	1316	2020	2186	2108	2145	2055	2615	2556	1570	1322	731	600	428
GGT	U/L	15—58			195		290		22.9	343		278			
CRP	mg/L	<10	44.5	44	49.7		30.2			19	9.9	5.3	<5	<5	<5
ESR	mm/h	<15								6					
Ferritin	ng/mL	7—137			1820										

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *LDH* lactate dehydrogenase, *GGT* gamma-glutamyl transferase, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate

Table 2 Epstein–Barr virus serology and plasma EBV-DNA

	Positivity	May 2018	January 2019	July 2019
EBV-IgM	> 40 UI/mL	18.1	> 160	> 160
EBV-IgG	> 20 UI/mL	23.5	101	156
EBV-DNA	> 350 copies/mL		3287	< 350

Dr. Bai

On admission, the patient complained fatigue and reduced appetite; the temperature was 39.5°C, and the pulse 105 beats per minute, blood pressure, and respiratory rate breathing ambient air were normal.

Laboratory data reported a progressive pancytopenia (anemia, leukopenia, and relative lymphocytosis), increase in aminotransferase levels, LDH, and ferritin, and a slight rise in CRP (Table 2)

The first microbiological analysis such as blood cultures, urine culture, and the rapid test for *Legionella* and *Pneumococcus* urinary antigens were also negative.

Serology for Toxoplasmosis, *S. typhi*, brucellosis, and rickettsiosis were negative.

Furthermore, we searched sexually transmitted diseases: both gynecological examination with vaginal swabs and blood tests for viral hepatitis (HAV, HBV, and HCV) and HIV (both antibodies and HIV-RNA) were negative; given the previous positivity for EBV and CMV IgG antibodies, blood CMV-DNA and EBV-DNA were measured.

Due to the persistence of fever higher than 39°C, the patient underwent a transthoracic echocardiography, which

was normal, and a total-body CT scan that revealed diffuse lymphadenopathy with nodes greater than 1 cm and a splenomegaly of 16 cm with a millimetric cystic lesion in the lower pole.

Given the negativity of the microbiological examinations, no antibiotic therapy was started at this moment.

Differential diagnoses and further investigations

Prof. d'Arminio Monforte: This 20-year-old girl, who has no clinically significant medical history, presents with the second episode of persistent fever, pancytopenia, lymphadenopathy, and splenomegaly.

The first diagnostic hypothesis was an infectious disease, but all our microbiological findings were negative for viruses or bacteria: serology analyses excluded the most common pathogens responsible for endothelial reticulum system diseases and the cultural examinations made on peripheral blood, urine, and vaginal/oral swabs were negative. Despite the reported unsafe sexual intercourse, the patient was not diagnosed with a sexually transmitted infection or a genital tract infection.

Considering the time of the year, we also searched for influenza virus, which, however, was negative.

According to the patient clinical history, we knew that the patient had had mononucleosis and CMV infection, so we looked for a reactivation of these viruses with molecular biology techniques.

Finally, we excluded “baggage malaria” given the recent travels around Europe and visceral leishmaniasis for the previous case in the patient’s family [1, 2].

At the same time, we considered alternative non-infectious hypothesis that included autoimmune diseases, late-onset metabolic or storage diseases, and oncological or hematological diseases.

Dr. Podda: The clinical case was also suggestive of an autoimmune disease, like systemic lupus erythematosus: the patient had 3 SLICC classification clinical criteria (serositis with pleural and pericardial effusions, leukopenia <4000/uL, and thrombocytopenia <100000/uL). Thus, we searched immunologic criteria but autoantibodies and the direct Coombs test were negative; furthermore, complement component C3 was found to be normal with increased C4 component.

Other autoimmune diseases were also considered, like adult-onset Still’s disease and Rheumatoid Arthritis (RA) or one of its variants: as regards adult-onset Still’s disease, we had several minor criteria (negativity for ANA and rheumatoid factor, increase in aminotransferases, splenomegaly, and enlarged lymph nodes), but just one major criterion (intermittent fever, higher than 39 °C for more than a week). RA and its variants were excluded because of the absence of arthritis symptoms and the negativity of antibodies anti-cyclic citrullinated peptide [3].

We also tested for the anti-streptolysin, anti-neutrophil antibodies, and agglutinins, but laboratory tests were inconclusive. Furthermore, paroxysmal nocturnal hemoglobinuria has been excluded.

Patients with metabolic or storage diseases may present with findings similar to those seen in this patient, but age of onset and absence of key symptoms rendered this diagnosis unlikely.

Dr. Ferrari

It was appropriate that the patient’s physicians were concerned about the possibility of an onco-hematological disease.

Clinical presentation with fever and profuse sweating, CT scan images with the evidence of splenomegaly and lymphadenopathy on both sides of the diaphragm, and laboratory findings with anemia, leukopenia, and thrombocytopenia associated with high levels of ferritin (1820 ng/mL) and a progressive increase of LDH (greater than 2000 U/L) could be consistent with a hematological disease, such as Hodgkin or non-Hodgkin lymphoma, or leukemia.

Moreover, fever could also have a paraneoplastic origin, due to the release of cytokines from a solid cancer, such as renal adenocarcinoma or hepatocellular carcinoma; however, total-body CT scan did not show densitometric alterations compatible with a solid cancer.

Based on these premises, considering the young age of the patient, we recommended positron emission tomography (PET) and the collection of histological samples to establish the diagnosis.

Prof. Marchetti

PET images showed focal fluorodeoxyglucose hyperaccumulation in the splenic tissue (maximum SUV 4.9) and in several lymph nodes (latero-cervical, parapharyngeal, axillary, and abdominal lymph nodes). The highest radiotracer accumulation was in nasopharynx (maximum SUV 9.2). The Nuclear Radiologist specialist concluded that these findings were probably compatible with a lymphoproliferative disease, but inflammatory or infectious illnesses could not be excluded.

Considering the radiotracer distribution, after collegial discussion, we decided to perform a nasopharynx and latero-cervical lymph-node biopsy.

Dr. Caberlon

The bone marrow examination was assessed to obtain a sample of bone marrow blood for culture examination considering the persistence of fever and the progressive pancytopenia of the patient. The peripheral blood smear had excluded abnormalities in the blood cells. A bone marrow examination was thus performed for microbiological as well as histological purposes. Leishmaniasis and other intracellular pathogens were excluded and the histological examination showed dyspoiesis of all cellular lines.

Chronic active Epstein–Barr Virus infection

Dr. Castoldi and Dr. Bai

Meanwhile, the fever persisted (two peaks around 40°C each day), with poor response to Acetaminophen. CBC test showed a worsening of pancytopenia: a reduction in hemoglobin (9.7 g/dL) and platelet count (lowest level reached 76000/uL) were observed in association with lymphocytosis (up to 71.1 %); other laboratory tests showed persistently abnormally elevated liver-function tests (AST/ALT ratio 302/425 U/L) and LDH (up to 2145 U/L). Therefore, on 4 January, therapy with indomethacin was started leading to a better control of fever.

After the introduction of indomethacin therapy, a slight improvement in the clinical condition of the patient was observed. At this moment, the positive result of plasma EBV-DNA was communicated (3287 copies/ml, normal value <350 copies/mL). We decided to repeat EBV serology and we found that the patient presented a higher title of IgG

antibodies compared to the previous analysis of May 2018, but also a positive title of EBV-IgM antibodies (that were negative in May 2018) (Table 2). We discussed the results with the oncologist, the hematologist, and the pathologist to carry out the differential diagnosis taking into consideration EBV-related lymphoproliferative diseases (Burkitt's lymphoma, EBV-positive Hodgkin lymphoma, low-grade B-cell lymphoma, nasopharyngeal carcinoma, NK/T lymphoma, X-linked lymphoproliferative disorders, chronic active EBV), EBV-related autoimmune diseases, and adult hemophagocytic syndrome (hemophagocytic lymphohistocytosis) [4].

Dr. Ferrari

Considering clinical presentation, the PET results, and EBV viremia, the hypothesis of EBV-related neoplasia became more consistent.

EBV is found in all cases of endemic Burkitt's lymphoma, but also in some sporadic cases. This lymphoma produces B symptoms, which were present in our patient, and, sometimes, involves the jaw or ileocecal region with a Bulky mass.

Hodgkin's lymphoma is also related to EBV infection, particularly the classical form, subtype mixed-cellularity. Our patient had many typical symptoms of this cancer, like fever, sweats, lymphadenopathy, and hepatosplenomegaly.

Our clinical case was also compatible with nasopharyngeal carcinoma, a solid cancer related to EBV and food consumption. Taking into consideration the PET results, we performed a rhino-pharyngeal biopsy to establish this diagnosis, even if this type of cancer shows a high prevalence in East Asia, and Central and South America and is a rare disease in Europe. [5]

Dr. Podda

Our patient could also be affected by hemophagocytic syndrome, an immune disorder caused by a dysfunctional natural killer and cytotoxic T-cell response, which is characterized by fever, pancytopenia, hepatosplenomegaly, and activated macrophage infiltration in hematopoietic organs [6]. This syndrome is more frequent in females (ratio male/female 1:7) and viral infections are the most common trigger, with EBV accounting for almost half of the cases. According to the diagnostic guidelines proposed by the HLH-2004 trial, a molecular diagnosis consistent with the hemophagocytic syndrome or five criteria could allow the diagnosis; our patient presented four criteria (fever, splenomegaly, pancytopenia, and increased ferritin levels). Even if there is no consensus regarding the optimal treatment for this syndrome, glucocorticoids, ciclosporin,

intravenous immunoglobulin therapy, and etoposide have been used in some cases with improved survival.

Dr. Moro

The latero-cervical lymph-node biopsy showed a morphology characterized by hyperplastic germinal centers into the cortex and an immunophenotype compatible with reactive lymphadenitis.

The nasopharynx biopsy showed adenoid tissue with maintained architecture, hyperplastic lymphoid follicles, and a blastic population. This was composed of mononuclear cells, scattered or clustered in microaggregates, with central nucleolus, predominantly immunoblastic morphology and immunophenotype CD15 -, CD3 -/+, CD30 +/-, PAX5 +/-, CD45 +, and MUM1 + (Fig. 1). A large amount of these cells was EBV/EBER-positive (Fig. 1). We also observed that immunoglobulin heavy chains had a polyclonal rearrangement pattern.

These findings were thus not suggestive of a lymphoproliferative disorder, but they seemed to be reactive to an infectious trigger.

As regards the diagnostic hypothesis of hemophagocytic syndrome, the key marker is the phagocytosis of hematopoietic cells by activated macrophages and the bone marrow is the main anatomical site to investigate the presence of this syndrome; 84% of patients diagnosed with this syndrome showed a positive bone marrow aspirate. However, our patient displayed hemophagocytosis neither in bone marrow nor in the lymph-node biopsy.

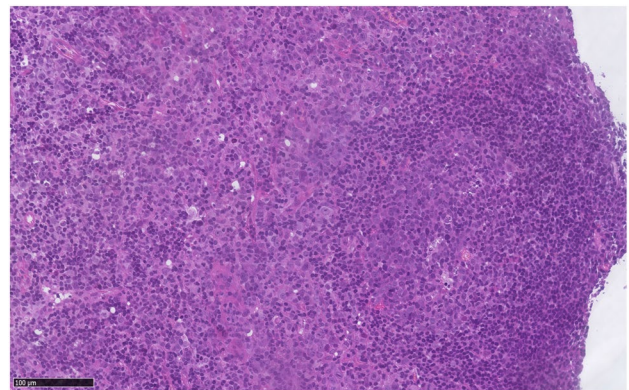


Fig. 1 Biopsy of the nasopharynx. The biopsy of the nasopharynx mucosa displayed the maintained architecture with cortical expansion and hyperplastic follicles, associated with a blastic population of large cells, scattered or in sheets, with prominent nucleoli, consistent with immunoblasts.

Prof. Marchetti

Excluding the most common EBV-related diseases, we established the diagnosis of chronic active Epstein–Barr virus disease (CAEBV), a rare disorder due to the inability of the immune system to control this pathogen. [7] CAEBV is characterized by persistent or recurrent mononucleosis-like symptoms with elevated EBV antibodies, presence of EBV-DNA, or of EBV-positive lymphocytes in histological findings; diagnosis also requires the absence of other EBV-related diseases that nevertheless can occur over time. [8–10]

Many etiopathogenetic hypotheses have been made for CAEBV, like a particularly virulent strain of EBV, a deficit in the production of EBV-related CTLs, a clonal expansion of CAEBV-lymphocytes, or a genetic predisposition, but this disease has probably a multifactorial pathogenesis. [11, 12]

Depending on disease severity, different therapeutic strategies have been described, but none of them can obtain a lasting resolution of the disease; only hematopoietic stem cell transplantation (HSTC) has been resolute in the most severe cases. [13–16]

Conclusions

Dr. Bai

Indomethacin therapy had reduced, but not resolved the febrile episodes; thus, on 9 January, we started high-dose glucocorticoid therapy for CAEBV (1 mg/Kg of prednisone). The patient's conditions got progressively better, fever and fatigue disappeared, and laboratory data improved; after 8 days of treatment, the patient was discharged and followed up at our outpatients' services. Her latest laboratory tests showed normalization of CBC and CRP with a reduction in liver-function tests and LDH (Table 1).

Plasma EBV-DNA was negative (<350 copies/mL) after 3 months. Steroid therapy was gradually tapered until its discontinuation in 3 week time.

A lymph-node and abdomen ultrasound showed a reduction of the latero-cervical lymph nodes to less than 1 cm and of splenomegaly to less than 14 cm after 5 months.

Finally, we planned for our patient a close follow-up because of the possibility of disease recurrence or progression; no disease recurrence has occurred so far and the patient is currently asymptomatic.

Prof. d'Arminio Monforte

CAEBV is a chronic multisystem disease, probably caused by a poor control over viral infections, with persistent or recurrent mononucleosis symptoms that can be particularly severe and that can last months or years. Several aspects of this disease are still unknown: the presence of a genetic predisposition or of

an underlying immune deficiency, the possibility of progression toward autoimmune diseases, hemophagocytic syndrome or lymphoproliferative diseases, and the optimal treatment [17]. Most cases of CAEBV are reported in Japan, Korea, and Taiwan, while the prevalence is lower in Latin America and rare in Western and African populations; children and adolescents are the most affected; in adults, the disease is rare, but the clinical progression could be more rapid and aggressive. The late onset, after 8 years of age, and liver impairment are the main risk factors for mortality [18].

In this clinical case, the patient had a primary EBV infection in pediatric age and two episodes of reactivation; the first had a spontaneous resolution, but was followed by an early recurrence with a more severe presentation that needed glucocorticoid therapy.

The patient had a complete recovery and suppression of peripheral EBV replication; a longer follow-up will allow us to early diagnose possible viral reactivations. The putative risk factors for EBV reactivation and which therapeutic strategies are necessary to prevent recurrences still need to be understood.

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Compliance with ethical standards

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Ethical approval We guaranteed anonymity to our patient.

Statement of human and animal rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed consent The patient provided informed consent.

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