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Original Article

Clinical differentiation of infectious mononucleosis that is caused by Epstein-Barr virus or cytomegalovirus: A single-center case-control study in Japan

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ABSTRACT

Introduction: Infectious mononucleosis (IM) is a common viral infection that typically causes fever, pharyngitis, and lymphadenopathy in young patients. The Epstein-Barr virus (EBV) is the most common cause of IM, followed by cytomegalovirus (CMV). Given that serological testing is associated with limitations regarding its accuracy, availability, and time to receive results, clinical differentiation based on symptoms, signs, and basic tests would be useful. We evaluated whether clinical findings could be used to differentiate EBV-IM from CMV-IM.

Methods: In this single-center retrospective case-control study, we evaluated >14-year-old patients with serologically confirmed EBV-IM or CMV-IM during 2006–2017. We compared the patients' symptoms, physical findings, blood counts, and serum biomarkers to create three regression models: model 1 (symptoms and signs), model 2 (model 1 plus sonographic hepatosplenomegaly and blood counts), and model 3 (model 2 plus hepatobiliary biomarkers).

Results: Among the 122 patients (72.6%) with EBV-IM and 46 patients (27.4%) with CMV-IM, the median age was 25 years and 82 patients (48.8%) were male. The median age was 10 years older in the CMV-IM group ($p < 0.001$) and the median interval from onset to visit was 5 days longer in the CMV-IM group ($p < 0.001$). Logistic regression revealed that EBV-IM was predicted by younger age, short onset-to-visit interval, lymphadenopathy, tonsillar white coat, hepatosplenomegaly, atypical lymphocytosis, and elevations of lactate dehydrogenase and gamma-glutamyl transferase. All regression models had areas under the curve of >0.9.

Conclusion: History and physical findings, especially when used with atypical lymphocytosis and sonographic hepatosplenomegaly, can help physicians differentiate EBV-IM from CMV-IM.

1. Introduction

Infectious mononucleosis (IM) is a common self-limiting viral infection that typically affects juvenile patients and presents with a classical triad: fever, pharyngitis, and lymphadenopathy [1]. Most cases of IM are caused by Epstein-Barr virus (EBV) and the second most common causative agent is cytomegalovirus (CMV), which causes 5–16% of cases [1,2]. Other conditions that must be considered in the differential diagnosis include toxoplasmosis, human herpes virus-6 (HHV-6) infection, human herpes virus-7 (HHV-7) infection, adenovirus infection, rubella, herpes simplex virus (HSV) infection, influenza/parainfluenza virus infection, rhinovirus infection, coronavirus infection, and acute human immunodeficiency virus (HIV) infection [1,3].

Relative to IM caused by EBV (EBV-IM), IM caused by CMV (CMV-IM) reportedly affects patients who are 10–15 years older and who present with milder lymphadenopathy and pharyngitis, but more frequent and serious hepatitis and thrombocytopenia. However, some reviews have concluded that EBV-IM and CMV-IM are nearly indistinguishable [4,5]. In this context, serological

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examination is useful for identifying the causative agent. Heterophile antibody tests (HAT), which are widely available and relatively rapid tests, are limited by their low sensitivity in the early phase and in young patients [3]. Although combinations of antibodies specific to EBV or CMV are more accurate [6], even these specific antibodies have several limitations. First, serological examination is not useful for the initial diagnosis, as it requires several days to obtain the results, which can limit its application to retrospective confirmation. Second, serological examination is expensive and not widely available, which are especially important issues in the primary care setting and in rural areas. Third, despite combinations of EBV-specific antibody tests having high accuracy, they do infrequently produce inaccurate results, such as false negatives [7] and cross-reactivity with CMV-IM [8]. Furthermore, there are questions regarding the accuracy of CMV-IgM for diagnosing primary acute CMV infection [9]. Thus, it would be helpful for primary care physicians to differentiate between EBV-IM and CMV-IM based on symptoms, clinical signs, and readily available laboratory data, such as complete blood counts (CBC). However, we are only aware of a few studies that have directly compared the clinical characteristics of EBV-IM and CMV-IM [2,10], and those studies were limited by their small size and the absence of multivariate analyses. Therefore, the present study aimed to determine whether clinical findings could be used to differentiate between EBV-IM and CMV-IM before serological confirmation could be obtained.

2. Patients and methods

2.1. Design and patients

This single-center retrospective case-control (case-case) study evaluated medical records of immunocompetent patients with serologically confirmed EBV-IM or CMV-IM from the Toho University Medical Center Omori Hospital, which has 948 beds and is located in the southern part of Tokyo, Japan. The center's ethics committee approved the study's retrospective protocol (M17295). Patients were included if they were >14 years old and received inpatient or outpatient treatment for clinically evident and serologically confirmed EBV-IM or CMV-IM at the General Medicine and Emergency Care department between January 2006 and December 2017. Serological confirmation of EBV-IM was based on a $<10\times$ result for EBV nuclear antigen (EBNA) plus a $\geq 10\times$ result for viral capsid antigen IgM (VCA-IgM), which were both measured using fluorescent antibody (FA) technique. Serological confirmation of CMV-IM was based on a $\geq 0.8\times$ result for CMV-IgM, which was measured using enzyme immunoassay. All tests were performed by SRL (Tokyo, Japan). We regarded the initial dilution concentrations, officially provided by the manufacturer (Fujirebio Inc. Tokyo, Japan), of each examination kit as cut-off values for EBNA and VCA-IgM; this was due to the methodological difficulty involved in setting clear cut-off values for tests that use the FA technique (we discussed this issue with a staff member of SRL). The CMV-IgM cut-off value was set as 0.8 based on the value provided by the manufacturer (Denka Seiken Co. Ltd., Tokyo, Japan) [11]. Although values between 0.8 and 1.2 were border-line, we selected the lowest value of the range to obtain the most sensitive results [11]. We intended to categorize patients who had diabetes mellitus, liver cirrhosis, end-stage renal diseases, or cancers or had received immunosuppressants or glucocorticoids as immunocompromised for exclusion. Pregnant women were not intentionally excluded. Doctors in charge of the patients included both resident doctors who had completed their 3rd post-graduate year at least and experienced attending physicians. All laboratory data were collected at the first visit.

2.2. Study variables

The patients' records were searched to collect data from their first visit regarding sex, age, interval from onset to the first visit, fever, sore throat, cervical lymphadenopathy, headache, abdominal pain, cough, facial edema, rash, throat redness, tonsillar white coat, abdominal tenderness, sonographic hepatosplenomegaly, leukocyte count, atypical lymphocytes (%), platelet count, serum C-reactive protein (CRP), serum total bilirubin, serum aspartate aminotransferase (AST), serum alanine transaminase (ALT), serum lactate dehydrogenase (LDH), serum alkaline phosphatase (ALP), and serum gamma-glutamyl transferase (GGT). Pharyngitis was identified based on sore throat and/or throat redness. The classical triad was defined as fever, pharyngitis, and lymphadenopathy. Fever was defined as a body temperature of $\geq$38.0 °C. Hepatosplenomegaly was identified based on ultrasound-based confirmation of hepatomegaly and/or splenomegaly. Leukocytosis was defined as a leukocyte count of >10,000/mm^3 [2] and thrombocytopenia was defined as a platelet count of <150,000/mm^3 [2]. For the analyses, some continuous variables were categorized: age ($\leq$30 years, 31–40 years, and >40 years) [5], atypical lymphocytes ($\leq$10%, 11–30%, and >30%) [5,12], ALT ($\leq$40 IU/L, 41–200 IU/L, and >200 IU/L) [2,12], LDH ($\leq$250 IU/L, 251–500 IU/L, and >500 IU/L), ALP ($\leq$350 IU/L, 351–500 IU/L, and >500 IU/L), and GGT ($\leq$50 IU/L, 51–300 IU/L, and >300 IU/L). The cut-off values for age, leukocytosis, thrombocytopenia, atypical lymphocytosis, and ALT level were selected based on previous relevant studies and the distribution of our data. We used original categories for LDH, ALP, and GGT levels based on previous studies and the distribution of our data [2,12,13].

2.3. Statistical analysis

Univariate analyses were performed to compare the characteristics of patients with EBV-IM or CMV-IM. Categorical variables were compared using the chi-square test and the Wilcoxon rank-sum test was used for all continuous variables because most variables had skewed distributions. Logistic regression analysis was subsequently performed based on the results of the univariate analyses. The differentiating abilities of history and physical findings (H&P), CBC, ultrasound-confirmed hepatosplenomegaly, and hepatobiliary biomarkers were tested using three models: model 1 (only H&P factors), model 2 (model 1 plus hepatosplenomegaly and CBC), and model 3 (model 2 plus hepatobiliary makers). However, given the relatively small sample size, we limited the number of explanatory variables by only carrying forward factors that were significant in the previous regression model (e.g., only significant H&P variables in model 1 were included in model 2). Thus, model 1 ultimately included age, interval from onset to visit, lymphadenopathy, pharyngitis, headache, facial edema, tonsillar white coat, abdominal tenderness, and the classical triad. Model 2 ultimately included age, interval from onset to visit, lymphadenopathy, tonsillar white coat, hepatosplenomegaly, leukocytosis, atypical lymphocyte percentage, and thrombocytopenia. Model 3 ultimately included age, lymphadenopathy, tonsillar white coat, hepatosplenomegaly, leukocytosis, atypical lymphocyte percentage, ALT, ALP, and GGT.

To evaluate multicollinearity, we examined the variance inflation factors of all regression models. Although sex and body temperature are important factors that are included in the classical triad, they were omitted from the models because no significant differences were detected in the univariate analyses. We also omitted AST based on multicollinearity with ALT and because ALT is generally considered a more specific liver biomarker.

Receiver operating characteristic (ROC) curves were created, and the differentiation abilities of the three regression models were compared using the methods of DeLong et al. [14]. All analyses were performed using Stata/IC software (version 15.1; Stata Corp, USA).

3. Results

3.1. Patients' characteristics and univariate analyses

The 168 eligible patients including 122 patients (72.6%) with EBV-IM and 46 patients (27.4%) with CMV-IM. The median age was 25 years and 82 patients (48.8%) were male. Pregnant women were absent. No patients subsequently underwent serological examinations for concurrent differential diagnosis of IM, including tests for toxoplasmosis, HHV-6, HHV-7, adenovirus, rubella, HSV, influenza/parainfluenza, rhinovirus, coronavirus, and acute HIV infection. No patients were eventually excluded due to immunocompromised status because no patients had diabetes mellitus, liver cirrhosis, end-stage renal diseases, or cancers or had received any immunosuppressants or glucocorticoids. The patients' characteristics and the results of the univariate analyses are shown in Table 1. The univariate analyses revealed that the median age was 10 years older in the CMV-IM group ($p < 0.001$) and that the median interval from onset to visit was 5 days longer in the CMV-IM group ($p < 0.001$). Headache was the only symptom that was more frequent in the CMV-IM group. The EBV-IM group was more likely to exhibit pharyngitis, the classical triad, tonsillar white coat, cervical lymphadenopathy, abdominal tenderness, hepatosplenomegaly, leukocytosis, atypical lymphocytosis, and elevated hepatobiliary enzymes. The platelet count was significantly lower in the EBV-IM group ($p < 0.001$), although the median platelet count was still >150,000/mm^3 (Table 1).

3.2. Logistic regression analysis

Model 1 revealed lower odds ratios (OR) for EBV-IM at older ages and for longer intervals to the first visit, with >40-year-old patients having an OR of 0.13. In contrast, significantly higher ORs for EBV-IM were observed for patients with cervical lymphadenopathy (OR: 11.01) and tonsillar white coat (OR: 4.53). None of the other factors were statistically significant in model 1 (Table 2). Model 2 revealed a lower OR for EBV-IM at older ages, as well as higher ORs for patients with cervical lymphadenopathy, hepatosplenomegaly, leukocytosis, and atypical lymphocytosis. Interval from onset to visit, tonsillar white coat, and thrombocytopenia were not significant factors in model 2 (Table 3). Model 3 revealed a lower OR for EBV-IM at older ages, as well as higher ORs for patients with cervical lymphadenopathy, tonsillar white coat, and mild-to-moderate elevations of atypical lymphocytosis (11–30%), LDH (251–500 IU/L), and GGT (51–300 IU/L). Extreme elevations of atypical lymphocytes, LDH and GGT, as well as hepatosplenomegaly, leukocytosis, ALT elevation, and ALP elevation, were not significant factors in model 3 (Table 4).

Table 2
Logistic regression model for predicting EBV-IM using only H&P factors (model 1).

	Odds ratio [95% CI]	p-value
Age		
<31 years	1	N/A
31–40 years	0.31 [0.094–1.04]	0.057
>40 years	0.13 [0.027–0.65]	0.013
Interval from onset to visit (days)	0.91 [0.85–0.99]	0.021
Cervical lymphadenopathy	11.01 [2.46–49.19]	0.002
Pharyngitis	2.82 [0.77–10.30]	0.12
Headache	0.48 [0.16–1.38]	0.17
Facial edema	1	N/A
Tonsillar white coat	4.53 [1.02–20.19]	0.047
Abdominal tenderness	1.23 [0.20–7.60]	0.82
Classical triad	0.31 [0.042–2.27]	0.25

CI, confidence interval; EBV-IM, infectious mononucleosis caused by the Epstein-Barr virus; H&P, history and physical examinations; N/A, not applicable.

Table 1
Patients' characteristics and results from the univariate analysis.

	All patients (n = 168)	EBV-IM (n = 122)	CMV-IM (n = 46)	p-value
Age (years)	25 [21–32]	24 [21–27]	34 [28–41]	<0.001
Male sex	82 (48.8)	56 (459)	26 (56.5)	0.22
Interval from onset to visit (days)	9 [6–13]	8 [6–10]	14 [9–18]	<0.001
Fever (≥38.0 °C)	41 (24.4)	32 (26.2)	9 (19.6)	0.37
Pharyngitis	114 (67.9)	96 (78.7)	18 (39.1)	<0.001
Cough	22 (13.1)	18 (14.8)	4 (8.7)	0.3
Headache	43 (25.6)	21 (17.2)	22 (47.8)	<0.001
Abdominal pain	16 (9.5)	12 (9.8)	4 (8.7)	0.82
Facial edema	14 (8.3)	14 (11.5)	0	0.016
Tonsillar white coat	66 (39.3)	63 (51.7)	3 (6.5)	<0.001
Cervical lymphadenopathy	103 (61.3)	94 (77.1)	9 (19.6)	<0.001
Abdominal tenderness	21 (12.5)	19 (15.6)	2 (4.4)	0.05
Classic triad	74 (44.1)	69 (56.6)	5 (10.9)	<0.001
Hepatosplenomegaly	101 (60.1)	83 (68.0)	18 (39.1)	0.001
Leukocyte count (10^3/mm^3)	9.05 [6.7–11.9]	10.05 [6.7–12.6]	7.99 [6.7–8.7]	0.0022
Atypical lymphocytes (%)	36.3 [18.0–54.5]	43.5 [27.0–58.5]	15 [3.0–37.0]	<0.001
Platelet count (10^3/mm^3)	172 [141.5–223.5]	166 [138.0–210.0]	195 [162.0–242.0]	<0.001
CRP (mg/dL)	0.7 [0.3–1.6]	0.6 [0.3–1.5]	0.9 [0.5–2.0]	0.13
Bilirubin (mg/dL)	0.65 [0.5–1.0]	0.7 [0.5–1.1]	0.6 [0.5–0.7]	0.059
AST (IU/L)	134.0 [61.0–239.0]	178.5 [85.0–290.0]	59.0 [43.0–86.0]	<0.001
ALT (IU/L)	154.0 [75.0–336.5]	237.0 [106.0–416.0]	76.0 [49.0–126.0]	<0.001
LDH (IU/L)	522.5 [391.5–695.0]	555.0 [428.0–738.0]	401.5 [312.0–503.0]	<0.001
ALP (IU/L)	412.5 [269.0–751.5]	538.0 [312.0–853.0]	285.0 [227.0–385.0]	<0.001
GGT (IU/L)	110.0 [48.0–214.0]	145.0 [65.0–245.0]	51.0 [32.0–111.0]	<0.001

The data are reported as median [interquartile range] or n (%). The univariate analyses were performed using Wilcoxon's rank sum test for continuous variables and the chi-square test for categorical variables.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CMV-IM, cytomegalovirus-related infectious mononucleosis; CRP, C-reactive protein; EBV-IM, Epstein-Barr virus-related infectious mononucleosis; GGT, gamma-glutamyl transferase; LDH, lactate dehydrogenase.

Table 3
Logistic regression model for predicting EBV-IM using H&P factors, imaging results, and blood counts (model 2).

	Odds ratio [95% CI]	p-value
Age		
<31 years	1	N/A
31–40 years	0.21 [0.045–0.98]	0.048
>40 years	0.024 [0.0024–0.24]	0.002
Interval from onset to visit (days)	0.92 [0.84–1.01]	0.074
Cervical lymphadenopathy	12.0 [3.09–46.59]	<0.001
Tonsillar white coat	6.80 [1.21–38.18]	0.029
Hepatosplenomegaly	5.65 [1.52–21.03]	0.01
Leukocytosis	8.11 [1.68–39.11]	0.009
Atypical lymphocytes		
<11%	1	N/A
11–30%	29.27 [2.86–299.25]	0.004
>30%	12.13 [1.92–76.57]	0.008
Thrombocytopenia	3.61 [0.83–15.80]	0.088

CI, confidence interval; EBV-IM, infectious mononucleosis caused by the Epstein-Barr virus; H&P, history and physical examinations; N/A, not applicable.

Table 4
Logistic regression model for predicting EBV-IM using hepatobiliary biomarkers (model 3).

	Odds ratio [95% CI]	p-value
Age		
<31 years	1	N/A
31–40 years	0.24 [0.049–1.24]	0.082
>40 years	0.029 [0.0036–0.24]	0.001
Cervical lymphadenopathy	7.13 [1.89–26.95]	0.004
Tonsillar white coat	12.20 [1.67–89.23]	0.014
Hepatosplenomegaly	2.51 [0.70–9.03]	0.16
Leukocytosis	3.01 [0.64–14.11]	0.16
Atypical lymphocytes		
<11%	1	N/A
11–30%	11.51 [1.03–129.01]	0.048
>30%	4.53 [0.67–30.57]	0.12
ALT		
<41 IU/L	1	N/A
41–200 IU/L	0.33 [0.043–2.57]	0.29
>200 IU/L	0.25 [0.013–4.95]	0.37
LDH		
<251 IU/L	1	N/A
251–500 IU/L	17.65 [1.08–289.53]	0.044
>500 IU/L	13.01 [0.62–274.83]	0.099
ALP		
<351 IU/L	1	N/A
351–500 IU/L	0.50 [0.062–4.06]	0.52
>500 IU/L	3.23 [0.30–34.3]	0.33
GGT		
<51 IU/L	1	N/A
51–300 IU/L	11.11 [1.46–71.48]	0.02
>300 IU/L	11.82 [0.41–337.04]	0.15

ALP, alkaline phosphatase; ALT, alanine aminotransferase; CI, confidence interval; CRP, C-reactive protein; EBV-IM, infectious mononucleosis caused by the Epstein-Barr virus; GGT, gamma-glutamyl transferase; LDH, lactate dehydrogenase; N/A, not applicable.

3.3. Comparison of the regression models

The mean variance inflation factors were 1.65 for model 1, 1.19 for model 2, and 1.61 for model 3. The methods of DeLong et al. [14] revealed that the AUC values were 0.91 for model 1, 0.96 for model 2, and 0.97 for model 3. All of the models were considered highly accurate, based on the AUC values all being >0.9 (Fig. 1).

4. Discussion

This single-center retrospective case-control (case-case) study evaluated whether widely available clinical findings and laboratory test results could be used to differentiate between EBV-IM and CMV-IM. Univariate and multivariate analyses were used to create and test various diagnostic models, which revealed that EBV-IM was significantly predicted by younger age, shorter interval from onset to hospital visit, cervical lymphadenopathy, tonsillar white coat, hepatosplenomegaly, atypical lymphocytosis, and moderate elevations of LDH and GTT.

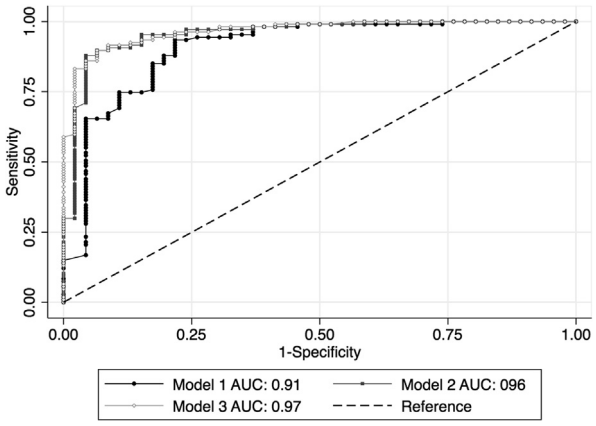

Fig. 1. Comparison of the regression models for differentiating between EBV-IM and CMV-IM. The ROC curves are shown for logistic regression model 1 (explanatory variables: age, interval from onset to visit, cervical lymphadenopathy, pharyngitis, headache, facial edema, tonsillar white coat, abdominal tenderness, classical triad), regression model 2 (explanatory variables: age, interval from onset to visit, lymphadenopathy, pharyngitis, headache, facial edema, tonsillar white coat, abdominal tenderness, classical triad, hepatosplenomegaly, leukocyte count, atypical lymphocyte percentage, and thrombocytopenia), and regression model 3 (explanatory variables: age, interval from onset to visit, lymphadenopathy, pharyngitis, headache, facial edema, tonsillar white coat, abdominal tenderness, classical triad, hepatosplenomegaly, leukocyte count, atypical lymphocyte percentage, alanine aminotransferase, alkaline phosphatase, and gamma-glutamyl transferase). AUC, area under the curve; CMV-IM, IM caused by CMV; EBV-IM, IM caused by EBV; ROC, receiver operating characteristic.

The present study revealed that 46 of 168 patients (27.4%) had CMV-IM, which is noticeably higher than the previously reported proportions of 5–16% [1,2]. Three factors may explain this discrepancy relative to the previous studies and clinical practice. First, limiting the patients to serologically confirmed EBV-IM and CMV-IM cases (i.e., excluding other differential diagnoses of IM) may have produced the higher prevalence of CMV-IM in our study. Second, differences in patient ethnicity and age may be relevant, as most of the previous studies have evaluated non-Asian or pediatric patients [1,2]. Third, a difference in the serological tests (i.e., HAT vs. EBV-specific antibodies) may explain this discrepancy. These issues in the context of serological diagnosis are discussed in the following paragraphs, as the characteristics of serological testing are relevant to the different prevalence of CMV-IM and the significance of our findings.

The HAT and EBV-specific antibody tests have been used for diagnosing EBV-IM, with HAT being the conventional method that is based on the fact that EBV-IM patients transiently develop antibodies that react to erythrocyte antigens from non-human mammals, such as sheep or horses [3]. In addition, HAT has been widely used to serologically support the diagnosis of EBV-IM because it is inexpensive, rapid, and widely available [7]. However, HAT can provide a negative result in 25% of EBV-IM patients during the first week of infection, and a positive result in only 25–50% of EBV-IM patients who are <12 years old [3]. Thus, given its low sensitivity, HAT is no longer recommended for the diagnosis of EBV-IM [7] and is not widely available in Japan. Given that CMV-IM was referred to as a differential diagnosis of “heterophile antibody negative mononucleosis” in a previous review article [3], previous studies have probably differentiated between EBV-IM and

CMV-IM based on HAT results. However, we used specific antibodies for EBV or CMV, instead of heterophile antibodies, and the differences in the serological testing methods may explain the different prevalences of CMV-IM.

The current recommendation is to use EBV-specific antibodies to diagnose EBV-IM because of its better diagnostic ability than that of HAT [7]. Combined use of VCA-IgM, VCA-IgG, and EBNA provides sensitivity of 95–100% and specificity of 86–90% [6], although the sensitivity of VCA-IgM during the first week is 85% [7]. Furthermore, cross-reactivity with CMV-IM has been reported in some patients [8]. Thus, even EBV-specific antibodies may produce inaccurate results. The reliability of CMV-IgM for diagnosing primary acute CMV infection is also problematic, as poor correlations have been reported between several commercial testing kits and false-positive results due to persistent elevation and reactivation [9]. In addition to these technological issues, specific antibody tests for EBV and CMV are expensive, difficult to use in the primary care setting, and require several days to obtain the results. Thus, although EBV-specific antibody tests are good tools for diagnosing EBV-IM, their limitations highlight the importance of not excessively relying on serological tests and to estimate the appropriate pre-test probability based on symptoms, signs, and rapidly available test results before the serological results are received.

Our results indicate that patients with CMV-IM had a longer interval from onset to visit. Previous studies have also revealed that CMV-IM is associated with prolonged fever [15] and elevations of hepatobiliary biomarkers during the post-diagnostic course [2,13]. Thus, the clinical characteristics of CMV-IM before and after the hospital visit appear to be milder but persist for longer than the characteristics of EBV-IM. It is possible that the more severe symptoms of EBV-IM, such as pharyngitis and painful lymphadenopathy, may motivate patients to seek medical treatment sooner than patients with CMV-IM.

Among the classical symptoms and signs, pharyngitis, tonsillar white coat, cervical lymphadenopathy, and hepatosplenomegaly were significantly associated with EBV-IM in the univariate analysis. Furthermore, younger age, lymphadenopathy, and tonsillar white coat were significant predictor of EBV-IM in all regression models. Facial edema has been reported as a specific symptom of EBV-IM, which is caused by disturbance of the orbital lymphatic drainage due to nasopharyngeal inflammation and cervical lymphadenitis [16,17]. In the present study, facial edema was only observed in EBV-IM cases (11/122 vs. 0/46; $p = 0.016$), although it was not a significant factor in any of the regression models. Nevertheless, facial edema in EBV-IM may not be as well-known as other common symptoms and may be underdiagnosed. Abdominal pain and tenderness have also been reported in cases of EBV-IM, which is likely related to remarkable distention of the liver and spleen, acalculous cholecystitis, or splenic infarction [18–20]. Our findings also revealed that abdominal tenderness was significantly more common among EBV-IM cases (15.6% vs. 4.4%; $p = 0.05$), although this factor was also not significant in the regression models.

Hepatosplenomegaly and leukocytosis were significant predictors of EBV-IM in model 2 but not in model 3. In this context, hepatosplenomegaly is a well-known sign of EBV-IM [3,5], although leukocytosis is not. Thrombocytopenia may be a predictor of CMV-IM [2,10], although the previous studies have reported inconsistent results and our findings indicate that thrombocytopenia was more severe, albeit not significantly, in EBV-IM cases. Although atypical lymphocytosis is assumed to be a non-specific reaction caused by various immune disturbances, we found that it significantly predicted EBV-IM in all regression models, and this result is compatible with the findings of previously studies [5,21].

Our analyses, including the multivariate regression models, revealed significantly higher hepatobiliary markers in the EBV-IM cases. Similar to our results, some previous studies of hepatobiliary markers in EBV-IM and CMV-IM cases have revealed significantly higher hepatobiliary biomarkers in EBV-IM patients [10,13]. However, other recent studies have indicated that the differences were not significant [2,22,23]. Our logistic regression analyses revealed that mild-to-moderate elevation of GGT and LDH were independent predictors of EBV-IM, and this finding may be useful when evaluating hepatobiliary biomarkers in IM patients. In this study, we compared hepatobiliary markers of patients with EBV-IM and CMV-IM at the time of first visit because we aimed to demonstrate the clinical differences between EBV-IM and CMV-IM that are recognizable before serological confirmation. However, the time of testing is important for interpretation because reportedly, elevation and recovery of hepatobiliary markers takes longer in CMV-IM cases than in EBV-IM cases. Thus, for precise interpretation, the interval between onset and testing should be considered for each case [2,13].

Comparison of the three regression models (Fig. 1) showed that model 1, which only used H&P factors, could precisely differentiate EBV-IM from CMV-IM (AUC = 0.91), and that model 2, which added hepatosplenomegaly and CBC, further improved the differentiation ability (AUC = 0.96). However, addition of the hepatobiliary biomarkers only improved the AUC value by 0.01 (model 3). Thus, physicians may be able to accurately differentiate between EBV-IM and CMV-IM using H&P factors with or without widely available test results (e.g., CBC and hepatosplenomegaly detection), rather than relying on serological examinations.

The present study has several limitations. First, the sample size was small (168 patients and only 46 CMV-IM cases) and there were relatively large number of explanatory factors, which might affect the odds ratio estimates of variables in the logistic regression models, especially for model 3. Nevertheless, our study was larger than previous similar studies [2,10]. We recognized that the small sample size could not allow us to estimate precise odds ratio values. However, we performed logistic regression for specific clinical and laboratory findings because we aimed to clarify the contribution of each clinical sign and laboratory finding independently. Second, our study had a potential risk of interaction among covariates in the model because clinical symptoms such as lymphadenopathy and hepatosplenomegaly and laboratory data such as lymphocytosis may have deep correlations as they have the same etiology. Although we excluded apparent multicollinearity by evaluating the variance inflation factor, the possibility of interaction and its negative impact on statistical reliability should be addressed. Third, insufficient exclusion of other differential diagnoses of IM could have reduced the reliability of our results. Because no participants (who were serologically confirmed to have EBV-IM or CMV-IM) underwent tests for serological exclusion of other differential diagnoses of IM including infection with toxoplasmosis, HHV-6, HHV-7, adenovirus, rubella, HSV, influenza/parainfluenza, rhinovirus, and coronavirus and acute HIV infection. Although simultaneous mixed infection with EBV or CMV and other IM-causing infectious agents is rare, routine serological exclusion might have improved reliability of the study findings. Given that our study was performed in the metropolitan city of Tokyo, our study findings may have been influenced by potential confounding from undiagnosed concurrent acute HIV infection. Our results should be cautiously applied for patients with clinical IM because the patient groups in the present study were limited to patients serologically diagnosed with EBV-IM and CMV-IM and not all patients with clinically diagnosed IM. Fourth, we could not access data regarding other important factors (e.g., history of present illness and common symptoms, such as fatigue, nausea,

vomiting, and the specific sites of the swollen lymph nodes). Fifth, variance in the clinical expertise of doctors in charge of each case may affect the results of physical examinations and reliability of the study. Sixth, we did not consider lymphocyte counts as we elected to use atypical lymphocytosis because it is more specific and significant than measured lymphocyte counts. It is possible that this approach limits the applicability of our findings, as automatic lymphocyte counts using flow cytometry are often more readily available than atypical lymphocyte counts in clinical practice [24]. Seventh, because we considered the distribution of our data, some cut-off values for the present study were not previously defined, and our values could be considered arbitrary. Further studies are needed to address these limitations, although we believe that our study provides important preliminary evidence that H&P factors and other clinical characteristics may be useful in the diagnosis of EBV-IM.

In conclusion, our retrospective case-control study revealed that younger age, shorter interval from onset to hospital visit, cervical lymphadenopathy, tonsillar white coat, hepatosplenomegaly, atypical lymphocytosis, and moderate elevations of LDH and GTT could be used to differentiate EBV-IM from CMV-IM. These findings imply that evaluating H&P factors, especially in combination with CBC and ultrasonography data, may allow physicians to differentiate between EBV-IM and CMV-IM before receiving serological confirmation.

Conflicts of interest

None.

Authorship statement

ICMJE statement: All authors meet the ICMJE authorship criteria.

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