Clinical characteristics and laboratory findings in Danish children hospitalized with primary Epstein-Barr virus infection

SOFIE KATHRINE TOPP¹, VIBEKE ROSENFELDT¹†, HANNE VESTERGAARD², CLAUS BOHN CHRISTIANSEN³ & MARIE-LOUISE VON LINSTOW¹

From the ¹Department of Paediatrics, Hvidovre University Hospital, ²Statens Serum Institut, Department of Microbiological Diagnostics and Virology, Division of Diagnostics and Infection Control, and ³Department of Clinical Microbiology, Rigshospitalet, State University Hospital, Copenhagen, Denmark

Abstract

Background: Epstein-Barr virus (EBV) positive infectious mononucleosis (IM) is a common disease in adolescents. However, IM is often considered a rare disease in early childhood. We aimed to describe the classical presentation of adolescent EBV-associated IM compared to EBV infection at younger age. Methods: All immunocompetent children hospitalized at Hvidovre University Hospital, Copenhagen between 2002 and 2013, who presented with clinical features that prompted a laboratory test for EBV, and who tested positive by presence of EBV-specific antibodies, heterophile antibodies or a positive EBV PCR were included (n = 95). Results: Children aged 1–2 years were the age group most commonly hospitalized with acute EBV infection (27% of the cohort), followed by teenagers aged 14–15 years (23%). Fever, cervical lymphadenopathy, tonsillitis and fatigue were the most common physical findings overall. Dividing the children into three age groups (0–4 years, 5–10 years and 11–15 years) revealed that the oldest age groups significantly more often suffered from headache, tonsillitis, sore throat, abdominal pain and nausea. Young children typically presented with a runny nose, fever, fatigue and cervical adenitis. Compared with children under 5, children aged 5–15 years more often showed lymphocytosis (84% vs 62%), elevated alanine aminotransferase (77% vs 33%) and lactate dehydrogenase (79% vs 44%). Conclusion: EBV infection is common in young children, and children less than 3 years of age constitute the largest group of hospitalizations for acute EBV infection. EBV-associated IM should be suspected in febrile children of all ages with tonsillitis, lymphadenopathy, lymphocytosis and elevated liver enzymes.

Keywords: Children, Epstein-Barr virus, symptoms, age, mononucleosis

Introduction

Infectious mononucleosis (IM) is most commonly caused by Epstein-Barr virus (EBV). A triad of fever, lymphadenopathy and pharyngitis characterizes classic IM, but there are diverse clinical features including headache, fatigue, exanthema, jaundice, and hepatomegaly and splenomegaly [1]. Additionally, IM is associated with lymphocytosis and elevated liver enzymes as well as rare complications like peritonsillar abscesses, airway obstruction, splenic rupture, and a wide range of haematological and neurological manifestations [2–4].

EBV is a herpes virus that replicates primarily in B lymphocytes, but also in the epithelial cells of the pharynx and parotid duct. Resting memory B lymphocytes are thought to be the site of EBV persistence within the body [5]. EBV is spread by saliva and the incubation period is 4–8 weeks [6,7]. EBV-caused IM is particularly common in children and adolescents. However, IM is often considered a rare disease in early childhood. This assumption is partly due to the lack of typical clinical findings, resulting in children not being tested. Furthermore, many young children do not develop heterophile antibodies following a primary EBV infection, so if the heterophile is the only test ordered, the diagnosis is missed [8–11]. Symptomatic infection is mostly seen in adolescents and through the adult years [12].
Following primary infection, the virus establishes lifelong latency, and 90–95% of adults are seropositive for EBV [3,13].

In Denmark, no recent studies of the clinical manifestations and laboratory findings of EBV infection in children have been conducted. In 1981, Krabbe et al. described nine children hospitalized with one or more symptoms of EBV infection [14]. In 1973 and 1983, seroepidemiological surveys based on measuring heterophile antibodies and viral capsid antigen (VCA) IgG, respectively, were performed [9,15]. The aim of the present study was to describe the clinical features and laboratory findings of children hospitalized in a large general paediatric ward with a diagnosis of EBV infection during a 12-year period, and to discriminate the classical presentation of adolescent EBV-associated IM from EBV infection at younger age.

Material and Methods

Study group

For this retrospective study, all children hospitalized at the Department of Paediatrics, Hvidovre University Hospital, Copenhagen between 1 January 2002 and 31 December 2013 and who tested positive for acute EBV infection were included. Patients with a compromised immune system due to an underlying chronic disease and/or immunosuppressive therapy were excluded from the study. Demographic variables, clinical features and laboratory findings were collected from medical records.

Laboratory methods

The EBV-specific serological tests and polymerase chain reaction (PCR) tests were performed by Statens Serum Institut. All samples were screened for EBV nuclear antigen (EBNA) IgG by qualitative enzyme immunosorbent assay according to the manufacturer’s instructions (Anti-EBV; Bio-Rad, Marnes-la-Coquette, France)). Samples with an optical density (OD) value equal to or higher than 750 OD were considered positive and no further tests were performed. Children below 18 months of age were tested for VCA IgM, VCA IgG and EBNA IgG simultaneously. The kit used for EBV testing was an anti-EBV EBNA IgG, VCA IgG and VCA IgM ELISA. The test is a specific capture enzyme immunoassay (EIA) detecting specific VCA EBV IgM and IgG antibodies directed towards the recombinant antigens p18 and p23 as well as EBNA-1 IgG in plasma or serum. The sensitivity of the test is 94–97% and the specificity is 96–98%. The heterophile antibody tests were carried out by a chromatographic immunoassay using a bovine erythrocyte glycoprotein (Clearview IM, Alere, Waltham, MA, USA).

From 2010 and onwards, PCR for EBV has been performed on EBV seronegative patients to explore if the samples were taken too early after clinical onset for the immunological response to generate measurable antibodies. However, this was not done systematically. Quantification of EBV DNA in serum or plasma was performed using an in-house real-time Taqman PCR with primers, an EBV probe and a negative control. The test was run similar to the accredited tests in the laboratory and was subject to external quality control panels. The detection limit was 500 copies/ml.

Primary EBV infection was defined by presence of EBV-specific antibodies, heterophile antibodies or a positive EBV PCR. The outcome of the antibody tests was evaluated according to Table I. In general, patients found to be positive for VCA IgM, either negative or positive for VCA IgG and negative for EBNA IgG were classified with primary EBV infection. Furthermore, patients with negative VCA IgM, positive VCA IgG, negative EBNA IgG and positive PCR were included, as PCR may become positive before detectable VCA IgM and the negative EBNA IgG makes reactivation unlikely [16,17]. Patients with negative VCA IgM and positive EBNA IgG and/or absence of heterophile antibodies were defined as not having an acute EBV infection and were not included in the study. Among the 95 EBV-positive children, serology was available for 79 children, heterophile antibodies for 45 and PCR for 24 children, respectively.

Statistics

Statistical analyses were performed using IBM SPSS Statistics Data Editor, version 22. Overview of clinical measurements was generated by frequency distributions and basic descriptive statistics. Clinical and laboratory data were analysed using cross tabulations.

Table I. Interpretation of Epstein-Barr virus (EBV)-specific serology.

| Result | VCA IgM | VCA IgG | EBNA IgG | Clinical situation |
|--------|--------|--------|----------|-------------------|
| −      | −      | −      | −        | Seronegative      |
| +      | +      | −      | −        | Acute infection   |
| +      | +      | −      | −        | Acute infection or non-specificity* |
| −      | −      | +      | −        | Acute or previous infection* |
| +      | +      | +      | −        | Acute infection (late phase) or reactivation* |
| −      | +      | +      | −        | Previous infection |
| −      | −      | +      | −        | Previous infection or non-specificity* |

EBNA, Epstein-Barr virus nuclear antigen; VCA, viral capsid antigen.

*Further analyses required.
and chi-squared tests. A \( p \) value \( \leq 0.05 \) was considered statistically significant.

**Results**

During the period 2002–2013, we identified 110 children diagnosed with acute EBV infection either by the presence of positive serology or heterophile antibodies or by a positive EBV DNA PCR. Fifteen of those patients were excluded due to immunosuppressive treatment and/or compromised immune system caused by chronic diseases like Crohn’s disease and ulcerative colitis, leaving 95 EBV-positive patients for analyses.

**Demographic features**

The demographic characteristics of the EBV-positive patients are shown in Table II. The study group consisted of slightly more males than females. The median age of the children was 7.0 years, the youngest child being 14 months old.

As seen in Figure 1, the patients most commonly hospitalized with acute EBV infection were the youngest children under 3 years of age, accounting for 27% of hospitalizations, followed by teenagers 14–15 years of age with 23% of hospitalizations. In all, 40% of the children were below the age of 5 years. Significantly more boys aged 2 years were hospitalized (12 boys vs 3 girls), while no sex difference was seen for the other age groups.

Most paediatric patients were tested positive for EBV infection in March (15.8%) and December (13.7%), but no clear seasonal variation in admissions was seen.

**Clinical features and laboratory findings**

Table III shows the clinical characteristics of the patients. Fever, cervical lymphadenopathy, tonsillitis and fatigue were the most common physical findings overall. By dividing the children into three age groups, 0–4 years, 5–10 years and 11–15 years, it appears that the oldest age groups significantly more often reported suffering from headache, sore throat, abdominal pain, nausea and myalgia/arthritis. Young children typically presented with a runny nose, fever, fatigue and cervical adenitis.

Table IV shows the laboratory values of the patients. Overall, 63% of children had elevated C-reactive protein, 75% had elevated lymphocyte count and more than half of the children had paracllinical signs of parenchymatous liver involvement. However, an age difference was observed, as children aged 5–15 years significantly more often had lymphocytosis and liver involvement than children below the age of 5.

**Special cases of antibody patterns**

Three of our patients presented with positive VCA IgM, VCA IgG and EBNA IgG, while PCR assays and heterophile antibody tests were not performed. Normally this result would have been categorized as a reactivation of a previous EBV infection, but two of the patients were less than 2 years old, making the probability of a reactivation very unlikely as maternal antibodies often protect infants from infection during the first months of life [18]. The third patient was a 5-year-old girl with hepatosplenomegaly, fever and lymphocytosis. In this case, the probability of a reactivation was unlikely because the child was immunocompetent and without any competing diseases to an EBV infection. Furthermore, low VCA IgG and EBNA IgG values were detected, consistent with a late primary infection.

Table II. Demographic characteristics among 95 children hospitalized with acute Epstein-Barr virus (EBV) infection at Hvidovre University Hospital, 2002–2013.

| Characteristic   | Results |
|-----------------|---------|
| Gender          |         |
| Male            | 52 (55%)|
| Female          | 43 (45%)|
| Caucasian       | 70 (74%)|
| No. of siblings | = 78    |
| 0               | 16      |
| 1               | 36      |
| 2               | 19      |
| ≥ 3             | 7       |
| Age (years), median (range) | 7.0 (1–15) |

Figure 1. Age distribution of 95 children with acute Epstein-Barr virus (EBV) infection hospitalized at Hvidovre University Hospital during 2002–2013.
Infectious mononucleosis in Danish children

and heterophile antibodies. The last patient had a negative EBNA IgG with the remaining tests being inconclusive. This patient presented with clinical features and a haematological profile characteristic of an EBV infection: fever, fatigue, tonsillitis, cervical lymphadenopathy and lymphocytosis. Three patients had a positive heterophile antibody test despite lacking a VCA IgM response. They were considered positive based on the absence of EBNA IgG and a positive EBV DNA PCR. Eight of the included children had detectable VCA IgM and VCA IgG but a negative heterophile antibody test.

**Complications**

The incidence of reported complications among the EBV-positive children was 28.4% (n = 27) and included beta-haemolytic streptococcal co-infections with group A, B or G (n = 11); pneumonia (n = 1); abdominal symptoms (n = 4) including infectious gastroenteritis, subacute pancreatitis, perforated appendicitis and rectal bleeding. Further complications were otitis media (n = 4); haematological findings (n = 3) like post infectious anaemia, thrombotic thrombocytopenic purpura and iron-deficiency anaemia; systolic heart murmur (n = 2) and proteinuria/haematuria (n = 2).

**Table III. Clinical manifestations according to age in 95 children hospitalized with acute Epstein-Barr virus (EBV) infection.**

| Manifestations                  | Age 0–4 years (n = 38) | Age 5–10 years (n = 26) | Age 11–15 years (n = 31) | p value |
|--------------------------------|------------------------|-------------------------|--------------------------|---------|
| Fever                          | 32 (84.2%)             | 22 (84.6%)              | 25 (80.6%)               | 0.90    |
| Fatigue                        | 25 (65.8%)             | 16 (61.5%)              | 23 (74.2%)               | 0.58    |
| Headache                       | 0 (0%)                 | 3 (11.5%)               | 9 (29.0%)                | 0.001   |
| Periorbital oedema             | 4 (10.5%)              | 5 (19.2%)               | 7 (23.2%)                | 0.38    |
| Conjunctivitis                 | 1 (2.6%)               | 0 (0%)                  | 1 (3.2%)                 | 0.67    |
| Runny nose                     | 21 (55.3%)             | 9 (34.6%)               | 6 (19.4%)                | <0.01   |
| Stomatitis                     | 3 (7.9%)               | 4 (15.4%)               | 4 (12.9%)                | 0.63    |
| Tonsillitis                    | 24 (63.2%)             | 21 (80.8%)              | 27 (87.1%)               | 0.06    |
| Sore throat                    | 9 (23.7%)              | 16 (61.5%)              | 25 (80.6%)               | <0.001  |
| Cough                          | 5 (13.5%)              | 1 (3.8%)                | 6 (19.4%)                | 0.21    |
| Cervical lymphadenopathy       | 34 (89.5%)             | 21 (80.8%)              | 29 (93.5%)               | 0.31    |
| General lymphadenopathy        | 12 (31.6%)             | 10 (38.5%)              | 10 (32.3%)               | 0.83    |
| Abdominal pain                 | 3 (7.9%)               | 11 (42.3%)              | 14 (45.2%)               | 0.001   |
| Nausea                         | 2 (5.3%)               | 11 (42.3%)              | 9 (29.0%)                | <0.01   |
| Hepatomegaly                   | 13 (34.2%)             | 6 (23.1%)               | 6 (19.4%)                | 0.34    |
| Splenomegaly                   | 5 (13.2%)              | 7 (26.9%)               | 4 (12.9%)                | 0.27    |
| Jaundice                       | 1 (2.6%)               | 1 (3.8%)                | 3 (9.7%)                 | 0.48    |
| Rash                           | 15 (39.5%)             | 4 (15.4%)               | 10 (32.3%)               | 0.12    |
| Myalgia/arthralgia             | 0 (0%)                 | 2 (7.7%)                | 6 (19.4%)                | <0.05   |
| Neurological symptoms          | 0 (0%)                 | 0 (0%)                  | 1 (3.2%)                 | 0.35    |

**Table IV. Laboratory findings in 95 children hospitalized with acute Epstein-Barr virus (EBV) infection.**

| Variable                  | n | Median | Range          | Age 0–4 | Age 5–15 | Total | p value |
|---------------------------|---|--------|----------------|---------|----------|-------|---------|
| Haemoglobin (mmol/L)      | 92 | 7.5    | 5.4–9.8        | 0       | 0        | 0     | –       |
| WBC (10^9/L)              | 93 | 12.6   | 3.5–39.8       | 0       | 0        | 0     | <0.05   |
| PLT (10^9/L)              | 91 | 217.0  | 12–520         | 44.7%   | 69.1%    | 59.1% | <0.05   |
| Granulocytes (10^9/L)     | 92 | 4.0    | 0.5–16.5       | 2.8%    | 1.8%     | 2.2%  | 1       |
| Lymphocytes (10^9/L)      | 92 | 6.4    | 1.3–23         | 62.2%   | 83.6%    | 75%   | <0.05   |
| CRP (mg/L)                | 92 | 16.0   | <1–172         | 51.4%   | 70.9%    | 63%   | 0.08    |
| ALT (U/L)^b               | 82 | 43.5   | 6–552          | 33.3%   | 77.3%    | 53.7% | <0.01   |
| LDH (U/L)^b               | 80 | 419.0  | 64–1850        | 43.8%   | 79.2%    | 65%   | <0.01   |
| Bilirubin (μmol/L)        | 75 | 5.0    | 0–82           | 0       | 15.2%    | 9.3%  | <0.05   |

ALT, alanine aminotransferase; CRP, C-reactive protein; LDH, lactate dehydrogenase; PLT, platelets; WBC, white blood cells.

*According to age-related normal values.

^bSixty children out of 82 (73%) had elevated ALT and/or LDH.

**Discussion**

In industrialized countries, EBV-associated IM is considered to occur most often in adolescents and young adults, while younger children often are described with a mild subclinical primary EBV infection [8,19]. Unexpectedly, a large proportion...
of the EBV-positive patients in this study were 4 years old or younger (40%), and 27% were below the age of 3. In line with this finding, Sumaya and Ench concluded that EBV IM is not uncommon among the very young children in industrialized countries [20]. In their prospective study including 113 children with acute primary EBV infection, a surprisingly large number of children (n = 47) were found to be less than 4 years old. In another study, Henke et al. described that even though IM was less frequent in preschool children than in older children, 25% from the youngest age group were hospitalized compared with only 2% among those aged 5–14 years and 17% of those aged 15–18 years [21], indicating that this infection constitutes a significant burden for the youngest children. The frequency of symptomatic EBV infection among this young age group has traditionally been considered quite low [22], but perhaps the decreased heterophile antibody response in young children [11] has resulted in underdiagnosing younger children with EBV in many centres.

As expected, the classic triad of fever, tonsillitis and lymphadenopathy were the most common symptoms among patients with acute EBV infection. However, IM is known for its variable nature of clinical presentations [1,7,12,23,24]. The present study demonstrates that in the youngest children, non-specific symptoms like a runny nose and a rash were frequently present, making a clinical suspicion of EBV infection less obvious. Tamir et al. stated that the younger age of the child the less typical symptoms of IM [10]. In particular, children less than 1 year of age suffered from diarrhoea, vomiting and upper respiratory tract infection, while older children had more typical IM symptoms [10]. An explanation for the age-related differences in clinical manifestations may be that some symptoms are difficult to explore objectively (headache, sore throat, nausea and myalgia) and therefore expected to be reported less frequently for the youngest children.

The main discrepancies regarding the classical symptoms in comparable studies of IM caused by EBV include the presence of fever and tonsillitis. Balfour et al. [7] and Rea et al. [1] reported that only 30% and 45% of EBV-positive patients presented with fever, respectively. Other studies, including the present study, found fever in approximately 80% of the EBV-infected patients [12,23,24]. Furthermore, Balfour et al. observed tonsillitis in all the patients with EBV [7], while Saldañá et al. only found tonsillitis in slightly more than half of the study group [12]. All the studies found lymphadenopathy in more than 80% of the EBV-positive patients, except Rea et al. where only 57% had lymphadenitis [1]. Of the comparable studies, some studied young adults [1,7,23] while others studied children younger than 18 years [12,24].

In the present study, splenomegaly and hepatomegaly were identified in 16.8% and 26.3% of the children, respectively. Physical examinations are quite inaccurate in detecting hepatosplenomegaly and only few patients in the present study underwent abdominal ultrasound. Dommerby et al. performed ultrasound on 29 patients with IM and found that only 17% of the enlarged spleens and 19% of the enlarged livers were discovered at the physical exam [23]. Absence of either splenomegaly or hepatomegaly by clinical examination should, therefore, not be used as evidence against EBV-associated IM [19,26]. Considering these reports, hepatosplenomegaly might have been more frequent in our cohort if ultrasound had been used for clarification.

Data from the present study suggest that the white blood cell (WBC) counts, including the lymphocyte counts, are significantly associated with EBV-positive IM, which is in line with previous reports [2,23,24]. Earlier studies have demonstrated an increased lymphocyte/WBC count ratio (L/WBC) among heterophile antibody-positive patients and even suggested the use of the L/WBC ratio in diagnostic matters [27,28]. Additionally, elevated levels of alanine aminotransferase (ALT) and lactate dehydrogenase (LDH), signifying liver dysfunction, were found in more than half of the patients, but less often in preschool children.

Considering co-infections, beta-haemolytic streptococcus was the most frequently found pathogen concurrent with the EBV infection. Those superinfections may occur due to a suppressed immune system caused by the virus infection, making the patients more susceptible to other pathogens. Another possibility is the coincidental finding of streptococci in the throat of a patient with EBV infection, as asymptomatic carriage of Group A streptococci has been reported to occur in up to 20% of healthy children [29].

It is well known that EBV serology presents with a high degree of variability and different interpretation criteria in addition to different immunohistochemical methods [17,30]. The ELISA kit used in this study has high sensitivities and specificities for EBV VCA IgM and IgG. False-positive results due to cross-reactivity to antibodies against varicella, toxoplasmosis, cytomegalovirus and hepatitis A are, therefore, rarely expected [31]. The diagnosis of EBV-associated IM is complex and is, in daily practice, based on medical history, clinical manifestations and haematologic findings, and then confirmed with positive serology or heterophile antibodies. However, despite the reliable ELISA test used in the present study, serologic diagnosis of EBV infection remains a challenge.
Three of our patients had detectable EBNA IgG, VCA IgG and VCA IgM. Simultaneous presence of VCA IgM and EBNA IgG in serum has previously been described in children with acute EBV-associated IM in the late phase [11,32]. Ten patients lacked VCA IgM antibodies, which can be explained by a late emergence of VCA IgM [7,20,32] or the fact that not all children with primary infection mount an IgM response [11,33]. As PCR can be positive before detectable antibodies, and as EBNA IgG was negative, these 10 patients were regarded as having an acute EBV infection [16,17]. Unfortunately, none of the children was followed up with later serum samples to monitor their immune responses.

Eight children lacked heterophile antibodies despite detectable IgM and IgG antibodies to VCA. The monospot assay is based on the observation in 1932 that sheep red cells will agglutinate when mixed with serum from patients with IM containing heterophile antibodies of IgM class [34]. It is a practical method for confirming the clinical diagnosis and, normally, more than 90% of patients with IM develop transient heterophile antibody responses, indicating that the illness is associated with EBV [35]. However, false-negative results of heterophile antibody assays are relatively common early in the course of infection [19]. Especially in children less than 4 years of age, the persistence of heterophile antibodies as well as VCA IgM is shorter when compared with older children [20,36]. This transient nature of the antibodies, therefore, can lead to false-negative results [37], which may explain the absence of heterophile antibodies among these eight patients. In a study from the United States, only 27.3% of children aged 10–23 months mounted a heterophile antibody response compared with 63.3% of children aged 26–48 months [38], and in a previous Danish study, none of eight children less than 5 years old with primary EBV infection had heterophile antibodies [14]. Therefore, importantly, the absence of heterophile antibodies as well as of VCA IgM does not exclude an acute EBV infection. Furthermore, the heterophile antibodies can persist up to 6–12 months after infection [11], so a positive test result does not always imply an acute EBV infection. For hospitalized children, a combination of EBV DNA in plasma and EBV-specific antibodies (VCA IgM, VCA IgG and EBNA IgG) provides the correct diagnosis in most cases. Monospot based on heterophilic antibodies should not be used due to the high number of both false negatives and false positives in paediatric patients.

The present study had some limitations. It was performed retrospectively on a selected group of hospitalized patients who underwent evaluations and examinations by multiple physicians. No follow-up was possible. Only 83% of the included patients were tested with EBV-specific serology, while the remaining children were included on the basis of heterophile antibody tests, with a larger possibility of false-negative results. Furthermore, it would have been preferable if the study had included a considerably larger number of patients.

In conclusion, this study contributes to the understanding of acute EBV infection in different age groups of hospitalized Danish children. It demonstrates that EBV-associated IM is common in young children and that symptoms in preschool children may be more non-specific than in older children. Importantly, acute EBV infection should be suspected in febrile children of all ages with tonsillitis, lymphadenopathy, fatigue, rash, lymphocytosis and elevated liver enzymes. A prospective study of EBV infection in young children including a systematic use of EBV serology and PCR is warranted to give a comprehensive evaluation of the disease burden in this age group.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. No funding was received for the study.

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