Journal of Clinical Virology 56 (2013) 312–315

Contents lists available at SciVerse ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Antiphospholipid antibodies during infectious mononucleosis and their long term clinical significance

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ARTICLE INFO

Article history:
Received 28 September 2012
Received in revised form 5 December 2012
Accepted 10 December 2012

Keywords:
Epstein-Barr virus
Antiphospholipid
Antibodies

ABSTRACT

Background: The prevalence of antiphospholipid antibodies (aPLs) during acute Epstein-Barr virus (EBV) infection may be as high as 30–60%. The role of these autoantibodies in the development of antiphospholipid syndrome (APS) is not clear.
Objective: To investigate the prevalence, persistence and clinical significance of aPLs in a series of patients diagnosed with acute EBV infection.
Study design: A cohort of 94 patients aged 15 or older, recently diagnosed with acute EBV was retrieved. Serum samples obtained during diagnosis were tested for the presence of aPLs and anti-β_2GP antibodies. Patients with positive sera for aPLs were assessed for the persistence of aPLs and the development of APS.
Results: The prevalence of aPLs among 94 patients with acute EBV was 37.2%. Five of 27 available serum samples were also positive for anti-β_2 glycoprotein (anti-β_2GP) antibodies. Repeat testing for aPLs after a median of 21 months post acute infection (range 13–50 months) was performed in 17 of the 35 patients with positive aPL test. All 17 patients were found negative for aPL-IgG antibodies. Two of them had positive aPL-IgM antibodies and positive anti-β_2GP antibodies. None of the patients who had positive aPLs experienced any manifestations of APS.
Conclusion: The disappearance of aPLs in the majority of the patients after acute EBV infection, along with the absence of consistent clinical findings, suggests that the detection of aPLs during acute EBV is not associated with the development APS over time.

1. Background

A 17-year-old female patient was admitted to our hospital due to fever, diffuse lymphadenopathy and jaundice. A diagnosis of acute Epstein-Barr virus (EBV) infection was made. During admission, the patient described mild pain in her left calf. Deep vein thrombosis (DVT) was clinically suspected. As part of a thrombophilia workup, the presence of antiphospholipid antibodies (aPLs) was tested (by request of a vigorous resident). Eventually, DVT was ruled out by Doppler ultrasonography, however the test for aPLs was positive. The patient had neither history nor clinical symptoms consistent with antiphospholipid syndrome (APS). Repeated testing at least 12 weeks apart of the first test was recommended. The patient planned a long distance flight a week after discharge and was concerned regarding the potential risk for thrombotic complications, carrying these antibodies.

The relationship between infections, specifically viral infections, and the emergence of autoimmune processes had been previously reported. Barzilai et al. reviewed the association of previous EBV or cytomegalovirus (CMV) infection with multiple autoimmune diseases including systemic lupus erythematosus (SLE), rheumatoid arthritis, multiple sclerosis, polymyositis and APS.[1]

APS (Hughes syndrome) was described in 1983 and was originally characterized by the presence of circulating aPLs, as well as thrombosis, repeated miscarriage and, occasionally, thrombocytopenia.[2] A review of the literature reveals numerous reports showing raised serum levels of aPLs in HIV, CMV, hepatitis B and C and other viral infections.[3–6] Sorice et al. demonstrated the presence of aPLs in 14 of 46 patients with infectious mononucleosis (IM),[7] nine of them had an acute EBV infection.

Yamazaki et al. described a 25-year-old woman who presented with an episode of left calf DVT and pulmonary embolism. She had antibodies for EBV, indicative of primary infection, and aPLs. Six

Abbreviations: aPLs, antiphopholipid antibodies; anti-β_2GP, anti-β_2 glycoprotein; APS, antiphospholipid syndrome; EBV, Epstein-Barr virus; CMV, cytomegalovirus; IM, infectious mononucleosis; VCA, viral capsid antigen; EBNA, EBV nuclear antigen; SLE, systemic lupus erythematosus; DVT, deep vein thrombosis; ELISA, enzyme-linked immunosorbent assay.

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http://dx.doi.org/10.1016/j.jcv.2012.12.011

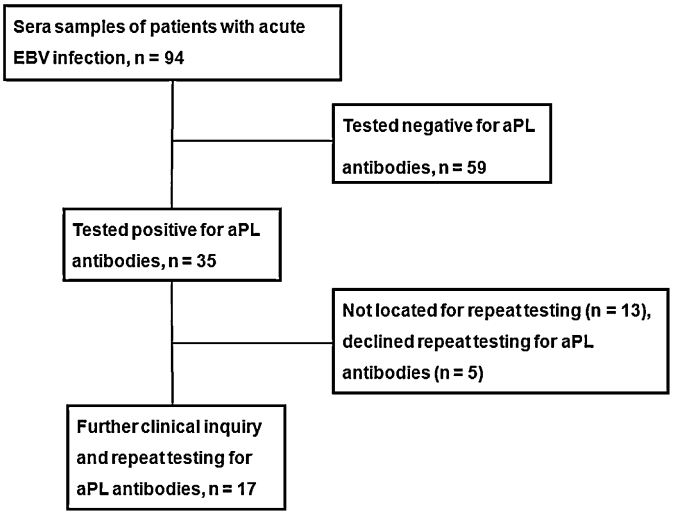

Fig. 1. Flow chart of study cohort.

months later, aPLs became negative, and EBV antibodies seroconversion was demonstrated.[8] Splenic infarction during acute EBV infection has been reported in association with aPLs in at least three cases.[9–11] In one patient, aPLs disappeared six months after acute infection without sequelae,[9] but in the other, aPLs, specifically anti-β_2 glycoprotein (β_2GP) antibodies, persisted on repeat testing four months post infection.[11]

The case described above provided the incentive for conducting this observational study.

2. Objectives

The aim of this study was to investigate the prevalence, persistence and clinical significance of aPLs in a series of patients diagnosed with acute EBV infection.

3. Study design

3.1. Prevalence of aPLs in acute EBV patients

A retrospective cohort of 94 patients aged 15 or older, diagnosed with acute EBV during 2007–2011 was retrieved from the archives of the two major hospitals in Jerusalem. Acute EBV infection was indicated if EBV viral capsid antigen (VCA) IgM and/or IgG was positive and EBV nuclear antigen (EBNA) IgG was negative, along with a clinical diagnosis of IM. IgG EBNA positive patients were excluded. Sera samples collected during diagnosis and stored at −20 °C were tested for aPLs using enzyme-linked immunosorbent assay (ELISA) (BL-Diagnostika GmbH, Germany). Cut-off values for positivity of aPL-IgG and IgM were above 10 U/ml and 7 U/ml, respectively. Positive sera for aPLs were further tested for anti-β_2 glycoprotein (anti-β_2GP) IgG and IgM antibodies (cut-off value for both antibodies was above 8 U/ml).

3.2. Persistence of aPLs after acute EBV

The persistence of aPLs was assessed at least 12 months after acute EBV infection. Blood samples were drawn and re-tested for the presence of aPLs after obtaining informed consent. Both samples (at diagnosis of acute EBV and repeated tests at least a year later) were tested in one laboratory by the same technician using the same method and kits. A flow-chart of the study cohort is presented in Fig. 1.

Patients were asked about any clinical manifestations of APS (i.e. thrombotic events, abortions). All samples were held at Hadassah-Hebrew University Hospital and at Shaare-Zedek Medical Center Laboratories, both in Jerusalem. Immunological assays were all performed at the Hadassah-Hebrew University Hospital Clinical Immunology Laboratory.

4. Results

4.1. Prevalence of aPLs in acute EBV patients

Ninety four sera samples of patients with acute EBV infection were collected and screened for aPL antibodies. 45.7% of the patients were females. The mean age was 26 years (median 23, range 15–64). The prevalence of aPLs was 37.2% (95% CI 27.5–47.8%). No age or gender differences were observed between aPL-positive and aPL-negative patients.

The majority of patients (33/35, 94.3%) tested positive for aPL-IgM (>7 U/ml). Two patients tested positive for IgG only (>10 U/ml) and six patients tested positive for both IgG and IgM.

Twenty seven sera samples (27/35, 77.1%) were available for further testing of anti-β_2GP antibodies. Median titers of IgG and IgM antibodies were 3.43 U/ml (range 1.2–12.5) and 4.6 U/ml (range 0.4–53.5), respectively. Five sera samples were positive for anti-β_2GP antibodies. Four of them were IgM positive (mean titer 16.8 U/ml, standard deviation 8.3) and one had both IgM and IgG antibodies positive (titers were 53.5 U/ml and 12.5 U/ml, respectively).

4.2. Persistence of aPLs after acute EBV infection

During February 2012, repeat testing for aPLs was performed. Seventeen patients (of the 35 patients initially tested positive for aPLs) agreed to be retested, after a median of 21 months (range 13–50) post acute EBV infection. Thirteen patients were not located and five declined repeat testing (see Fig. 1). Eight subjects were female (8/17, 47%). The median age was 25 (range 17–64) (Table 1).

All 17 patients were found negative for aPL-IgG antibodies after repeat testing. Two of the patients had positive aPL-IgM antibodies (patients 8 and 15). The sera of these two healthy subjects were further tested for anti-β_2GP antibodies and were found to be positive. Patient 8 had an IgM titer of 11.5 U/ml. Patient 15 had an IgM titer of 18.9 U/ml. IgG titers were normal.

None of the patients with aPLs positive serum, had experienced any manifestations of APS. Specifically, neither abortions nor thrombotic events were reported.

5. Discussion

The prevalence of IgG-aPLs and IgM-aPLs, in healthy subjects may be as high as 6.5% and 9.4%, respectively.[12] The prevalence of aPLs during acute EBV infection is higher, and range between 30 and 62%.[7,13] In our cohort, it reached 37.2% of the tested samples (35/94). Twenty years ago, Misra et al. suggested that the generation of aPLs in IM was attributed to the appearance of antigenic epitopes on EBV-transformed lymphocytes.[14] The mechanism of molecular mimicry between viral/bacterial peptides and self antigens had been widely proposed as an explanation for the development of these autoantibodies.[15–17] Lieby et al. suggested that during primary IM, EBV infected and expanded a pre-existing memory B cells, among them a discrete pool of aPLs memory cells able to produce mutated forms of antibodies.[18]

Although previous reports have demonstrated the occurrence of aPLs during acute EBV infection, only few have assessed the persistence of those antibodies.[9,10]