



## Multiple Interferences of Serologic Studies in an Epstein–Barr Virus-Related Infectious Mononucleosis

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Epstein–Barr virus (EBV) infection is a common infectious disease in young adults and children. A 4-year-old boy was presented with enlarged tonsils and purulent discharge. Sudden airway collapse and desaturation were noted, and endotracheal tube intubation was performed. The laboratory data demonstrated positive results for EBV immunoglobulin M (IgM). Polyclonal B-cell activation that led to positive results for cytomegalovirus (CMV) IgM and herpes simplex virus (HSV) IgM was also detected by the interference of heterophilic antibodies. A low-dose intravenous immunoglobulin (IVIG) infusion was performed, and an IVIG-related false-positive result for CMV immunoglobulin G (IgG) was observed. After the aggressive medical care, the boy was discharged under a stable condition. Herein, we report the important features of EBV-related infectious mononucleosis that harvested multiple interferences. First, the heterophilic antibodies caused due to polyclonal B-cell activation resulted in the false-positive result for CMV IgM and HSV IgM. Second, the IVIG interpreted the CMV IgG. We must be aware of the multiple interferences that could misinterpret our laboratory data.

**Key words:** Cytomegalovirus, Epstein–Barr virus, herpes simplex virus, heterophilic antibody, intravenous immunoglobulin

### INTRODUCTION

Epstein–Barr virus (EBV) infection is a common infectious disease in young adults and children. EBV, also known as human herpesvirus 4 (HHV-4), is a ubiquitous double-stranded DNA virus that belongs to the  $\gamma$ -herpesvirus subgroup of the HHV family. Approximately 95% of the world's population would have been infected during their childhood.<sup>1</sup> Only when the immune status is weak, the virus undergoes replication cycles and causes the disease. The most well-known disease related to EBV infection is infectious mononucleosis (IM), with the clinical manifestations comprising fever, acute tonsillitis with purulent discharge, and hepatosplenomegaly.<sup>2</sup> The laboratory tests indicate lymphocytosis with >50% lymphocytes in

the total leukocyte population and presenting with atypical lymphocytes in >10% of the lymphocytes.<sup>3</sup> We report important laboratory results of IM that harvested multiple interferences. All the interferences, of which we must be aware, may interpret our clinical judgment.

### CASE REPORT

A 4-year-old boy, who had a history of sleep apnea, suffered from a runny nose for 2 weeks. He was brought to our pediatric emergency department-emergency department (PED-ED) due to shortness of breath. Physical examination revealed enlarged bilateral tonsils with purulent discharge. Laboratory examinations indicated leukocytosis with a lymphocyte

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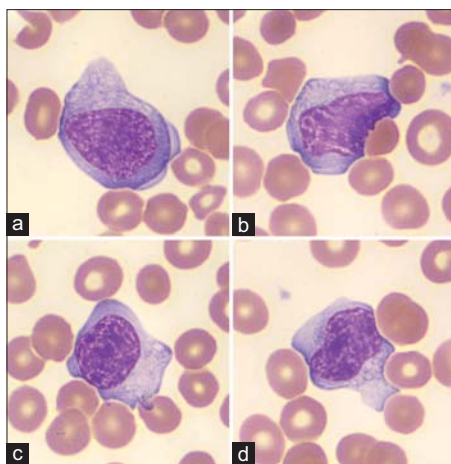
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proportion of up to 48.2% [Table 1]. The results of the influenza rapid test, Group A streptococcus antigen test, and throat culture were negative. In addition, the boy's shortness of breath aggravated with stridor 1 day later. Therefore, he was again brought to the PED-ED. Plain film of the neck soft tissue revealed no thumb sign of acute epiglottitis. However, there was a sudden drop in SpO<sub>2</sub> (lowest 77%) during the treatment with a bronchodilator, after which emergent intubation was performed. The patient was then admitted to the PED intensive care unit. The initial laboratory tests revealed positive results for herpes simplex virus (HSV) immunoglobulin M (IgM), EBV IgM, and cytomegalovirus (CMV) IgM and negative results for CMV immunoglobulin G (IgG) [Table 2]. Peripheral blood smear examination demonstrated atypical lymphocytes [Figure 1]. Abdominal sonography revealed hepatosplenomegaly [Figure 2]. Due to persisting sepsis, intravenous immunoglobulin (IVIG) infusion (1 g/kg, totally 17 g/day and dripped slowly for more than 10 h/day) was prescribed for 2 days. Extubation was performed after the IVIG infusion, and then the boy was transferred to the ordinary ward. However, he suffered a fever episode again 1 day later. Repeat laboratory evaluations showed positive results for EBV IgG and CMV IgG [Table 2]. After treatment, the patient was discharged under a stable condition 4 days later.

However, the rate of coinfection of the three different viruses has been reported to be very low (<2.5%).<sup>4</sup> We performed a polymerase chain reaction (PCR) of the three viruses from the patient's plasma, and only one showed positive results for EBV (23,128 copies/mL). The results for the other two viral IgM were false positive.



**Figure 1:** (a-d) Atypical lymphocytes in the peripheral blood smear. They showed blue cytoplasm with irregular cytoplasmic membrane and nuclear membrane. Clumping of the chromatin was also seen

## DISCUSSION

Our case exhibited a typical presentation of IM. However, elevated levels of EBV IgM, CMV IgM, and HSV IgM were observed at the same time. After treatment, the CMV IgM subsided and changed into the elevation of CMV IgG. PCR of the three viruses demonstrated positive results only for

**Table 1:** Initial complete blood count data

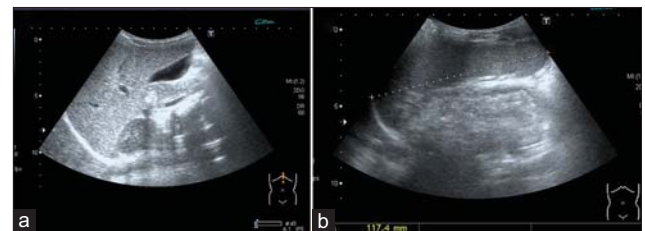
| Items          | Results                   |
|----------------|---------------------------|
| WBC            | 18.05×10 <sup>3</sup> /μL |
| RBC            | 5.02×10 <sup>6</sup> /μL  |
| Hb             | 14 g/dL                   |
| HCT            | 39.1                      |
| MCV            | 77.9 fL                   |
| MCH            | 27.9 pg                   |
| MCHC           | 35.8 g/dL                 |
| Platelet       | 171×10 <sup>3</sup> /μL   |
| Neutrophil (%) | 47.8                      |
| Lymphocyte (%) | 48.2                      |
| Monocyte (%)   | 3.5                       |
| Eosinophil (%) | 0.3                       |
| Basophil (%)   | 0.2                       |

WBC=White blood cells; RBC=Red blood cell; Hb=Hemoglobin; HCT=Hematocrit; MCV=Mean corpuscular volume; MCH=Mean corpuscular Hb; MCHC=Mean corpuscular Hb concentration

**Table 2:** The viral serology results of serum samples from the initial to the following date

| Date test item | October 26, 2018 (initial) | November 6, 2018 (following) | Unit  | Reference range |
|----------------|----------------------------|------------------------------|-------|-----------------|
| HSV IgG        | 3.04                       | Not performed                | Ratio | <1.00           |
| HSV IgM        | 1.92                       | Not performed                | Ratio | <1.00           |
| EBV IgG        | 6.15                       | 49.92                        | S/CO  | <1.00           |
| EBV IgM        | 2.54                       | 0.90 (equivocal)             | S/CO  | <1.00           |
| CMV IgG        | 1.0                        | 139.9                        | AU/mL | <6.00           |
| CMV IgM        | 1.4                        | 0.56                         | S/CO  | <0.85           |

HSV=Herpes simplex virus; EBV=Epstein-Barr virus; CMV=Cytomegalovirus



**Figure 2:** Abdominal sonography studies. (a) Enlarged liver size. (b) Enlarged spleen size (Max size: 117.4 mm)

EBV. However, no published studies have investigated the clinical significance of triple positivity of EBV IgM, CMV IgM, and HSV IgM antibodies in a primary EBV infection. Alternately, only a few case reports have discussed about the dual positivity of EBV IgM and CMV IgM and suggested that the cross reaction of serum antibodies is a possible mechanism in children with a primary EBV infection.<sup>5,6</sup> A recent study reported that 40 (26.8%) among 149 children with a primary EBV infection exhibited serum EBV IgM and CMV IgM dual positivity, but a true CMV infection was confirmed only in one child (2.5%) with a positive CMV PCR result.<sup>4</sup> If we are not aware of these interferences, we may prescribe acyclovir for HSV and ganciclovir for CMV that may do more harm to the patient, such as causing diarrhea, nausea, or vomiting. Our laboratory studies were under multiple interferences, and we have discussed them in four parts.

#### **Initial herpes simplex virus immunoglobulin M (Anti-herpes simplex virus-1/2-pool ELISA, EUROIMMUN)**

The initial value of HSV IgM was 1.92, which indicated HSV infection in theory. However, there was no evidence of HSV infection in the patient's gingiva or body. EBV is a B-lymphocyte mitogen that induces several different types of B lymphocytes to produce various classes of antibodies. Besides the specific anti-EBV response in IM, several strong antibodies are found, most of which are IgM.<sup>7</sup> This condition has been termed as the so-called polyclonal B-cell activation.<sup>8</sup> Some of the various immunoglobulins were IgM formed against HSV and detected by our laboratory test that led to the false-positive result for HSV IgM.

#### **Initial herpes simplex virus immunoglobulin G (Anti-herpes simplex virus-1/2-pool ELISA, EUROIMMUN)**

As HSV infections are extremely common in the Taiwan population, it was suspected that the findings could have been due to previously acquired immune responses.

#### **Initial cytomegalovirus immunoglobulin M (ARCHITECT i1000<sub>SR</sub><sup>TM</sup>, ABBOTT)**

First, like the polyclonal B-cell activation, some of the IgM forms would be detected. Second, according to the package insert of the CMV reagent, the heterophilic antibodies would interfere the data. Reviewing the life cycle of EBV virus, when the time of decreasing EBV IgM crossing with elevating EBV IgG, there was a production of the heterophilic antibodies.<sup>9</sup> This is because after being infected with EBV virus, some plasma cells are induced to multiply and produce immunoglobulins,

including IgM. These IgM antibodies do not react with EBV-specific antigens but recognize antigenic determinants on sheep, horse, and cattle erythrocytes. These IgM antibodies are the so-called heterophilic antibodies because they react with antigens other than EBV viral proteins. In addition, the heterophilic antibodies could cross react with HSV and CMV viral antigens on the microparticle of the reagents in the Abbott architect system.<sup>10</sup> We rechecked the CMV IgM using the DiaSorin XL system, and the result showed 20.8 U/mL that was within the borderline range of ~18–22 U/mL. This result demonstrated that CMV IgM levels are elevated by heterophilic antibodies as confirmed by the two detection systems, i.e., Abbott and DiaSorin.

#### **Following cytomegalovirus immunoglobulin G and Epstein–Barr virus immunoglobulin G (ARCHITECT i1000<sub>SR</sub><sup>TM</sup>, ABBOTT)**

Based on the cross reaction from the acute phase of EBV IgM to EBV IgG, the elevated level of EBV IgG could be explained. However, why did the level of CMV IgG also elevate? Tracing back to the patient's history, IVIG infusion was performed. The IVIG was a passive immune preparation containing structurally and functionally intact immunoglobulin molecules with a normal proportion of IgG subclasses. Most of them were isolated from pooled human plasma (1000–10,000 donors).<sup>11</sup> Hence, various classes of the IgG form were contained in the IVIG product, and parts of them containing EBV IgG and CMV IgG. According to a previous study, these antibodies could be detected just like endogenous antibodies.<sup>12</sup>

In conclusion, we must be aware of the multiple interferences of EBV infection in the diagnostic methodology, such as polyclonal B-cell activation that could produce several immune responses, especially IgM antibodies. Furthermore, heterophilic antibodies could interfere CMV IgM. Moreover, IVIG infusion could elevate the levels of IgG. Our case had multiple interferences that we should be aware of and should not make incorrect diagnoses.

#### **Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understand that his name and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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