CASE REPORT

Acquired Aplastic Anaemia Due to Epstein-barr Virus Infection Aggravated by Bacteremia: A Case Report and Review of Literature

Athirah Mohammad[1,2], Salfarina Iberahim[2,3], Aziati Azwari Annuar[2,4], Zeti Norfidiyati Salmuna[1,2]

[1] Department of Medical Microbiology & Parasitology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia
[2] Hospital Pakar Universiti Sains Malaysia, 16150, Kota Bharu, Kelantan, Malaysia
[3] Department of Haematology, School of Medical Sciences, Universiti Sains Malaysia, 16150, Kota Bharu, Kelantan
[4] Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, 16150, Kota Bharu, Kelantan

ABSTRACT

One of the infectious causes of aplastic anaemia (AA) is EBV. A comprehensive range of examinations are required to discover the causes of aplastic anaemia. We describe a case of a one-year-and-three-month-old boy who presented with pancytopenia and marrow hypocellularity due to EBV infection that maybe aggravated by bacteremia. The case was effectively treated with a combination of cyclosporine and anti-thymocyte globulin (ATG), and the diagnosis was confirmed serologically. This case is supported by a recent review of the literature.
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Keywords: EBV infection, Aplastic anaemia, Pancytopenia, Marrow hypocellularity, Bacteremia

Corresponding Author:
Zeti Norfidiyati Salmuna, MPath
Email: zetifidiyati@usm.my
Tel: +609-7676252

INTRODUCTION

As a member of the Herpesviridae family, Epstein-Barr virus is a double-stranded DNA virus. Body fluids, including saliva, blood, and semen during sexual contact, organ transplants, and inanimate things like toothbrushes or glasses used by infected individuals are all potential ways for this virus to spread. EBV infection can present as infectious mononucleosis with these symptoms: fever with rash, inflamed throat, lymphadenopathy, splenomegaly and hepatomegaly. Once infected with EBV, someone can spread the virus for weeks before the symptoms appear before it becomes latent in the host's B lymphocytes. EBV can reactivate if the immune system is weakened and cause virus spreading as well.

One of the frequent, yet poorly understood causes of acquired AA is EBV infection. There are many causes of AA such as autoimmune, chemicals or drugs induced, hereditary (i.e: Fanconi's anaemia), idiopathic, radiation, infections [ie: Sepsis, (viruses: EBV, CMV, seronegative hepatitis, HHV-6, HIV, parvovirus B19, VZV)] and miscellaneous (i.e: hypopituitarism, pregnancy, thymoma, anorexia nervosa, paroxysmal nocturnal hemoglobinuria). Diagnosis of AA is based on marrow hypocellularity with two or more cytopenias.

A T-cell-mediated autoimmune disorder of the haematopoietic system causes AA by severely depleting haematopoietic stem and progenitor cells (HSPCs). T cells are basically hyperactive and incapable of identifying autoantigens, which leads to an imbalance in the ratios of Th helper cells (Th) 1/Th2, Th17/regulatory T-cells (Treg), and CD8+ T-cell subsets cytotoxic-T (Tc) 1/Tc2 .

Patients with EBV infection typically have liver dysfunction, splenomegaly, and fever. Anaemia, thrombocytopenia, lymphadenopathy, rash, hemophagocytic syndrome, coronary artery aneurysms, hypersensitivity to mosquito bites, diseases of the central nervous system, calcification of the basal ganglia, mouth ulcers, interstitial pneumonia, and lymphoma are additional manifestations . Hematological diseases associated with EBV infection are autoimmune hemolytic anaemia, pure red cell aplasia, lymphoma, leukemia, severe aplastic anaemia, immune thrombocytopenic purpura, thrombotic thrombocytopenic purpura, hemolytic uremic syndroem and hemophagocytic lymphohistiocytosis. However, in this patient, no

evidence of hemolysis found from full blood picture. It is believed that the enhanced inflammatory response brought on by the hypersecretion of proinflammatory cytokines such IFN-g, TNF-a, IL-6, IL-10, and macrophage colony-stimulating factor is responsible for the clinical features of severe EBV infection. Meanwhile in M. pneumoniae infection, hemolytic anemia and thrombocytopenia are due to presence of cold agglutinins and cross-reactive antibodies to the platelets (1).

We present a case of acquired aplastic anaemia mainly induced by EBV infection and possibly aggravated by bacteremia, as demonstrated by positive EBV IgM in both the boy and his mother, first ever reported in Malaysia which was successfully treated with a combination of anti-thymocyte globulin (ATG) and cyclosporine.

CASE REPORT

A one-year-and-three-month-old child, was brought to the hospital complaining of persistent swelling and bruises around his forehead and periorbital area, following a history fall from steps around a week earlier. Two days before to admission, his parents also noticed paleness on both upper and lower limbs and widespread petechiae.

He looked pale and had no signs of jaundice or fever. The vital signs remained stable. An examination of the abdomen revealed hepatomegaly without splenomegaly. Multiple lymph nodes swelled over the right cervical and inguinal areas. A full blood count revealed pancytopenia with severe anaemia, thrombocytopenia and neutropenia (refer to Table II). Throughout his hospital stay, only ALP was elevated but his ALT, ALT and bilirubin level were all in normal ranges (refer to Table II).

Despite the low absolute neutrophil count, the white blood cell count (9.17 x 10^9/L) was normal. A full blood picture (FBP) showed a leucoerythroblastic blood film with circulating aberrant mononuclear cells, as illustrated in Figures 1 a, b, and c (arrow). The results of the first trephine biopsy and bone marrow aspiration were ambiguous due to poor sample quality. Flowcytometry revealed the possibility of infection with normal T, B, and natural killer cells. No overexpression of CD 8 found from flowcytometry.

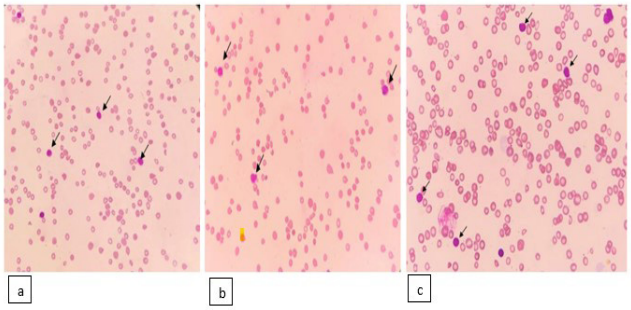

Figure 1: A full blood picture (FBP) showed a leucoerythroblastic blood film with circulating aberrant mononuclear cells.

His antinuclear antibody (ANA) test was negative upon admission, and his C3 / C4 levels were all within normal limits. EBV VCA IgM showed positive result with the titre of 1937 units/ml with second result 2 weeks apart showed the titre of 1879 units/ml. Table I summarises the results of both the patient and his mother. His hemoglobin and platelet levels improved after multiple packed cells and platelet transfused in the ward, (refer to Table II). His blood culture after day twelve of admission grew Acinetobacter baumanii, which was susceptible to gentamicin and imipenem but resistant to piperacillin-tazobactam. He had IV Amikacin and piperacillin tazobactam for four days before changed to IV imipenem for one week after receiving the blood culture result. He was discharged after 21 days in the ward. Repeat EBV VCA IgM showed positive result but with declining titre level to 622 units/ml. The chromosomal breakage test revealed no increase in spontaneous or mitomycin-C induced chromosome breakage frequencies as compared to normal controls, ruling out Fanconi's anaemia.

Table I: Patient's and his mother serological investigations

Patient's serological investigations	**Result**	
Dengue serology		
NS1 Antigen	Negative	
Dengue IgM	Negative	
Dengue IgG	Negative	
Infective screening HbS Antigen Anti-HCV Syphillis ECLIA HIV Ag/Ab combo ECLIA	All: Non-reactive	
Mycoplasma IgM	Negative	
Parvovirus B 19	IgM: negative IgG: positive	
EBV IgM (EBV-VCA: viral capsid antigen)	Positive (1st sample:1937 units/ml) Positive (2nd sample- 2 weeks apart: 1879 units/ml) Positive (3rd sample- 1 month apart: 622 units/ml)	
TORCHES (Toxoplasma, CMV, Herpes simplex I & II)	1st sample	2nd sample sent 2 weeks apart
Toxoplasma IgM	Non-reactive	Non-reactive
Toxoplasma IgG (Possible latent toxoplasma infection in view of no evidence of seroconversion or 4-fold rise in toxoplasma IgG titre)	Reactive (84.37 IU/mL)	Reactive (84.37 IU/mL) Non-reactive Reactive (116.8 U/mL)
CMV IgM	Non-reactive	
CMV IgG (Possible latent CMV infection in view of no evidence of seroconversion or 4-fold rise in toxoplasma IgG titre)	Reactive (99.83 U/mL)	Reactive Non-reactive
HSV I IgG	Reactive	
HSV II IgG	Non-reactive	
Mother's serological investigations	**Result**	
TORCHES (Toxoplasma, CMV, Herpes simplex I & II)		
Toxoplasma IgM	Non-reactive	
Toxoplasma IgG	Non-reactive	
CMV IgM	Non-reactive	
CMV IgG	Reactive	
HSV I IgG	Reactive	
HSV II IgG	Non-reactive	
EBV IgM (EBV-VCA: viral capsid antigen)	Positive	

Table II: Biochemical and hematological parameters

	Admission from 2nd April 2021 to 23rd April 2021												Admission from 5th July 2021 to 19th July 2021					
Date	**4/4**	**5/4**	**9/4**	**11/4**	**13/4**	**14/4**	**15/4**	**16/4**	**17/4**	**20/4**	**22/4**	**23/4**	**5/7**	**7/7**	**8/7**	**9/7**	**14/7**	**17/7**
	Full blood count (FBC) parameters																	
WBC	9.13	7.36	4.08	6.3	6.4	3.21	5.57	5.17	4.71	5.54	6.17	5.43	3.26	-	6.06	7.66	9.32	6.15
Hb	3.8	5.8	8.6	8.4	8.2	6.7	10.2	9.3	8.9	9.2	9.3	8.3	6.5	-	13.7	10.8	8.1	9.9
MCV	79.1	80.6	82	81.6	82.5	82.4	82.4	83.1	82.7	81.4	83.8	82.2	73	-	75.2	73.6	76.6	79.3
MCH	28.4	26.7	27.7	28.1	28.2	28.2	29	28.5	29	28	27.9	28.4	26.2	-	26.5	26	26	27.7
Hct	10.6	17.5	25.5	24.4	24	19.6	29	27.1	25.4	26.7	27.9	24	18.1	-	38.9	30.6	23.9	28.3
Plt	2	108	80	4	23	2	65	37	24	22	4	101	2	-	13	41	4	5
Neutrophil #	0.57	0.33	0.15	0.12	0.27	0.16	0.00	0.00	0.01	0.03	0.03	0.04	0.10	-	0.09	0.06	0.11	0.05
Reticulocyte #	-	2.45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Full blood picture (FBP) report: 4/4/24																	
Morphology description: Red blood cell: Anemia. Normochromic normocytic red cells, some hypochromic microcytic. Anisopoikilocytosis. Presence of nucleated red blood cells. White blood cell: Neutropenia. Toxic changes of neutrophils. Presence of two populations of lymphocytes, some lymphocytes show moderate in size. Platelet: Thrombocytopenia. No platelet clumping. Interpretation: suggest for BMAT, Immunophenotyping, Molecular and cytogenetic study.																		
	BMAT, cytogenetic																	
BMA	4/4/2021: This BMA is inconclusive due to suboptimal sample												7/7/2021: Adequate fragments were available for interpretation. Hypocellular marrow suggestive of aplastic anemia. However, need to exclude secondary causes of hypocellular marrow (ie: infection, drugs induced, autoimmune disease etc). To correlate with other clinical and laboratory findings.					
Trephine	Hematopoiesis: Very few heterogeneous population of hematopoietic cells seen. Scattered erythroid and granulocytic precursors present. Suboptimal sample, inconclusive for interpretation.												- Cellularity: Hypocellular marrow that consisted of 10% cells and 90% fat with peripheral blood contamination and crushed. - Hematopoiesis: All hematopoietic cells were suppressed. Erythroid precursors were markedly reducedand not in colony with evidence of negative glycophorin A. granulocytic series were reduced. (MPO positive for histiocytes only). No obvious megakaryocytes seen evidenced by negative CD61. Blast cells were not increased. - Lymphopoiesis: There were predominantly lymphocytes with T>B cells, evidenced by positive CD3 and CD20. - Others : Scattered plasma cells with few histiocytes and dendritic cells evidenced by CD68 positivity. Hypoplastic marrow compatible with aplastic anaemia					
Cytogenetics	Normal												Normal					
	4/4	5/4	9/4	11/4	13/4	14/4	15/4	16/4	17/4	18/4	21/4	24/4	5/7	6/7	8/7	9/7	14/7	17/7
CRP	-ve	-	-	-	-	92	-	-	28	55	43	29	-	69	73	-	-	-

Table II: Biochemical and hematological parameters (CONT.)

	Admission from 2nd April 2021 to 23rd April 2021								Liver function test (LFT)			Admission from 5th July 2021 to 19th July 2021				
Date	4/4	5/4	9/4	11/4	13/4	14/4	15/4	16/4	17/4	18/4	21/4	23/4	5/7	6/7	8/7	9/7	15/7	17/7
AST	-	28	-	23	-	14	16	-	-	-	-	-	14	-	-	-	20	18
ALT	-	11	-	11	-	9	12	-	-	-	-	-	7	-	-	-	14	8
ALP	-	192	-	194	-	154	167	-	-	-	-	-	174	-	-	-	162	165
LDH	-	-	-	-	-	-	317	-	-	-	-	-	-	-	-	-	-	-
TB	-	9	-	7	-	8	3	-	-	-	-	-	17	-	-	-	9	8

WBC: white blood cell count (N: 3.8-9.7 X 10^9/L)
Hb: Hemoglobin (N: 13.5-17.4 g/dL)
MCV: Mean corpuscular volume (N:78.9-95.7 fl)
MCH: Mean corpuscular hemoglobin (N: 25.4-31.1 pg)
Hct: Hematocrit (N: 37.5-49.8%)
Plt: Platelet (N: 167-376 X 10^9/L)
Neutrophil # (N: 1.58-5.94 X 10^9/L)
Reticuloyte (N: 0.46-1.34%)
BMAT: bone marrow aspiration and trephine biopsy
CRP: C-reactive proteins (N: <10mg/L)
AST: Aspartate transaminase (N: 5-34 U/L)
ALT: Alanine transaminase (N: <45 U/L)
ALP: Alkaline phosphatase (N: 53-128 U/L)
LDH: Lactate dehydrogenase (N: 120-300 U/L)
TB: Total bilirubin (N: 3.4- 17.1µmol/L)

A repeat FBP at 3 months showed pancytopenia without blast cells, and a repeat BMAT indicated hypocellular marrow, confirming aplastic anemia. HLA typing found no matches. The patient had multiple admissions for anemia and thrombocytopenia, requiring numerous transfusions. He received IV ATG and started on Cyclosporine, with the dosage increased and monitored through TDM.

DISCUSSION

Epstein-Barr virus (EBV) is a human herpes virus that can cause lytic or latent infections, primarily affecting B cells. It often infects children from lower socio-economic backgrounds with poor hygiene, typically without symptoms. In adolescents or adults, it may cause infectious mononucleosis. EBV initially infects epithelial cells, then naıve B cells, which become activated and proliferate. In persistent infections, memory B cells can carry the virus, which can be spread through saliva.

EBV infection can cause acquired aplastic anemia. In this patient, tests for HIV, hepatitis B and C, parvovirus B19, and Mycoplasma were negative. TORCHES screening indicated possible latent CMV or toxoplasma infection without significant changes in IgG titres or seroconversion. No CMV DNA nucleic acid testing sent for this patient. Although this patient and his mother tested positive with EBV VCA IgM, a genetic aetiology cannot be ruled out.

To diagnose EBV infection, antibodies to viral capsid antigen (VCA), early antigen (EA), EBV nuclear antigen (EBNA), and the monospot test are used as reported by CDC in 2020. The monospot test is unreliable for children under four due to its low sensitivity and negative predictive value. Anti-EA IgG is not specific, being present in 20% of healthy individuals. Antibodies to EBNA only appear two to four months after symptoms start. As a result, in this patient, anti-VCA IgM, which emerges early in EBV infection and was also positive in his mother, is used for diagnosis. During an EBV acute phase infection, anti-VCA IgM will first appear. It will peak two to four weeks after infection and last for 4 to 8 weeks before anti-VCA IgG, which will last a lifetime, replaces it. . In comparison to nucleic acid amplification testing (NAAT), antibody detection, namely anti-VCA IgM, is superior in EBV diagnosis. In this patient, no EBV DNA nucleic acid testing was sent.

The diagnosis of Fanconi's anaemia relies either on chromosomal breakage test by using mitomycin C or diepoxybutane to stimulate DNA breakage in absence of DNA repair system or cell cycle analysis flowcytometry. Fanconi's anaemia was ruled out because there was no evidence of mitomycin-C-induced chromosomal breakage in this patient when compared to normal control.

The patient received piperacillin tazobactam for bacteremia for only a few days. Prolonged use of this antibiotic (over 10 days) can cause reversible myelosuppression (2), but bacteremia itself might also contribute to myelosuppression.

Neutropenia is defined as an ANC < 1,000/mL. This patient had severe anemia, thrombocytopenia, low ANC, and hypocellular marrow, confirming aplastic anemia. By referring to Table III, two out of three cases of EBV associated with AA presented with hepatitis evidenced by liver enzymes derangement, but not for this patient. EBV infections not associated with hepatitis like in this patient can happen like in previous case report (4).

Table III : Recent cases of acquired aplastic anaemia secondary to EBV infection

	Authors	Journal	Year	Age	Sex	Hematological profiles	Liver function test	Other infectious screening	Markers for diagnosis of EBV infection	Treatment	Status
1	Khan et al.(3)	Journal of Blood disorders and Transfusion	2013	3	Female	Pancytopenia; no BMAT done	Result: not available	Parvo virus B19 DNA negative; CMV IgM and IgG: negative	EBV- VCA IgM (-);EBV- VCA IgG (+); EBV EBNA IgG: titre >750; EBV EA IgG >150; EBV DNA viral load : 8613 copies/ml.	Prednisolone Acyclovir Anti-thymocyte globulin (ATG) followed by cyclosporine	Alive
2	Wasekar et al.(5)	Journal of Experimental and clinical Medicine	2020	49	Female	Pancytopenia; BMAT: hypocellular marrow with relative lymphocytosis	Elevated liver enzymes with indirect hyperbilirubinemia	CMV and parvovirus B19 screening not done: financial constraint	EBV DNA viral load: 2250 copies/µl	Eltrombopag, anti-thymocyte globulin (ATG) followed by cyclosporine and intravenous immunoglobulin (IVIG)	Alive
3	Zhang WJ et al.(4)	World Journal of Clinical Cases	2022	30	Male	Pancytopenia; BMAT: hyperplastic marrow with 70% granulocytes and 16% erythrocytes	Elevated liver enzymes and bilirubin level	CMV DNA: negative. HBV DNA: below detection limit	EBV DNA viral load : 1.91 x 10^4 copies/ml.	Eltrombopag, anti-thymocyte globulin (ATG) followed by cyclosporine	Alive

BMAT: bone marrow aspiration and trephine

Malaysia has not previously reported a case of acquired aplastic anemia caused by EBV infection. However, other case reports have documented such cases (3-5). ATG combined with cyclosporine is recommended for treatment, as it has been widely used since 1984 to improve survival in severe aplastic anemia. Patients unresponsive to immunosuppressive treatment may benefit from eltrombopag, an oral thrombopoietin-receptor agonist (4,5).

CONCLUSION

This patient is a case of acquired aplastic anaemia mainly induced by EBV infection and possibly aggravated by bacteremia that was successfully treated with an anti-thymocyte globulin and cyclosporine combination.

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