Correlates of illness severity in infectious mononucleosis

John Odame1, Joan Robinson MD FRCPc2, Nasser Khodai-Booran PhD3, Simon Yeung BSc1, Tony Mazzulli MD FRCPc3, Derek Stephens MSc4, Upton D Allen MBBS MSc FRCPc1,4

INTRODUCTION: Understanding the spectrum and frequencies of Epstein-Barr virus (EBV) complications and markers of illness severity in immunocompetent patients with primary EBV infection will inform management of patients with EBV-related illnesses.

OBJECTIVES: To determine the clinical and laboratory correlates of illness severity among infants, children and youth with infectious mononucleosis (IM).

METHODS: Study subjects with confirmed IM were prospectively enrolled. Illness severity was assessed at baseline and at six weeks using a scoring tool. Peripheral blood viral loads served as a measure of viral burden.

RESULTS: Among 32 children and young adults with IM, the median age was 16 years (range two to 24 years). The predominant clinical findings were lymphadenopathy (23 of 32 [72%]), pharyngitis (16 of 32 [50%]), fever (nine of 32 [28%]) and splenomegaly (six of 32 [19%]). With respect to symptoms or signs that persisted to at least six weeks after illness onset, the predominant complaint was lymphadenopathy in 35% of subjects available for reassessment. Devanged liver function tests were present at presentation in up to 44% of subjects. Patients with the highest viral loads at presentation had significantly higher illness severity scores at baseline and at six weeks.

CONCLUSION: In IM, viral loads are not necessarily correlated with illness severity, with the exception of fatigue. EBV-related hepatitis is common in IM, confirming the status of this virus as a relatively common cause of transient hepatitis in children and youth. This entity is not necessarily a marker of disease severity.

Key Words: Epstein-Barr virus; Hepatitis; Mononucleosis; Viral load

I

fectious mononucleosis (IM) results from primary infection by the Epstein-Barr virus (EBV). EBV is a double-stranded DNA gamma herpesvirus that was first isolated in 1964 from Burkitt lymphoma tissue (1). Humans are the only source of EBV and the virus has a worldwide distribution, with seropositivity rates of 90% among adults.

In most nonindustrialized communities, primary EBV infection is usually asymptomatic and occurs within the first three years of life (2-7). In industrialized countries, infection is often delayed until the second decade of life or later (4,8), where clinical illness is typically characterized by IM. This entity is usually asymptomatic and occurs within the first three years of life (2-7). In industrialized countries, infection is often delayed until the second decade of life or later (4,8), where clinical illness is typically characterized by IM. This entity is usually asymptomatic and occurs within the first three years of life (2-7). In industrialized countries, infection is often delayed until the second decade of life or later (4,8), where clinical illness is typically characterized by IM. This entity is usually asymptomatic and occurs within the first three years of life (2-7). In industrialized countries, infection is often delayed until the second decade of life or later (4,8), where clinical illness is typically characterized by IM. This entity is usually asymptomatic and occurs within the first three years of life (2-7). In industrialized countries, infection is often delayed until the second decade of life or later (4,8), where clinical illness is typically characterized by IM. This entity is usually asymptomatic and occurs within the first three years of life (2-7). In industrialized countries, infection is often delayed until the second decade of life or later (4,8), where clinical illness is typically characterized by IM. This entity is usually asymptomatic and occurs within the first three years of life (2-7). In industrialized countries, infection is often delayed until the second decade of life or later (4,8), where clinical illness is typically characterized by IM. This entity is usually asymptomatic and occurs within the first three years of life (2-7). In industrialized countries, infection is often delayed until the second decade of life or later (4,8), where clinical illness is typically characterized by IM.

In the above context, the goal of the present study was to determine the clinical and laboratory correlates of illness severity among infants, children and youth with IM.

1Division of Infections Diseases, Hospital for Sick Children, Toronto, Ontario; 2Division of Infections Diseases, Stollery Children’s Hospital, University of Alberta, Edmonton, Alberta; 3Mount Sinai Hospital, University of Toronto; 4The Research Institute, Hospital for Sick Children, Toronto, Ontario

Correspondence: Dr Upton D Allen, Division of Infections Diseases, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8. Telephone 416-813-8129, fax 416-803-8404, e-mail upton.allen@sickkids.ca

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METHODS

Study design and subject identification
The present prospective study enrolled subjects who met the following criteria: >1 month to 25 years of age; serologically confirmed acute IM using EBV-specific serology; and a positive test for immunoglobulin M (IgM) antibodies to viral capsid antigen. Subjects were identified from inpatients admitted because of IM as well as outpatients. The latter were identified for inclusion in the study by physicians who were aware of the study when the subjects presented to the emergency departments of the two hospitals, local university health centres and physicians’ offices. Information about the study was provided by the Ontario Public Health Laboratory to physicians whose patients had EBV serology consistent with acute EBV infection. Subjects were also recruited via radio messages.

Clinical assessments
All subjects underwent clinical assessments at enrollment and follow-up visits. A list of symptoms known to be associated with IM was used to grade severity of illness. This assessment strategy was modified from Rea et al (13), as originally derived from the Symptom Checklist – 90 (14). A focus was placed on symptoms and signs; measurement of health-related functional status, including but not limited to general health, mental health, social functioning and emotional well-being, were not included (13). The items and the scores used were as follows: fatigue, sore throat, painful lymph nodes, fever, somnolence, headache, sore muscles, nausea, sore joints, cough and rash. Severity was ranked from 1 to 5, where 1 = not at all, 2 = a little bit, 3 = moderately, 4 = quite a bit and 5 = extremely. Symptom scores ≥3 were regarded as clinically significant. The maximum possible score was 55 (11 items × 5 levels). All subjects were followed by weekly telephone contact, with a follow-up visit at six weeks. Additional visits were arranged as clinically indicated.

Laboratory measurements
Virological assays: Antibodies to viral capsid antigen (immunoglobulin G [IgG] and IgM), early antigen (IgG) and Epstein-Barr nuclear antigen (IgG) were performed using ELISA methodology (DiaSorin Inc, USA) to confirm the diagnosis (15). For viral load quantitation, specimens consisting of 3 mL of anticoagulant (EDTA)-treated blood were processed and DNA extracted from whole blood samples using the NucliSENS easyMag system (bioMérieux, France). Real-time polymerase chain reaction for the measurement of viral load was performed using the RealStar EBV PCR Kit (Altona Diagnostics, Germany) and the RotorGene instrument (Corbett Life Science, Australia) (16,17). The analytic sensitivity of the assay is 1.1 copies/µL and the detection threshold is 275 genome copies/µL of whole blood. Based on extensive experience with viral load measurements in organ transplant patients (18), viral loads of ≤6000 copies/mL were classified as low, >6000 to 30,000 as low to intermediate, >30,000 to 300,000 as intermediate to high and >300,000 copies/mL as high. The viral load assays were performed in one centre (Dr Mazzulli, Mount Sinai Hospital, Toronto).

Statistical methods
Data were managed using Microsoft Access database and Excel (Microsoft Corporation, USA). Data were summarized using descriptive statistics and, whenever appropriate, means or medians were compared using Student’s t or a nonparametric test, respectively. Proportions were compared using χ2 or Fisher’s exact test as appropriate.

RESULTS

Descriptive characteristics
Among 32 children and young adults with acute IM, the median age was 16 years (range two to 24 years), and the male-female ratio was 1:2.6. The study subjects were symptomatic for a mean (± SD) of 16.9±22.9 days before initial assessment (interquartile range 15.5 days).

Clinical assessments
Table 1 summarizes the spectrum of symptoms and signs. The predominant clinical findings at presentation were lymphadenopathy (23 of 32 [72%]), pharyngitis (16 of 32 [50%]), splenomegaly (six of 32 [19%]) and fever (nine of 32 [28%]). Somnolence and fatigue were documented in 53% (17 of 32) and 66% (21 of 32) of subjects, respectively. Among subjects who were available for follow-up assessment at six weeks, the predominant complaints that persisted were lymphadenopathy in seven of 20 subjects (35%) and fatigue in four of 25 subjects (16%). For the vast majority of subjects, splenomegaly either was not present at enrollment or had resolved by six weeks; persistence of splenomegaly was observed in 10% (two of 20) at six weeks.

The illness severity scoring showed the expected improvement over time, as presented in Figure 1. The median score at enrollment was 27.5 out of a maximum score of 55 (range 12 to 41), while at six weeks, the median was 12 (range 11 to 27); (P<0.0001). As shown in Figure 1, the highest illness severity scores at baseline were primarily attributed to fatigue, somnolence, sore throat and headaches.

Nonvirological laboratory assessments
Table 2 summarizes the results of liver function tests. Blood samples at six weeks were obtained for 20 (62.5%) of the 32 subjects. Sixteen percent of children <11 years of age and 44% of older children/youth had deranged liver function tests during the acute phase of IM. Based on the age cut-offs for normal values, among subjects >1 year of age,
the median alanine transaminase (ALT) level was 198 IU/L (range 61 IU/L to 458 IU/L), while the median aspartate transaminase (AST) level was 179 IU/L (range 51 IU/L to 771 IU/L). Thirty-one percent of subjects had ALT values that were at least twice the upper limit of normal at presentation, while the corresponding percentage for AST was 6% and 22% for subjects one to <11 and ≥11 years of age, respectively. At six weeks, one of 20 evaluable patients still had transaminase levels that were at least twice the upper limit of normal. One subject was identified who developed autoimmune hepatitis that was triggered by EBV and which led to a liver biopsy and treatment with corticosteroids after initial treatment with acyclovir.

Assessment of hematological parameters at initial presentation indicated neutrophil counts <1×10^9/L in 12.5% (four of 32) of patients and lymphocyte counts <1.5×10^9/L in 15.6% (five of 32) patients. Thrombocytopenia (platelet count <150×10^9/L) was observed in four (12.5%) of 32 subjects; the median platelet count was 227×10^9/L (range 1×10^9/L to 380×10^9/L). These hematological abnormalities had resolved among the subjects who were available for testing at the six-week visit.

### Viral loads in relation to clinical and laboratory variables

The median viral load at the initial visit for all subjects was 5180 copies/mL (range 0 copies/mL to 926,014 copies/mL). This median viral load value would be categorized as low based on the assay. There was no significant relationship between the age of the study subjects and their viral loads at the time of enrollment (r=−0.25; P=0.24). The relationship between viral loads (log_{10}) at enrollment and laboratory indexes indicated no significant correlation between loads and the following: hemoglobin level (r=0.35; P=0.09), white blood cell count (r=−0.24; P=0.26), neutrophil counts (r=0.18; P=0.39), lymphocyte counts (r=−0.29; P=0.17), platelet counts (r=−0.12; P=0.52), AST level (r=−0.10; P=0.66) and ALT level (r=−0.01; P=0.95).

Among 27 patients with complete pairs of viral load values and severity scores, there was only weak correlation (r=0.29) between viral loads at baseline and symptom severity scores for fatigue (Figure 2). However, subjects with intermediate to high levels of viral load (>30,000 copies/mL) were more likely to have fatigue symptom scores of 4 or 5, compared with those with lower viral loads (P=0.02).

DISCUSSION

We have documented the spectrum of clinical and laboratory manifestations in a cohort of children and youth with primary EBV infection. We assumed that our study centres were likely to have attracted individuals with the more severe end of the spectrum of primary EBV infection because individuals with milder degrees of infection would

**TABLE 2**

<table>
<thead>
<tr>
<th>Liver function tests</th>
<th>Initial visit (n=32)</th>
<th>Six-week follow-up visit*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>Mean</td>
</tr>
<tr>
<td>ALT (&gt;50 IU/L)</td>
<td>15 (47)</td>
<td>188.4</td>
</tr>
<tr>
<td>AST (&gt;45 IU/L, age 1 to &lt;11 years)</td>
<td>4 (13)</td>
<td>259.5</td>
</tr>
<tr>
<td>AST (&gt;36 IU/L, age ≥11 years)</td>
<td>13 (41)</td>
<td>131</td>
</tr>
<tr>
<td>Alkaline phosphatase (&gt;555 IU/L)</td>
<td>1 (3)</td>
<td>582</td>
</tr>
<tr>
<td>Total bilirubin (&gt;19 µmol/L)</td>
<td>5 (16)</td>
<td>50.6</td>
</tr>
<tr>
<td>Gamma-glutamyl transferase, IU/L</td>
<td>12 (38)</td>
<td>49.8</td>
</tr>
</tbody>
</table>

*Data availability at six weeks (denominators): Alanine transaminase (ALT), n=20; aspartate transaminase (AST), n=19; alkaline phosphatase n=16; bilirubin, n=14; gamma-glutamyl transferase, n=7

Figure 2) Baseline viral loads in relation to illness severity scores associated with fatigue. Patients with with intermediate to high levels of viral load were more likely to have fatigue symptom scores of 4 or 5, compared with those with lower viral loads (P=0.02)

**DISCUSSION**

We demonstrated the presence of hepatitis in a significant proportion of patients at enrollment. Hepatitis is known to be a complication of IM, although the magnitude is not well documented in prospective studies that incorporate viral load measurements. The condition is usually benign, but fulminant hepatitis has been documented (21,22). Our findings are similar to a prospective study by Rea et al (13), in which 31% and 61% of their patients had normal ALT and AST levels at their initial visits. Typically, the abnormal transaminase values are in the mildly elevated category (13,23-28). However, as shown in our study, these values may be moderate or more significantly elevated. While in both studies, liver function tests typically normalized by six weeks, prolonged abnormalities may occur. Such prolonged liver function abnormalities may herald the onset of such prolonged liver function abnormalities may herald the onset of such
autoimmune hepatitis, as was documented in our study. Typically, subjects recovered without antiviral therapy. In our cohort, one patient had hepatitis that was deemed sufficiently severe to warrant antiviral therapy (and subsequently corticosteroids for autoimmune hepatitis).

The kinetics of viral load in IM was studied during primary infection by Balfour et al. (23). These investigators demonstrated that the median half-life of viral elimination from whole blood was three days (mean 3.4 days, range 1.8 to 6.6 days). This was consistent with our findings that indicated the majority of subjects had low or undetectable viral loads by the time they presented with a clinical diagnosis of IM. The vast majority of subjects became asymptomatic within six weeks. However, given that oropharyngeal shedding of EBV is known to persist beyond the acute phase of primary EBV, even if the virus has been cleared from the peripheral blood (23), it is likely that asymptomatic study subjects were still shedding virus well into the convalescent period.

In the above context, persistence of detectable viral loads in the peripheral blood may help to define a subgroup of patients who are less able to contain viral load. In such situations, the occurrence of prolonged high viral loads could be considered to represent an abnormal state that is not consistent with what would be expected in an individual with the ability to contain EBV replication. This notwithstanding, prolonged illness may occur despite containment of viral load (29). In our study, subjects with the highest viral load were more likely to have higher illness severity scores relating to fatigue. However, while those with detectable viral loads experienced improvement in such loads over time, the loads were not correlated with the magnitude of the overall illness severity scores. This is consistent with the situation that commonly occurs in immunocompromised hosts, who may have prolonged periods of high viral loads following primary EBV infection without such loads being correlated with the severity of their symptoms (30,31).

We have summarized the characteristics of the more severe forms of primary EBV encountered in our study. For example, we have documented prolonged thrombocytopenia, neutropenia and significantly elevated transaminase levels. We were unable to document an association between these variables and viral loads, but we do acknowledge that the sample size was a limitation of our study. A larger study would also enable the determination of the full spectrum of the more extreme clinical presentation of IM, and would be better able to detect some of the more uncommon severe complications of IM. This notwithstanding, we have determined the more common clinical and laboratory correlates of primary EBV infection deemed sufficiently severe to present to centres such as ours. These findings will help with the assessment of immunocompromised patients in whom these clinical and laboratory markers of primary EBV (eg, hepatitis) may be more profound. We plan to perform additional studies to determine the genotypic correlates of the more severe presentations of primary EBV in immunocompetent hosts. An understanding of such markers of severity would also be of relevance to some immunocompromised hosts in whom the effects of intrinsic susceptibility to severe EBV infection may be exaggerated by iatrogenic immunosuppression (30,31).

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REFERENCES