1	Research	article

 $\mathbf{2}$ The aim of the measurement of Epstein-Barr virus DNA in hydroa vacciniforme and hypersensitivity to mosquito bites 3 4 Tomoko Miyake, MD, PhD¹, Keiji Iwatsuki, MD, PhD¹, Yoji Hirai, MD, PhD¹, Takenobu $\mathbf{5}$ Yamamoto, MD, PhD^{1,2}, Toshihisa Hamada, MD, PhD^{1,3}, Kazuyasu Fujii, MD, PhD^{1,4}, 6 Hideaki Imamura, MD⁵, Shin Morizane, MD, PhD¹. 7 Departments of Dermatology, ¹Okayama University, Graduate School of Medicine, Dentistry 8 and Pharmaceutical Sciences, Okayama, Japan, ²Kawasaki Medical School, Okayama, Japan, 9 ³ TAKAMATSU Red Cross Hospital, Kagawa, Japan, and ⁴Kagoshima University, 10 Kagoshima, Japan. ⁵Departments of Pediatrics, Faculty of Medicine, University of Miyazaki, 11 12Miyazaki, Japan. *Corresponding author: Shin Morizane, Department of Dermatology, Okayama University 1314Graduate School of Medicine, Dentistry and Pharmaceutical Sciences. 2-5-1 Shikata-cho, Kita-ku, Okayama, 700-8558, Japan. (zanemori@cc.okayama-u.ac.jp) 15A running head: The meaning of EBV DNA load for HV and HMB. 16Manuscript word count: Text; 2733 words. Abstract word count: 150. References: 27. 17

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94	

26 Abstract

27	Epstein-Barr virus (EBV) DNA load in the blood increases in post-transplant
28	lymphoproliferative disorders and chronic active EBV infection. In this report, we analyzed
29	the EBV DNA load in the peripheral blood mononuclear cells (PBMCs) and plasma of
30	patients with hydroa vacciniforme (HV) and/or hypersensitivity to mosquito bites (HMB) to
31	understand the clinical significance of EBV DNA load. All 30 patients showed high DNA
32	loads in the PBMCs over the cut-off level. Of 16 plasma samples, extremely high in two
33	samples obtained from patients with hemophagocytic lymphohistiocytosis (HLH). The
34	amount of cell-free DNA in plasma were correlated to the serum levels of lactate
35	dehydrogenase, and inversely correlated to platelet counts. These results indicate that the
36	EBV DNA load in PBMCs can provide one of the diagnostic indicators for HV and HMB and
37	marked elevation of cell-free EBV DNA in plasma might be related to cytolysis such as that
38	observed in HLH.

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41 Keywords: Epstein-Barr Virus DNA load; hydroa vaccniforme; hypersensitivity to mosquito
42 bite; hemophagocytic lymphohistiocytosis.

43 Introduction

44Epstein-Barr virus (EBV)-associated T/NK lymphoproliferative disorders (LPDs) include chronic active EBV infection (CAEBV), hydroa vaccniforme (HV) and hypersensitivity to 45mosquito bite (HMB).^{1,2} HV was first reported as a benign photosensitivity disorder of 46 47childhood, characterized by vesiculopapules on sun-exposed areas. In contrast to this classic type of HV (classic HV: cHV), patients with a severe form of HV associated with edematous 4849face, fever, liver damage, and serious hemophagocytic lymphohistiocytosis (HLH) have been 50reported mainly from the Eastern Asia and Central America under the name of systemic HV (sHV).^{3,4} The patients of sHV was listed in the WHO classification as HV-like LPD. On the 51other hand, patients with HMB, or SMBA in the WHO classification², are characterized by 5253skin ulcers and swelling at the sites of mosquito and other insect bites or injection sites for vaccinations, and frequently show fever and lymphadenopathy. 5-7 Patients with HMB are 5455also encountered in the endemic areas similar to those of sHV/HV-like LPD and CAEBV. 56Unlike patients with cHV, patients with sHV, HMB or CAEBV show a progressive disease with often fatal outcomes.^{4.8} Jeffrey I. Cohen et all reported that in the WHO classification, 5758CAEBV is divided into two major forms: systemic CAEBV and cutaneous CAEBV; overlap 59occurs with some patients with cutaneous CAEBV exhibiting clinical and pathological evidence of systemic EBV infection. The two main forms of cutaneous CAEBV include HV-60 like LPD and severe mosquito bite allergy (SMBA).¹ 61

62	Patients with cHV and HMB usually have a dominant clone of EBV-infected $\gamma\delta T$ cells
63	and NK cells, respectively.9 Patients with sHV without HMB are classified into two groups:
64	$\gamma\delta$ T-cell-dominant or $\alpha\beta$ T-cell-dominant types. Since the patients may have different subsets
65	of EBV-infected T/NK cells in the blood, the clinical manifestations of HV, HMB and
66	CAEBV often overlap with each other in the clinical course. ³
67	The increase of EBV DNA load in the peripheral blood is closely related to the
68	occurrence of EBV-associated LPDs in recipients with organ or hematopoietic stem cell
69	transplant. ^{10.11} Such EBV-associated LPDs are mainly caused by EBV-infected B cells.
70	However, little is known about the clinical significance of EBV DNA load in patients with
71	EBV-associated T/NK LPDs such as HV and HMB. Previously, Kimura et al. and our group
72	reported that the EBV DNA load in the peripheral blood showed no relation to the survival
73	rate of the patients with CAEBV, HV or HMB. ^{4.8}
74	As reported previously, EBV is latently infected in the patient's peripheral blood
75	mononuclear cells (PBMCs) in an episomal fashion in most cases, ¹² and cell-free EBV DNA
76	fragments are mainly detected in the plasma. ¹³⁻¹⁵ Therefore, the EBV DNA loads measured
77	separately in the PBMCs and plasma may have different meanings in different disease
78	conditions. For instance, the EBV DNA load in plasma is generally high in patients with
79	EBV-related hemophagocytic lymphohistiocytosis (HLH) or infectious mononucleosis (IM).
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81	In the present study, we measured the EBV DNA load separately in PBMCs and plasma
82	samples obtained from patients with HV and HMB, and compared the results with clinical
83	manifestations such as the disease subtype and disease severity, and laboratory data including
84	blood chemistry tests, the major subset of EBV-infected lymphocytes and its cell numbers.
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100 **1. Material and Method:**

101 **1.1. Patients**

- 102 A total of 30 patients including 12 patients with cHV, 9 with sHV, 5 with HMB only,
- 103 and 4 with HMB associated with HV-like eruptions were enrolled (Table 1). The control
- 104 peripheral blood samples were obtained from 4 patients with IM and 24 healthy individuals.
- 105 This study was approved by our ethical committee (the institutional review board of
- 106 Okayama University Hospital) in accordance with the 1975 Declaration of Helsinki.
- 107 **1.2. Real-time PCR**
- 108 DNA was extracted from PBMCs of these patients at our hospital first visit or during their
- 109 clinical courses using a QIAamp[™] Blood Kit (Qiagen, Netherlands), and polymerase chain
- 110 reaction (PCR) amplification was performed using QuantiTect[™] Probe PCR (Qiagen,
- 111 Netherlands) by a Roche light cycler (Roche, Switzerland). The PCR primers for this assay
- 112 were selected in the *Bam*HI M region. We detected the EBV DNA loads in PBMCs and
- 113 plasma using this method. ^{3.4,9,13}

114 **1.3.** Detection of cell-free EBV DNA with DNase treatment

115 Cell-free EBV DNA consists of encapsidated virions or naked genomes derived from dying

- 116 cells. Naked viral DNA was degraded by DNase I, but the viral capsid protects encapsidated
- 117 virions from DNase I-digestion. After incubation of plasma samples (200µL) with or without
- 118 25µL DNase I (Promega, Madison) for 1hr at 37°C, DNA was extracted with QIAamp DNA

- above. EBV virion levels in plasma were calculated by the ratio of DNase I-treated EBV
- 121 DNA load to DNase I-untreated EBV DNA load. Our previous study confirmed that the cell-
- 122 free EBV DNA in the plasma of 7/9 patients with HV and/or HMB (77.8 %) exclusively
- 123 consisted of the naked form of EBV genomes sensitive to DNase treatment.¹³
- 124 **1.4. Reverse transcriptase (RT)-PCR**
- 125 RNA was extracted from the samples with TRIZOLTM reagent (Gibco BRL, Gaithersburg,
- 126 MD), and the cDNA was generated with random hexamer (Takara). The EBV reactivation
- 127 marker, BZLF1 was amplified by RT-PCR, using BZLF1-specific outer primers as previously
- 128 described. ^{17.18} The integrity of the RNA was checked by the parallel amplification of beta-2-
- 129 microglobulin (β 2-MG).

130 **1.5. Statistical analysis**

- 131 We analyzed the relation of the characteristic skin symptoms, such as vesiculo-papules,
- 132 erythema and scale crusts, and the DNA load during each patient's clinical course. The
- 133 DNA load at the first visit was evaluated in relation to the routine laboratory test results and
- 134 outcomes of patients using SPSS Windows version 20.0 software. Fisher's test and the
- 135 Mann-Whitney t-test were used to evaluate differences among groups (P < 0.05).
- 136 Relationships between the DNA load in PBMC and plasma, or the DNA load and routine
- 137 laboratory tests were evaluated using Pearson's test.

138 **2. Results**

139Cellular and cell-free EBV DNA load in patients with IM, HV and HMB 2.1. 140 The majority of healthy adults are known to possess a small number of EBV-infected B cells 141 in the peripheral blood. Thus, we measured EBV DNA loads in PBMCs (n=30) and plasma 142samples (n=21) obtained from healthy individuals. In these individuals, the EBV DNA load 143 ranged from 0.5 to 108 copies/ μ g DNA in the PBMCs, and from 50 to 100 copies/mL in the 144plasma samples. The EBV DNA load in plasma (4/4 cases) were exclusively composed of 145cell-free EBV DNA fragments, sensitive to DNase-treatment. Based on these findings, we 146 determined the cut-off values as follows: 120 copies/ μ g DNA (the mean+3SD) for PBMCs, 147and 130 copies/mL (the mean+3SD) for plasma samples. 148 Then, we measured EBV DNA loads in 30 samples of PBMCs and 16 plasma samples 149 from patients with EBV-associated T/NK LPDs including HV and HMB. The EBV DNA 150loads in these PBMCs ranged from 446 to 140,000 copies/µg DNA (the mean: 28,486 copies/ 151 μg DNA), all of which were higher than the cut-off value. The EBV DNA loads in the 152plasma samples ranged from 0 (detection limit) to 50,000 copies/mL (the mean: 8,446 copies/mL), and 3 of the 16 samples were below the cut-off value (Figure 1). Of the 16 153154plasma samples, two samples obtained from patients with active HLH showed extremely high 155levels of cell-free DNA (50,000 and 28,600 copies/mL, respectively) (Figure 1). There was 156no relation between PBMC and plasma levels of the patients with HV and HMB (r=0.21).

157	The EBV DNA load in PBMC or plasma showed no relation to the survival rate of the
158	patients with HV and HMB (Figure 2.). The 5 of 6 patients with sHV died. The main cause of
159	death was one patient from bowel bleeding, one from HLH, one from multiorgan failure,
160	which showed invasion of EBV positive T cells in the esophagus, gastrointestinal tract and
161	myocardium, and two from unknown. The 2 of 9 patients with HMB only and HMB+HV
162	died. The main cause of death was related to HLH in one HMB patient and was unknown in
163	one HMB+HV (Table 1).
164	In patients with IM characterized by primary infection of EBV to B cells (n=4), all patients
165	except one had higher levels of EBV DNA loads in the PBMCs than the cut-off value,
166	ranging from 13.3 to 2,680 copies/ μ g DNA, but the mean EBV DNA load in the PBMCs of
167	the IM group (mean: 1,783 copies/ μ g DNA) was much lower than that of the HV and/or
168	HMB group (mean: 28,486 copies/µg DNA) (Figure 1).
169	Correlation of EBV DNA load to the disease subtype, severity, and other laboratory
170	data
171	Of the 30 PBMC samples examined, the PBMC EBV DNA loads ranged from 770 to 73,000
172	copies/ μ g DNA (mean: 15,948) in patients with cHV, 446 to 101,000 copies/ μ g DNA
173	(mean: 37,736) in patients with sHV, and 1,900 to 140,000 copies/ μ g DNA (mean; 41,653)
174	in patients with HMB with or without HV. There was no correlation between each clinical
175	subtype, even though the dominant EBV-infected lymphocyte subsets and their numbers

176	varied. Our series of patients with cHV (patient 2 in Table 1), who were in a stable disease
177	condition, had high EBV DNA loads (73,000 copies/ μ g), comparable to those of other
178	subtypes, sHV and HMB, which had more serious disease or fatal outcome.
179	When cellular EBV DNA loads were monitored in the same patient with HV-like lesions,
180	there was no clear correlation to the severity of cutaneous manifestations. The expression of
181	the EBV reactivation marker, BZLF1 mRNA, is not related to the DNA load in PBMC or
182	plasma (Table 1). The cellular EBV DNA loads in the PBMCs (n=30) showed no correlation
183	to the following laboratory test results: white blood cell (WBC) counts, lymphocyte numbers,
184	hemoglobin (Hb), platelet (PLT) counts, lactate dehydrogenase (LD), aspartate
185	aminotransferase (AST), alanine transaminase (ALT) (Figure 3), or lymphocyte numbers,
186	atypical lymphocyte numbers, and ferritin (data not shown). However, there was a relation
187	between the cellular EBV DNA loads in the PBMCs and soluble interleukin-2 receptor (sIL-
188	2R) (r=0.74) (Figure 3). Therefore, the results of EBV DNA loads in the PBMCs did not
189	differentiate the benign subtype from the serious subtypes or represent the severity of the
190	clinical course.
191	In contrast, the amounts of cell-free EBV DNA in the plasma samples (n=16) were
192	correlated to elevated levels of serum LD and ALT, respectively (r=0.90 and 0.66), and
193	reversely correlated to the PLT counts (r=-0.68) (Figure 3). The two patients with
194	extremely high levels of cell-free EBV DNA in the plasma (patients 15 and 26 in Table 1)

195 showed high levels of LD (764 and 575 IU/L), and AST (48 and 17 IU/L), and low PLT

196 counts (3.1 and 8.4 x $10^4/\mu$ L).

197 2.2. Patients with extremely high levels of cell-free EBV DNA in the plasma

- **Table 1: patient 15 (the same patient as in Ref. 19)**
- 199 A 7-year-old girl of sHV presented with an abrupt onset of fever of 38 °C, discharge blood and 200 aphtha. The blood counts revealed white blood cells at 20500/ μ L, hemoglobin (6.9 g/dl), Plt 201 (28.5 x 10⁴/ μ L), LD (204 IU/L), AST (22 IU/L), and ALT (12 IU/L). Her colon lesions showed
- 202 many CD8-positive cells and EBER-positive cells. After 4 months, she presented with vesicle-
- 203 papules and scarring on her leg. Her skin lesions were waxed and waned and were treated
- with steroid pulse, cyclophosphamide or cyclosporine.
- 205 After about 21 months, she had a high-grade fever, and her blood counts revealed white
- blood cells at 900/ μ L, thrombocytopenia at 3.1 x 10⁴/ μ L, and an increased percentage
- 207 (86.3%) of CD8 cells. The elevated serum levels of LD (764 IU/L), AST (48 IU/L), ALT

208 (75 IU/L), sIL-2 receptor (4,500 U/mL) and ferritin (371.3 ng/mL), and the increase of

- nucleated cells (130000/ μ l) and blood phagocytosis in bone marrow test, together with
- thrombocytopenia, suggested the occurrence of HLH.
- 211 The EBV DNA loads in PBMCs and plasma revealed $80,000 \text{ copies}/\mu g$ DNA and 50,000
- 212 copies/mL, respectively. She was diagnosed as having HLH associated with CAEBV, and
- was initially treated with methylprednisolone pulse (20mg/kg/day×3 day) and intravenous

214 immunoglobulin, followed by cord blood transplantation with a successful outcome (Table

215 2).

216 Table 1: patient 26 (the same patient as in Ref. 20) 217A 15-year-old Japanese boy with CAEBV had a 12-year history of HMB. His complete blood 218 counts revealed a normal range of white and red blood cells, thrombocytopenia (8.4 x 219 $10^4/\mu$ L), and an increased percentage (40%) of NK cells with a CD2+, CD3-, CD4-, CD8-, 220CD16-, CD56+ phenotype. The elevated serum levels of LD (575 IU/L), AST (17 IU/L), 221ALT (79 IU/L), sIL-2R (4,760 U/mL) and ferritin (268 ng/mL), together with 222thrombocytopenia, suggested the occurrence of HLH. The patient was treated with a daily 223dose of prednisolone, 10 mg, and the dosage was increased to 40 mg/d when systemic 224symptoms, such as a high grade fever and splenomegaly, appeared. The cellular EBV DNA 225load in the PBMCs was moderately elevated (1,900 copies/ μg DNA), while the cell-free 226 EBV DNA in plasma had increased up to 28,600 copies/mL. A bone marrow transplant was 227 prepared from an HLA-matched donor because of disease progression, but unfortunately the 228patient died of a serious attack of HLH (Table 2). 229

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233 **Discussion**

234The present study showed that all 30 patients with HV and HMB had high EBV DNA loads 235in the PBMCs, well over the cut-off value (120 copies/ μg DNA), while only 3 of 16 patients 236 contained cell-free EBV DNA in the plasma at levels over the cut-off value (130 copies/ml). 237 Our data indicate that the measurement of EBV DNA load in the PBMCs provides a reliable 238 diagnostic indicator for HV and HMB, as compared with the detection of cell-free EBV DNA in plasma. This is the same result of the patients with CAEBV by Ito and Kimura et al.^{21.22} 239240But this is the opposite result of the previous observations in a largely immunocompromised and hospitalized cohort by Kanakry et al: ²³ In their study, the cell-free EBV DNA in plasma 241242performs better than the cellular EBV DNA load in PBMCs. The discrepancy must derive 243from differences in the host immunological conditions and the cell lineage with EBV 244infection: mainly B-cells in immunocompromised hosts, and T/NK cells in HV and HMB. 245There was no correlation between the EBV DNA loads and the clinical features of HV 246and HMB, including the disease subtype, disease severity, prognosis, major EBV-infected 247lymphocyte subset, and cell numbers. But the monitoring of the cellular EBV DNA load in 248PBMCs does not necessarily provide useful information for disease progression and the 249occurrence of serious complications. The observation periods of some patients are relatively 250short, so we need more follow up times for prognosis.

Among 16 plasma samples from HV and HMB, two plasma samples revealed extremely

253	Our results corresponded well with previous observations that patients with EBV-associated
254	HLH or IM generally showed high EBV DNA loads in PBMCs and plasma. ²⁴ Regarding the
255	pathogenesis of IM and HLH, EBV-infected cells are induced to cell death by host immune
256	responses or apoptosis, and cellular EBV DNA is released from the cells. ¹³⁻¹⁵ Our previous
257	data showed that the cell-free EBV DNA in plasma exclusively consisted of naked EBV
258	DNA derived from cytolysis in patients with HV and HMB. ¹³ Furthermore, Kawada <i>et al</i>
259	showed that the plasma DNA load of the patients with CAEBV were significantly high
260	during the active diseases. ²⁵ Thus, we compared the amounts of cell-free EBV DNA with
261	blood test results related to cytolysis. The results showed that the amount of cell-free DNA
262	was correlated to the serum levels of LD, and inversely correlated to the PLT counts in our
263	series (Figure 3.). These results were strongly affected by the two patients with HLH.
264	We investigated the plasma of the patient 15, treated with or without DNase-treatment by
265	using real-time PCR method. DNase-treatment resistant EBV DNA ratio (the DNA treated
266	with DNase-treatment/ the DNA treated without DNase-treatment) in the plasma were
267	13.85% before HLH, and 0% during HLH. Therefore, increased levels of cell-free EBV DNA
268	in the plasma might be related to cytolysis of EBV-infected cells and an alert for serious
269	complications such as HLH.

271 Conclusion

- 272 The measurement of cellular EBV DNA load in PBMCs provides a diagnostic indicator
- 273 for HV and HMB but does not reflect the clinical subtypes or outcomes. The amount of cell-
- 274 free DNA in plasma is less useful as a diagnostic indicator, but its marked elevation might be
- 275 related to serious complications such as HLH.

$= \cdot \cdot = \mu_{+} \cdot \cdot \cdot = \mu_{+} \cdot \cdot$	277	Epstein-Barr virus	(EBV),	lymphoproliferative	disorders (LPDs),	hydroa vaccniforme	(HV)
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- 278 hypersensitivity to mosquito bite (HMB), peripheral blood mononuclear cell (PBMC),
- 279 classical HV (cHV), systemic HV (sHV), hemophagocytic lymphohistiocytosis (HLH),
- 280 chronic active EBV infection (CAEBV), infectious mononucleosis (IM), polymerase chain
- 281 reaction (PCR), platelet (PLT), lactate dehydrogenase (LD), aspartate aminotransferase
- 282 (AST), alanine transaminase (ALT), soluble interlukin-2 receptor (sIL2R), bone marrow
- 283 transplantation (BMT)
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287 **Conflict of interest:** The authors have no conflict of interest to declare.

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381	Figure legends.

Figrue 1. The EBV DNA load in PBMCs, but not in plasma, has a diagnostic value for

383 HV and HMB.

- All 30 HV/HMB patients showed significantly higher levels of DNA in the PBMCs than
- those of healthy individuals. Patients with sHV (\Box) and HMB (O) showed HLH.

Figure 2. The relation of outcome and EBV DNA load or the EBV DNA load in PBMC
and plasma.

- 388 There were no correlations between the EBV DNA load and the patient's outcome or the
- 389 EBV DNA load in PBMC and plasma (r=0.21).

Figure 3. The relations of EBV DNA loads in PBMCs and plasma and routine

- **391** laboratory tests results.
- 392 The EBV DNA load in plasma showed a close correlation with PLT, LD and ALT,
- 393 respectively (r= -0.68, 0.90 and 0.66).
- 394 Figure1-3 Abbreviation : HV: hydroa vaccniforme, HMB: hypersensitivity to mosquito bite,
- 395 IM: infectious mononucleosis PBMC: peripheral blood mononuclear cell, HLH:
- 396 hemophagocytic lymphohistiocytosis, WBC: white blood cell, Hgb: hemoglobin, Plt:
- 397 platelet, LDH: lactate dehydrogenase, AST: aspartate aminotransferase, ALT: alanine
- 398 transaminase, sIL2R: soluble interlukin-2 receptor.
- 399
- 400 **Table legend.**
- 401 **Table 1. The list of 30 patients with HV and/or HMB.**

Table 2. These patients with sHV or HMB of HLH.

403 These patients showed extremely high levels of EBV DNA load in the plasma during HLH.

Table 1.

patient No./ Sex/ Age at onset (y)	clinical subtype	follow up time (y)	WBC (/µl)	Ly/Aty (/µl)	Hgb (g/dl)) Plt (10 4/μl)	LDH (IU/L)	AST/ALT (IU/L)	sIL2R (IU/L)	Ferritin (ng/mL)	outcome	EBVDNA load plasma/PBMC	BZLF mRNA	subset	gd (/µl)	ab (/µl)	NK (/µl)
1/f/5	cHV	10	5050	1398.9/0	12.5	20.6	193	17/9	ND	ND	Α	ND/1100	-		ND	ND	ND
2/m/3	cHV	4	14490	7389.9/144.9	12.8	27.1	244	30/16	1010	18	Α	ND/73000	-		ND	ND	ND
3/f/4	cHV	7	8200	3690/0	12.8	29.1	213	25/11	ND	ND	Α	ND/11000	-		ND	ND	ND
4/m/1	cHV	2	7440	3742/0	11.8	36.4	257	34/12	ND	ND	Α	2120/12000	-	γδ	409.2	1647.3	242
5/m/5	cHV	4	6980	2959/0	12.7	27.6	239	28/11	ND	ND	Α	ND/1500	-	γδ	548.1	1428.5	228
6/m/5	cHV	5	7000	3350/0	13.6	36.2	393	22/17	ND	ND	Α	ND/770	-		ND	ND	ND
7/m/4	cHV	10	5100	892.8/0	12	24.3	425	27/18	323	ND	Α	1060/3610	-	γδ	154	1354	234
8/f/5	cHV	9	4900	2205/0	ND	30.6	255	28/15	ND	ND	Α	ND/12000	-		ND	ND	ND
9/f/6	cHV	1	5420	ND/ND	12.5	17.1	218	47/49	ND	ND	Α	ND/1000	-	γδ	ND	ND	ND
10/m/5	cHV	3	6410	3200/0	12	26.4	292	27/13	ND	ND	Α	1275/29300	-	γδ	240	2030	356
11/f/17	cHV	3	4720	2200/0	14.9	19.5	161	20/13	ND	ND	Α	0/ 36100	-	γδ	299	1335	173
12/f/8	cHV	4	6150	3198/0	13	25.7	229	53/51	ND	ND	Α	1070/10000	-	γδ→ΝΚ	187.9	922.4	721.9
13/m/6	sHV	4	5700	3169.2/0	14.3	24.4	238	21/14	ND	ND	А	ND/46000	-		ND	ND	ND
14/f/17	sHV	7	3900	819/0	11.4	18.7	200	24/26	ND	115.1	Α	ND/3500	-		ND	ND	ND
15 ※ /f/5	sHV	6	1100	583/22	8.5	3.1	764	48/75	4500	371.3	A	50000/80000	-		ND	ND	ND
16/f/17	sHV	7	3940	1059.8/0	10.6	21.2	308	36/28	ND	ND	D	811/446	+		ND	ND	ND
17/f/25	sHV	9	12800	2560/0	12	29.1	189	30/35	ND	65.6	D	1460/101000	+	αβ	0	12416	3200
18/f/10	sHV	8	5800	2204/0	12.9	17.5	124	20/17	14.6	ND	D	800/35000	ND		ND	ND	ND
19/m/13	sHV	4	5170	ND/ND	13.9	22.4	200	17/9	ND	ND	А	0/3160	-	αβ	46	1506	66
20/f/68	sHV	14.1	NC	ND/ND	ND	ND	ND	ND/ND	ND	ND	D	0/6520	+	αβ	ND	ND	ND
21/m/74	sHV	4.5	6200	ND/ND	11.3	9.2	300	216/116	ND	ND	D	13000/64000	+	αβ	7.75	1444.6	511.5
22/m/6	HMB	5	3700	1258/37	12.4	5.1	318	30/22	108	ND	Α	ND/5700	+	NK	ND	1157	1182
23/f/8	HMB	3	8800	7110/0	12.1	11.6	400	221/400	55	ND	Α	ND/28000	-	NK	ND	ND	ND
24/m/6	HMB	3	4870	1821/0	12.2	18.2	251	26/10	ND	ND	Α	368/5580	-	NK	70	597	931
25/m/5	HMB	3	5200	ND/ND	11.1	16.9	454	52/62	ND	ND	Α	ND/140000	ND	NK	ND	ND	ND
26 ×/m/3	HMB	4	8380	ND/ND	15.2	8.4	575	17/79	4760	268	D	28600/1900	+	NK	ND	ND	1681
27/m/20	HMB+HV	14	4900	1900/0	15.3	19.6	166	21/19	ND	ND	D	1070/31700	+	NK	ND	ND	1681
28/f/8	HMB+HV	2	7780	4745.8/0	12	35.7	266	38/34	34.8	531	Α	8160/21000	-	NK	ND	465	1788
29/f/4	HMB+HV	19	3310	1095/0	12.6	27.5	243	23/10	ND	ND	Α	ND/78700	ND	NK	ND	409	726
30/f/2	HMB+HV	1	5600	ND/ND	12.7	23.8	386	130/151	ND	ND	Α	ND/11000	ND	NK	ND	ND	ND

Abbreviation : f: female, m: male, cHV: classical hydroa vaccniforme, sHV: systemic hydroa vaccniforme, HMB: hypersensitivity to mosquito bite, CAEBV: chronic active Epstein-Barr virus infection, WBC: white blood cell, Ly: lymphocyte, Aty: atypical lymphocyte, Plt: platelet, LDH:

Table 2.

	Patien	t 15. A sHV p	atient with H	Patient 26. A HMB patient with HLH				
	day-10	day0	day13	day29	day -11	day 0	day 163	
AST (IU)	43	99	138	53	4	106	34	
ALT (IU)	93	109	279	189	40	265	78	
LDH (IU)	413	992	476	278	571	980	615	
Plt × 10 ³ (/ μ I)	166	24	57	285	116	38	78	
Ferritin (IU)	ND	2368.5	ND	25.1	129.5	393.3	114.5	
EBV DNA load (plasma) (copy/ml)	ND	500000	300	600	3680	28600	6590	

Abbreviation : HMB: hypersensitivity to mosquito bite, sHV: systemic hydroa vaccniforme, HLH: hemophagocytic lymphohistiocytosis, AST: aspartate aminotransferase, ALT: alanine transaminase, LDH: lactate dehydrogenase, Plt: platelet, ND: not done, day0 : the occurrence of HLH.