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Urinary β2-microglobulin as an early marker of infantile enterovirus and human parechovirus infections

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Abstract

Enterovirus and human parechovirus (HPeV) are RNA viruses belonging to the family *Picornaviridae* that frequently infect infants. These infections show a wide variety of clinical manifestations, from mild to severe. However, there are no known early clinical markers for diagnosis and prediction of disease severity. The aim of this study was to examine the clinical utility of urinary beta 2-microglobulin (β2MG) for the early detection and prognosis of infantile enterovirus and HPeV infections.

This retrospective study included 108 full-term infants younger than 60 days of age, including 15 with enterovirus or HPeV-3 (enterovirus/HPeV-3), 22 with respiratory syncytial virus (RSV), and 24 with bacterial infections. Laboratory data and clinical characteristics were compared among these 3 groups. Of the 15 patients with enterovirus/HPeV-3, 6 were treated with intravenous immunoglobulin (IVIG subgroup) because of severe clinical conditions.

Urinary β2MG to creatinine ratio (β2MG/Cr) was significantly higher in the enterovirus/HPeV-3 group compared to bacterial and RSV infection groups (both $P < .001$). In the enterovirus/HPeV-3 group, mean peak urinary β2MG/Cr was observed on day 1 or 2. Urinary β2MG/Cr values were significantly higher in the IVIG subgroup than the non-IVIG subgroup ($P < .001$).

Increased urinary β2MG/Cr in early-stage infection may be a useful clinical marker for the detection and prediction of infantile enterovirus and HPeV infection severity.

Abbreviations: β2MG = beta 2-microglobulin, AST = aspartate aminotransferase, CK = creatine kinase, Cr = creatinine, CRP = C-reactive protein, HPeV = human parechovirus, IVIG = intravenous immunoglobulin, LDH = lactate dehydrogenase, MHC = major histocompatibility complex, PLT = platelets, rRT-PCR = real-time reverse transcriptase polymerase chain reaction, RSV = respiratory syncytial virus, VP = viral protein, WBC = white blood cells.

Keywords: enterovirus, human parechovirus, intravenous immunoglobulin, urinary β2 microglobulin

1. Introduction

The *Picornaviridae* family RNA viruses enterovirus and human parechovirus (HPeV) are frequent causes of febrile infections in infants.[1] Enterovirus and HPeV infections show a variety of clinical manifestations ranging from mild gastrointestinal symptoms to potentially fatal diseases such as meningitis and sepsis.[1–3] Therefore, the diagnosis and prediction of disease severity in the early stage of infection may aid in guiding therapeutic intervention to prevent the development of serious conditions, unnecessary treatments, and prolonged hospitalization. The gold standard for diagnosing enterovirus and HPeV infections is real-time reverse transcriptase polymerase chain reaction (rRT-PCR);[4] however, it is not a routine diagnostic procedure available in many clinical settings.

Beta 2-microglobulin (β2MG) is a low molecular weight protein that constitutes the light chain of class I major histocompatibility complex (MHC-I) proteins present on the surface of almost all nucleated cells.[5] Expression of MHC-I is particularly high on the surfaces of activated lymphocytes and macrophages during infection.[6,7] Increased serum β2MG has also been reported in several viral infections, suggesting utility as a marker of lymphocyte activation and associated immune reactions.[8–12] However, the clinical significance of β2MG and the implications of β2MG upregulation for viral infections remain unclear.

In this study, we examined the clinical utility and implications of urinary β2MG in infants with enterovirus, HPeV, and other viral or bacterial infections.

2. Patients and methods

2.1. Study population and sample collection

This retrospective study included 108 otherwise healthy full-term (37–41 weeks) infants younger than 60 days of age hospitalized for fever ≥ 37.8°C at Minho City Hospital between November 2014 and August 2017.

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Sepsis workup and rapid viral testing were conducted in all the 108 patients based on the seasonal spread of respiratory syncytial virus (RSV), rotavirus, influenza, and adenovirus infections. Among the cases in which these infections were excluded, those with suspicious clinical presentations were screened for enterovirus and HPeV by RT-PCR after obtaining oral informed consent from legal guardians. The ethics committee of Osaka Institute of Public Health approved the screening protocol. Control data were obtained from patients diagnosed with RSV infection by rapid viral testing and from patients with bacterial infections based on positive urine, serum, or cerebrospinal fluid culture or serum C-reactive protein (CRP) above 2 mg/dL.

2.2. Clinical and laboratory data analysis

The clinical characteristics and laboratory data were examined and compared among the following patient groups: enterovirus or HPeV (enterovirus/HPeV), RSV, and bacterial infection. In addition, we compared maximum urinary β2MG to creatinine ratio (β2MG/Cr) among the enterovirus/HPeV cases and the other groups. The patients in the enterovirus/HPeV group were classified further according to intravenous immunoglobulin (IVIG) treatment into an IVIG subgroup (patients 1–6) and a non-IVIG subgroup (patients 7–15) for comparison of clinical characteristics and laboratory data. Laboratory data including white blood cells (WBCs), platelets (PLTs), aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), and CRP at the time of peak urinary β2MG/Cr were included in the analysis. Finally, ferritin levels were examined in the IVIG subgroup.

2.3. Screening and genotyping of enterovirus and HPeV based on the viral genome

The MagDEA Viral DNA/RNA 200(GC) kit (Precision System Science Co., Japan) was used for RNA extraction according to the manufacturer's instructions. The RT-PCR screen for detection of enterovirus RNA[13] and HPeV RNA[14] was performed using StepOnePlus (Applied Biosystems, Foster City, CA). The samples were classified as genome-positive for enterovirus[15] or HPeV[16] according to the sequences of the enterovirus viral protein 4-2 region (VP4-2) and the HPeV VP1 region retrieved from GenBank using the Basic Local Alignment Search Tool (BLAST).

2.4. Urinalysis and urinary β2MG measurement

Urine samples were obtained by the usage of catheter or urine collection bags several times during hospitalization. Urine blood, glucose, protein, and uric acid were included in urinalysis for screening urinary system diseases. Urinary β2MG was determined by latex immunoassay (Wako, Osaka, Japan) and normalized to urinary Cr levels.

2.5. Statistical analysis

The differences among the enterovirus/HPeV, RSV, and bacterial infection groups were evaluated by the Kruskal–Wallis analysis followed by the Steel–Dwass test for pair-wise comparisons. The Mann–Whitney *U* test was used to compare the IVIG subgroup to the non-IVIG subgroup. However, given the small sample sizes, statistical power analysis was first employed to assess whether statistical significance was correct. JMP software version 12.0 (SAS Institute, Cary, NC) was used for all the statistical analyses except for statistical power analysis. A p value $< .05$ was accepted as significant. SPSS version 23.0 (IBM Corp, Armonk, NY) was used for statistical power analysis. Significance was considered correct when the power was 0.80 or higher.

3. Results

3.1. Detection of viral and bacterial pathogens

Of 108 patients examined, RSV was detected in 22 and bacterial infections in 24. Among the 24 patients with bacterial infections, urinary tract infections were found in 10, meningitis in 3, and other infections in 11 patients. Of the 20 patients evaluated by RT-PCR, 15 were positive for enterovirus or HPeV according to at least one throat swab, fecal sample, or serum sample. The detected viruses were coxsackievirus B5 in 5 patients, HPeV type 3 (HPeV-3, recently renamed parechovirus A3) in 4, echovirus 9 in 3, and echovirus 18, echovirus 25, and coxsackievirus B1 in 1 patient each. Of the remaining 47 patients, 4 were infected with rotavirus, 3 with influenza virus, and 1 each with adenovirus and cytomegalovirus. No specific pathogen could be found in the other 38 patients.

3.2. Clinical characteristics of the patients with enterovirus or human parechovirus infections

Table 1 summarizes basic characteristics of patients with enterovirus or HPeV-3 infection. Viruses were detected in the fecal samples of all the patients, in throat swabs of 12 out of 15 patients, and in serum samples of 9 out of 12 patients. Rash and diarrhea were observed in 11 out of the 15 enterovirus/HPeV-3 patients. Abdominal distention was observed in 3 patients with HPeV-3 infection and 1 patient with echovirus 9 infection. In addition, apnea was observed in 2 patients with HPeV-3 infection. IVIG was administered to 6 patients because of poor general clinical condition. None of the patients with enterovirus or HPeV-3 infection had sequelae.

Maximum temperature and heart rate were significantly higher in the enterovirus/HPeV group compared to the bacterial and RSV infection groups (Table 2, Fig. 1A and B). Furthermore, the maximum temperature and respiratory rate were significantly higher and the duration of fever was significantly longer in the IVIG subgroup than the non-IVIG subgroup (Table 3).

3.3. Laboratory findings

Maximum urinary β2MG/Cr was significantly higher in the HPeV-3 infection group than the enterovirus group, and significantly higher in both enterovirus and HPeV-3 groups than the other groups (Table 2 and Fig. 1D and E). LDH levels were significantly higher in the enterovirus/HPeV group than in the RSV infection group (Fig. 1C). The WBC and CRP levels were significantly higher in the bacterial infection group than the enterovirus/HPeV and RSV infection groups (Table 2). In the enterovirus/HPeV group, the mean maximum urinary β2MG/Cr was observed on day 1 or 2 and tended to decrease gradually thereafter (Fig. 2A), a trend that was more marked in the IVIG subgroup (Fig. 2B). Both LDH and maximum urinary β2MG/Cr were significantly higher in the IVIG subgroup than the non-IVIG subgroup (Table 3). Evaluation of the laboratory data over time revealed that the highest mean AST, LDH, CK, and ferritin levels were observed on day 3 or 4 (Fig. 2C–F). In the enterovirus/HPeV

Table 1

Clinical characteristics and laboratory findings of the patients with enterovirus and human parechovirus.

No.	Age, days, Sex	Virus	Isolation site	Maximum U-β2MG/Cr (μg/gCr)	Maximum fever, °C, heart rate, beats per minute, respiration rate, per minute	Minimum systolic blood pressure, mm Hg	Treatment	Fever duration, days	Clinical features
1	60/ male	HPeV3	T(+), Fe(+), S(+)	day 2 202760	40.6, 212, 62	76	Days 2–3 IVIG	5	Rash, diarrhea, seizure, distended abdomen
2	40/ female	HPeV3	T(+), Fe(+), S(+)	Day 2 195755	39.8, 204, 58	74	Days 2–3 IVIG	4	Rash, diarrhea, distended abdomen
3	18/ male	HPeV3	T(+), Fe(+), S(+)	Day 3 184525	39.8, 199, 60	70	Days 1–2 IVIG	4	Rash, diarrhea, apnea, distended abdomen
4	23/ male	E18	T(−), Fe(+), S(n/d)	Day 3 177780	39.8, 190, 56	70	Day 4 IVIG	5	Rash, diarrhea
5	6/ male	HPeV3	T(+), Fe(+), S(n/d)	Day 1 174400	40.1, 191, 58	74	Day 4 IVIG	4	Rash, diarrhea, apnea
6	43/ male	CVB5	T(+), Fe(+), S(n/d)	Day 7 73319	39.6, 200, 48	63	Day 3 IVIG	4	Rash, diarrhea
7	8/ female	CVB5	T(+), Fe(+), S(+)	Day 1 99718	38.7, 179,46	94		5	Rash, diarrhea
8	13/ male	CVB1	T(+), Fe(+), S(+)	Day 3 53340	39, 180, 57	84		4	Rash
9	29/ male	E25	T(+), Fe(+), S(+)	Day 3 42300	39.6, 185, 48	92		2	Diarrhea
10	25/ female	E9	T(+), Fe(+), S(+)	Day 1 48533	38.5, 185, 44	90		3	Diarrhea
11	23/ male	E9	T(+), Fe(+), S(+)	Day 2 39486	39.1, 206, 44	74		3	Rash, distended abdomen
12	24/ female	CVB5	T(+), Fe(+), S(−)	Day 2 37467	38.7, 195, 50	n/d		3	Rash
13	16/ male	E9	T(+), Fe(+), S(+)	Day 1 34200	39.1, 178, 56	88		3	diarrhea
14	21/ female	CVB5	T(−), Fe(+), S(−)	Day 2 19550	38.4, 172, 48	66		3	(−)
15	41/ female	CVB5	T(−), Fe(+), S(−)	Day 2 1000	38.4, 183, 49	106		3	Rash

CVB = coxsackievirus B, E = echovirus, Fe = feces, HPeV = human parechovirus, IVIG = intravenous immunoglobulin, n/d = not done, S = serum, T = throat swab, U-β2MG/Cr = urinary beta 2-microglobulin/creatinine. Day 1 indicates hospitalization date.

group, 2 patients exhibited elevated urinary glucose and one patient showed elevated urinary protein (patients 3 and 5).

4. Discussion

The current retrospective study revealed elevated urinary β2MG/Cr in infantile enterovirus and HPeV-3 infection patients compared to RSV and bacterial infection patients. Furthermore, early urinary β2MG/Cr elevation was even greater in more severe cases, suggesting utility as a marker of disease presence and severity.

MHC-I molecules are heterodimers of 2 polypeptide chains, α-microglobulin and β2MG. Newly synthesized MHC-Iα chains assemble with β2MG in the endoplasmic reticulum lumen and are transported to the external membrane.[17] The primary function of MHC-I is to present exogenous antigens such as viral proteins to cytotoxic T cells. Consequently, β2MG is upregulated during the early stage of infection to enhance antigen presentation by MHC-I. Increased MHC-I expression leads to elevated serum β2MG levels because β2MG can dissociate from membrane-associated MHC-I.[18] Serum β2MG is freely filtered through the glomerular basement membrane, and up to 99.9% of β2MG that passes through glomeruli is reabsorbed at proximal renal tubules.[19] Therefore, urinary β2MG levels increase in renal tubular dysfunction or when serum β2MG levels rise above the threshold of tubular reabsorption.[19] However, renal tubular dysfunction is unlikely to explain our findings. While the proximal tubule may be susceptible to hypoxia,[20] only 2patients with apnea in the enterovirus/HPeV group required oxygen administration during hospitalization. Furthermore, we did not

Table 2

Comparison of the clinical characteristics and laboratory findings among the groups of enterovirus or human parechovirus infections, bacterial infections, and respiratory syncytial virus infections.

	Enterovirus/HPeV (n = 15)	Bacterial infections (n = 24)	Respiratory syncytial virus (n = 22)	*P*
Sex (male)	10 (67)	15 (63)	12 (55)	.74
Age, days	23 (16–40)	33 (20–51.8)	41 (24–54)	.086
Maximum temperature, °C	39.5 (38.7–39.8)	38.5 (37.9–39.1)	38 (37.8–38.2)	< .001
Maximum heart rate, beats per minute	191 (185–204)	180 (173–186.8)	180.5 (175–186.3)	.009
Maximum respiratory rate, times a minute	50 (46–58)	52 (48–60)	49 (47–57)	.42
WBC, /μL	7,100 (6,600–9,800)	12,650 (7,700–17,000)	8,150 (6,900–8,875)	.005
PLT, $\times 10^4$/μL	40.4 (31–45.4)	42 (33.2–49.5)	42 (34.8–48.5)	.64
AST, IU/L	36 (22–41)	25 (20.3–33.8)	27.5 (23.5–40)	.14
LDH, IU/L	306 (283–347)	257 (239.3–322.3)	271 (234.8–295.3)	.013
CK, IU/L	75 (51–113)	82 (73–120)	84 (63–99)	.35
CRP, mg/dL	0.25 (0.1–0.49)	4.05 (2.3–6.6)	0.13 (0.1–0.66)	< .001
Maximum u-β2MG/Cr, μg/gCr	53,340 (37,467–177,780)	8188 (1,470–33,925)	15,430 (6,116–26,154)	< .001

AST = aspartate aminotransferase, CK = creatine kinase, CRP = C-reactive protein, HPeV = human parechovirus, LDH = lactate dehydrogenase, PLT = platelets, U-β2MG/Cr = urinary β2 microglobulin/creatinine, WBC = white blood cells.

Values are expressed as medians (range) or numbers (%), unless otherwise indicated.

Statistical analysis was performed using the Kruskal–Wallis test.

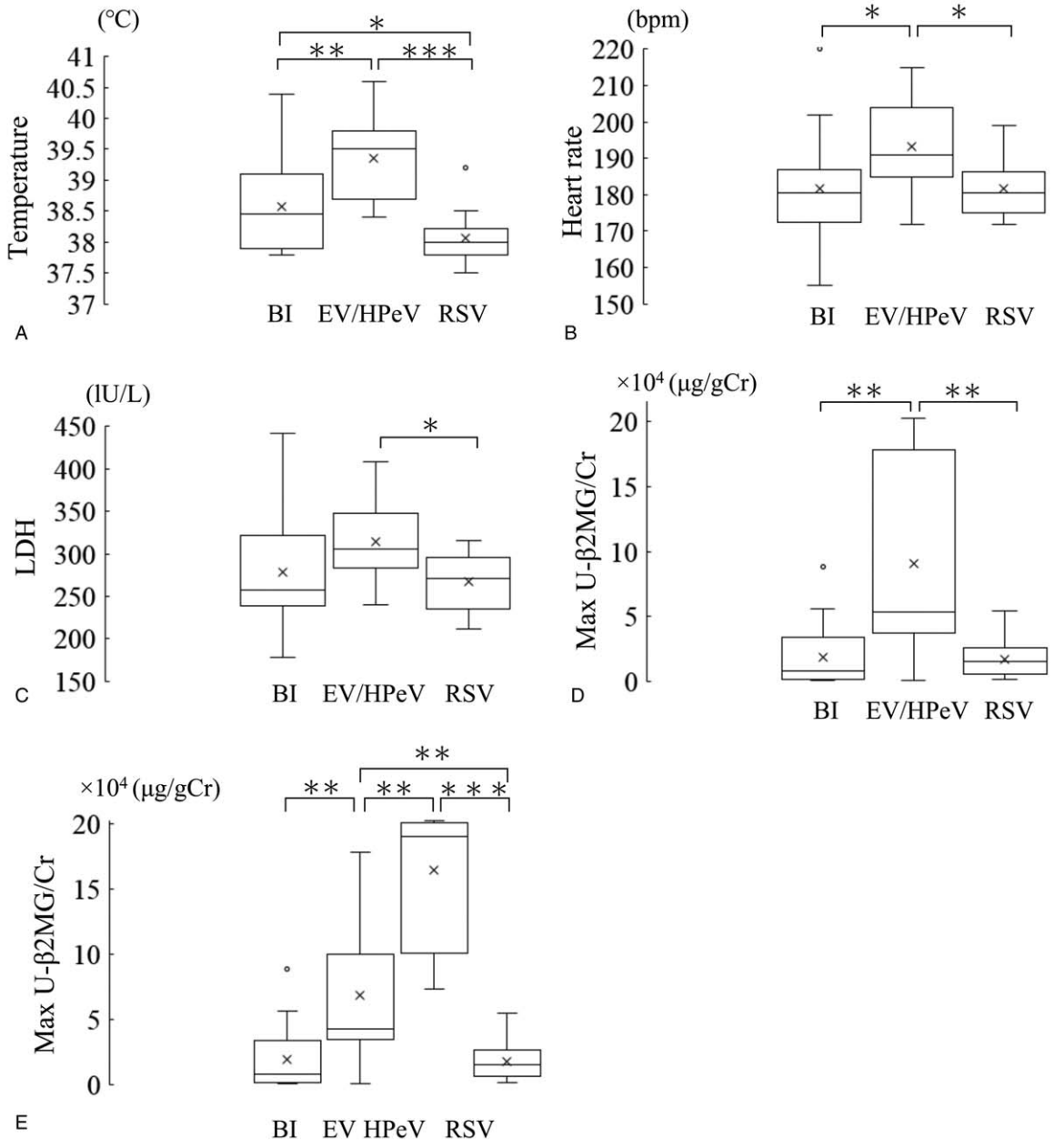

Figure 1. Significant differences in clinical markers, including urinary beta 2-microglobulin to creatinine ratio, among patient groups with enterovirus or human parechovirus infection, bacterial infection, or respiratory syncytial virus infection. In box-whisker plots, box bottom and top indicate the first and third quartiles, respectively, and the band inside the box is the second quartile (median). Ends of the whiskers are minimum and maximum values, and cross marks indicate mean values. Groups were compared using the Steel–Dwass test. $*P < .05$; $**P < .01$; $***P < .001$. BI = bacterial infections, EV = enterovirus, HPeV = human parechovirus, LDH = lactate dehydrogenase, Max = maximum, RSV = respiratory syncytial virus, U-β2MG/Cr = urinary beta 2-microglobulin to creatinine ratio.

observe an increase in urinary glucose or uric acid levels indicative of proximal tubule dysfunction in most of the study cases. Thus, we suggest that elevated urinary β2MG levels reflect increased protein production for MHC-I-mediated antigen presentation.

In neonatal enterovirus infections, the initial sites of virus replication are the pharynx and terminal ileum. Replication gives rise to transient viremia, which leads to hematogenous spread of the virus to lymphoid tissue throughout the body, followed by viral replication at these sites and secondary viremia.[1] These processes coincide with the onset of symptoms and result in viral spread throughout the entire body. Several studies have reported that the highest viral loads in blood occur in the early stage of enterovirus infection and decrease gradually thereafter.[21,22] Low levels of maternal antibodies against enterovirus and HPeV-3 may allow for high viral replication in the early stage of infection.[23,24] Of note, these changes in viral load paralleled the rise in urinary β2MG observed in study patients with enterovirus or HPeV-3 infection (Fig. 2A and B). In fact, β2MG was reported previously to be positively correlated with viral load in patients infected with human immunodeficiency virus or Epstein-Barr virus.[9,12]

Table 3

Comparison of the clinical characteristics and laboratory findings between IVIG and non-IVIG groups in patients with enterovirus and human parechovirus infections.

	IVIG group (n = 6)	Non-IVIG group (n = 9)	*P*	Power
Sex (male)	5 (83)	4 (44)	.12	.074
Age, days	31.5 (15–47.3)	23 (14.5–27)	.44	.213
Maximum temperature, (°C	39.8 (39.8–40.2)	38.7 (38.5–39.1)	.002	.999 *
Maximum heart rate, bpm	190.8 (190–200)	183 (179–190)	.018	.77
Maximum respiratory rate, times a minute	58 (54–61)	48 (45–53)	.015	.83 *
Minimum systolic blood pressure, mm Hg	72 (68.3–76)	89 (76.5–93.5)	.033	.74
Fever duration	4 (4–5)	3 (3–3.5)	.013	.84 *
WBC, /μL	11,150 (7050–13,175)	6,700 (6,500–8,350)	.033	.216
PLT, $\times 10^4$/μL	25 (22.8–33.8)	42 (33.5–45)	.025	.762
AST, IU/L	95 (36.5–189.5)	32 (22.5–40.5)	.045	.602
LDH, IU/L	505.5 (337.8–860.8)	290 (277.5–308.5)	.003	.854 *
CK, IU/L	125 (87.5–210)	77 (68.5–96)	.059	.639
CRP, mg/dL	0.1 (0.1–1.24)	0.35 (0.1–0.66)	.62	.062
Maximum U-β2MG/Cr, μg/gCr	181,152 (149,130–197,506)	39,486 (26,875–50,937)	.002	1.000 *

AST = aspartate aminotransferase, CK = creatine kinase, CRP = C-reactive protein, IVIG = intravenous immunoglobulin, LDH = lactate dehydrogenase, PLT = platelets, U-β2MG/Cr = urinary β2 microglobulin/creatinine, WBC = white blood cells.

Values are expressed as medians (range) or numbers (%), unless otherwise indicated.

Statistical analyses were performed using the Mann–Whitney *U* test followed by statistical power analysis. A *P* value $< .05$ was accepted as significant. Additionally, significance was considered correct when the power was 0.80 or higher*.

The innate immune system is the first line of host defense against pathogens, and interferon-α signaling may be particularly important for responses to viral infections and upregulation of both β2MG and MHC-I.[25] Furthermore, effective immunity against pathogens is dependent on the initial recognition of infectious agents. Increased levels of several cytokines/chemokines involved in the early detection of viral pathogens have been reported during both enterovirus and HPeV infections, although many types of inflammatory immune responses, including interleukin-6 and ferritin[26] responses, differ between HPeV and enterovirus infections.[27] Based on these findings, we suggest that the early rise in urinary β2MG/Cr during enterovirus and HPeV-3 infections may reflect increased viral load and the ensuing innate immune response.

Maximum urinary β2MG/Cr levels were significantly higher in the HPeV-3 group than the enterovirus group (Fig. 1E), and higher in both enterovirus and HPeV-3 groups compared to all other groups (Fig. 1D and E). Several factors might explain these observed differences in urinary β2MG/Cr levels in the present study. First, viral RNA concentrations tend to be higher during HPeV infection than enterovirus infection.[28] Second, RSV is highly restricted to the respiratory epithelium, so only low-level viremia is expected. Indeed, only 2 studies have detected the RSV genome in blood samples;[29,30] thus, viral load during RSV infection may be substantially lower than during enterovirus or HPeV-3 infection. Finally, in bacterial infections, antigen-presenting cells such as macrophages and dendritic cells detect and present bacterial antigens to helper T cells using MHC-II; thus, the immune system against bacterial infections is not primarily dependent on MHC-I.

Most enterovirus and HPeV infections are self-limited and do not require specific therapy. However, IVIG has demonstrated benefits in more severe cases.[23,31] Most notably, Yen et al[32] reported that early IVIG therapy may enhance survival from severe neonatal enterovirus infections. In the present study, we observed that patients with higher urinary β2MG/Cr levels tended to have more severe disease (Tables 1 and 3), and IVIG therapy resulted in prompt improvement of clinical symptoms. Several studies have reported that enterovirus and HPeV loads in blood or cerebrospinal fluid are correlated with disease severity,[21,22,28,31] which could possibly explain the tendency for more severe disease presentation in patients with higher urinary β2MG/Cr. The highest mean urinary β2MG/Cr levels were observed on day 1 or 2, whereas peak AST, LDH, CK, and ferritin levels were not observed until day 3 or 4 (Fig. 2). Therefore, we suggest that the marked elevation of urinary β2MG/Cr in the early stage of infection is a strong candidate indicator of disease severity and that early therapeutic intervention using IVIG may be effective for patients with clinically severe infantile enterovirus or HPeV-3 infection.

However, the increase in urinary β2MG/Cr may not be specific to enterovirus and HPeV-3 infections, but rather extend to other viral infection with viremia due to robust viral replication and ensuing strong immune responses. Thus, it may be possible to predict severe infantile viral infections based on high urinary β2MG/Cr elevation. In usual clinical settings, PCR is not a routine diagnostic procedure because it is not covered by medical insurance and is expensive. Consequently, measurement of urinary β2MG/Cr may be superior to PCR due to low cost, ease of performance, and noninvasiveness.

There were several inherent limitations in the present study. First, this was a retrospective case study. Second, the exact pathophysiology underlying the elevation in urinary β2MG/Cr levels during the early stage of infection remains unclear, although we suggest that the increase reflects viral load and the innate immune response against viral replication. Third, chemokine/cytokine levels and viral loads were not measured. Thus, further studies are necessary to clarify the relationships among β2MG, chemokines/cytokines, and viral load.

In conclusion, this retrospective analysis revealed that the increase in urinary β2MG/Cr may be a useful marker for infantile enterovirus and HPeV-3 infections. Additionally, an unusually large elevation in urinary β2MG/Cr in early-stage infantile enterovirus or HPeV-3 infection may be predictive of severe disease and an indicator for IVIG therapy.

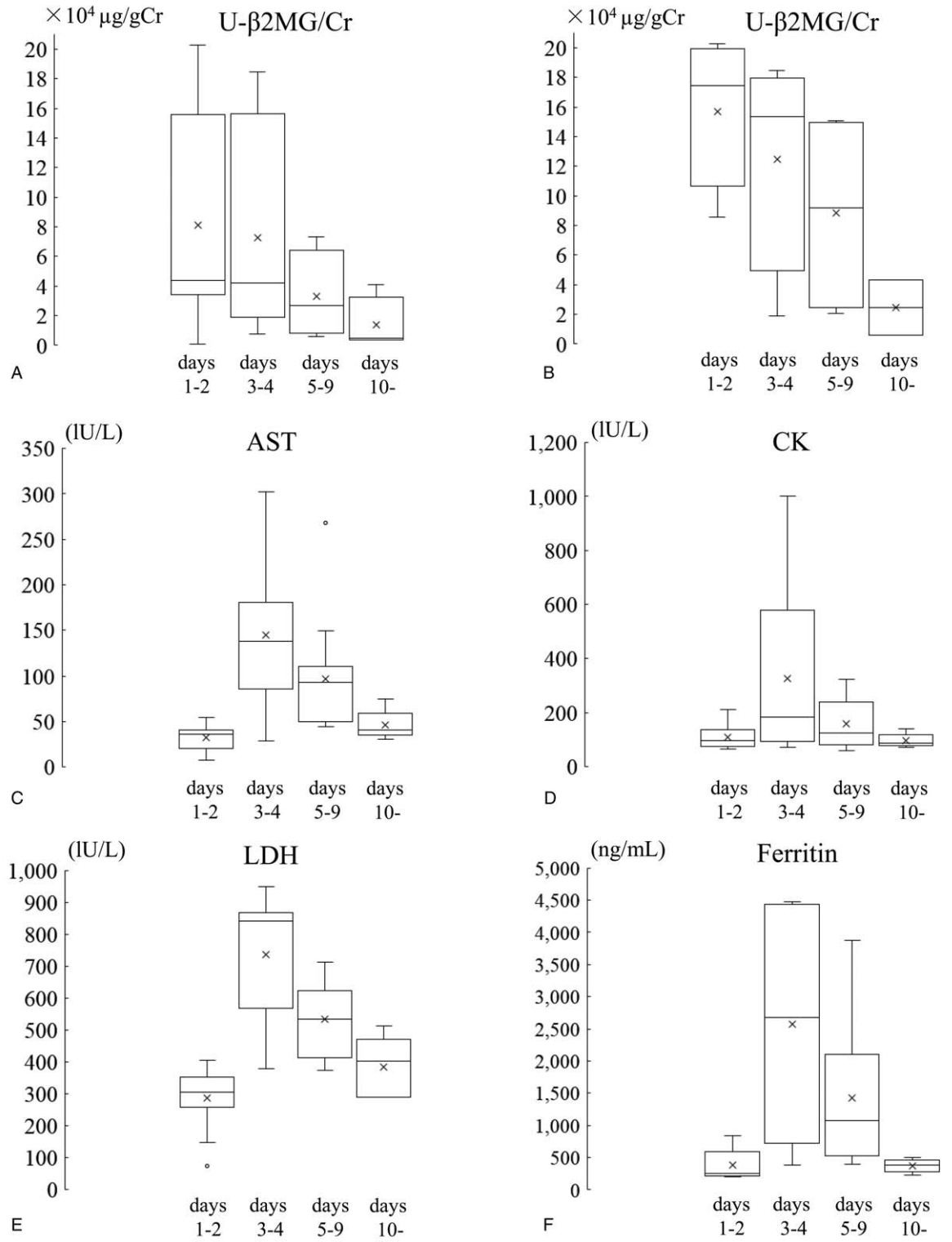

Figure 2. Temporal differences in peak clinical parameters among infection groups. Day 1 indicates the day of hospitalization. See Figure 1 for box-whisker plot definitions. (A) Changes in U-β2MG/Cr levels among patients with enterovirus or human parechovirus infection. (B) U-β2MG/Cr changes in the intravenous immunoglobulin subgroup. (C-F) AST, CK, LDH, and ferritin level changes in the intravenous immunoglobulin subgroup. AST = aspartate aminotransferase, CK = creatine kinase, LDH = lactate dehydrogenase, U-β2MG/Cr = urinary beta 2-microglobulin/ creatinine.

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