Check for updates

Clinical Outcomes of Japanese Encephalitis after Combination Treatment of Immunoglobulin, Ribavirin, and Interferon-α2b

Hyoshin Son
Jun-Sang Sunwoo
Sang Kun Lee
Kon Chu
Soon-Tae Lee

Department of Neurology,
Seoul National University Hospital,
Seoul, Korea

Background and Purpose Japanese encephalitis (JE) is caused by the JE virus of the Flaviviridae family and is spread by mosquito bites, and no specific antiviral treatment for it exists. Here we describe the clinical presentations, laboratory findings, clinical outcomes, and adverse events after combination treatment of immunoglobulin, ribavirin, and interferon-α2b administered to patients with JE.

Methods Data were collected and reviewed from a prospective cohort of encephalitis patients admitted to Seoul National University Hospital between August 1, 2010 and October 31, 2019. We reviewed the medical records of the patients diagnosed with JE and treated either with supportive care only or with combination treatment of intravenous immunoglobulin, oral ribavirin, and subcutaneous interferon-α2b.

Results Eleven patients were diagnosed with laboratory-confirmed JE based on the diagnosis criteria of JE. The median age was 61 years, and five patients were male. Eight patients were treated with the combination therapy, while three patients received supportive management only. Four of the eight patients (50%) treated with the combination therapy showed partial recovery, while one patient (12.5%) showed complete recovery. Two patients experienced hemolytic anemia related to ribavirin and febrile reaction to immunoglobulin, respectively. Among the three patients who received supportive management only, one (33.3%) showed partial recovery and the other two (67.7%) did not show improvement.

Conclusions Combination treatment of immunoglobulin, ribavirin, and interferon-α2b was found to be tolerable in JE in this study. Further studies of appropriate designs and involving larger numbers of patients are warranted to explore the efficacy of this combination therapy.

Key Words Japanese encephalitis, Japanese encephalitis virus, flavivirus, intravenous immunoglobulin, ribavirin, interferon.

INTRODUCTION

Japanese encephalitis (JE) is one of the most devastating viral encephalitis caused by Japanese encephalitis virus (JEV), which is a single-stranded, positive-sense RNA virus that is mainly spread by mosquito (Culex tritaeniorhynchus) bites.[1] Currently there is no specific antiviral treatment for JE, and JEV causes 35,000−50,000 cases of JE and 10,000 deaths per year.[2] Approximately 30% of JE patients die, and half of the patients who survive suffer from severe neurological sequelae that cause functional disability.[3] Several treatment regimens have been suggested, including steroids, immunoglobulin, antiviral drugs, and interferon, but none of them have been demonstrated to be sufficiently effective to cure the disease.[3,4] There is an urgent need for an effective treatment for JE, especially in endemic areas including South Korea, where the incidence of JE has increased since 2010 and

was highest during 2015, especially among those older than 40 years who were not candidates for a nationwide vaccination program. Considerable economic and social resources of the Korean public health system are used to deal with the disease burden of JE despite the presence of a well-established vaccination program.[5]

In cases of West Nile virus, which is a member of the Flaviviridae family and *Flavivirus* genus, and similar to JEV, several antiviral agents including purine analogs (e.g., ribavirin) and interferon have been found to exhibit some efficacy in the laboratory setting,[6] with immunoglobulin showing efficacy in animal studies.[7] Among other microorganisms that are also very challenging to treat, combination treatment of ribavirin and interferon had been the standard treatment of choice for hepatitis C virus (HCV) infection until 2016, and there are several reports of combination treatment of ribavirin and interferon being associated with improved survival and clinical outcomes in MERS-CoV.[8,9]

Considering the urgent need to manage the fatal infectious disease JE in the absence of standard guidelines for treatment, we aimed to determine the outcomes of applying combination treatment of immunoglobulin, ribavirin, and interferon-α2b to patients with JE. Here we describe the clinical presentations, laboratory findings, clinical outcomes, and adverse events after combination therapy in patients with JE.

METHODS

Patient enrollment

Data were collected and reviewed from a prospective cohort of encephalitis patients who were admitted to Seoul National University Hospital between August 1, 2010 and October 31, 2019. The patients had all undergone diagnostic studies, including routine neurological examinations, brain magnetic resonance imaging (MRI) (or computed tomography if MRI was not available), workups for infectious and metabolic etiologies, and lumbar punctures. We identified patients in the cohort data set as being diagnosed with JE if laboratory tests confirmed the presence of JEV-specific immunoglobulin M antibody in serum or cerebrospinal fluid (CSF) samples. The test for the antibody was conducted by the Korea Disease Control and Prevention Agency (KDCA). The patients diagnosed with JE were considered candidates for combination treatment of immunoglobulin, ribavirin, and interferon-α2b, and their clinical presentations, laboratory findings, clinical outcomes, and adverse events were analyzed retrospectively for this study. The study protocol was approved by the Seoul National University Hospital Institutional Review Board and followed the principles of the Dec-

laration of Helsinki (IRB number: 1910-132-1072).

Treatment regimens

The use of combination treatment of intravenous immunoglobulin (IVIG), oral ribavirin, and subcutaneous interferon-α2b was initiated as soon as the KDCA report suggested laboratory confirmation of the diagnosis of JE and if the neurological symptoms and signs including altered mental state, confusion, and brainstem signs such as diplopia could not be explained by an etiology other than JEV infection. Another infectious etiology (another virus, bacterium, or fungus) and vascular, inflammatory, and tumorous etiologies also needed to be ruled out to diagnose with JE.

Patients with JE received intravenous human immunoglobulin G for 5 consecutive days at a dosage of 400 mg/kg/day. Ribavirin (Viramid, Ilsung Pharmaceuticals, Seoul, Korea) was administered orally at a dosage of 400 mg every 12 h for 14 consecutive days. Interferon-α2b (Intron A, Merck & Co, Kenilworth, NJ, USA) was given by subcutaneous injection at a dosage of 3 million units per day for 2 weeks. Some of the patients with JE did not receive all components of the combination therapy due to medical contraindication, economic issues, or personal rejection. All patients received appropriate supportive management as required, including intravenous hydration, nutritional support, oxygenation, mechanical ventilation, or antibiotics, and intensive care.

Outcomes

The responses to combination treatment of IVIG, oral ribavirin, and subcutaneous interferon-α2b were categorized into three subgroups: complete, partial, and no recovery. Complete recovery was defined as complete recovery of the patient to their previous functional status. Partial recovery was defined as any improvement in functional status as measured by the modified Rankin Scale (mRS) score at discharge compared with the initial mRS score or as any improvements in the level of consciousness measured by the Glasgow Coma Scale (GCS), symptoms, and signs confirmed by neurological examinations performed by two neurologists independently during the hospital stay. No recovery was defined as no improvement, aggravation of the functional status as measured by the mRS, or death.

Adverse events

Adverse events related to IVIG, oral ribavirin, or subcutaneous interferon-α2b were identified and assessed based on the Common Terminology Criteria for Adverse Events (version 5.0).[10]

RESULTS

Eleven patients diagnosed with JE were included in the study (Table 1). The brain MRI findings of the patients corresponded with those of JE (Figs. 1A, B, C, G, H, and I), and the diagnosis of JE in those patients was confirmed by the antibody test conducted by the KDCA. The median age of the patients was 61 years (range 47−87 years), and five patients were male. Eight patients received combination treatment of IVIG, oral ribavirin, and subcutaneous interferon-α2b according to the common regimen described in the Methods; these patients also received supportive management. Three patients received supportive management only due to their guardians' decision or medical contraindication to the combination therapy.

The clinical features, laboratory findings, and functional outcomes are listed in Table 1. The most common initial symptom was confusion, while fever, headache, and altered mentality were also observed as initial manifestations. The initial median mRS score was 5 (range 1−5), and the median mRS score at discharge was 4 (range 1−5). Follow-up data at discharge from the hospital were obtained for all patients, and the median hospital stay was 27 days (range 13−181 days).

Four of the eight patients (50%) treated with the combination therapy showed a partial response, while one patient (12.5%) whose initial symptoms and signs included drowsiness, headache, and fever showed complete recovery. These five patients had mRS scores ranging from 0 to 5 at discharge, and the patient with complete recovery had no neurological sequelae at discharge. Among those who showed partial recovery, Patients 2, 4, and 7 showed improved mRS scores, and the level of consciousness of Patient 6 improved during the hospital stay, from comatose (GCS score=E1VtM1) to stupor (GCS score=7, E2V1M4). Specifically, there were improvements in alertness from comatose to a drowsy state in Patient 4 and in awareness in Patient 7, and Patient 2 showed not only an improved mRS score but also improvements in CSF results and follow-up MRI findings. Additionally, radiological improvement was achieved in two patients (Patient 2 and 5) in serial follow-up MRI examinations compared with their initial MRI examinations (Figs. 1D, E, F, J, K, and L). Even though the other three patients (37.5%) showed no recovery, there were no deaths during the hospital stay. During combination treatment of immunoglobulin, ribavirin, and interferon-α2b, two patients (Patient 4 and 8) experienced hemolytic anemia of grade 3 that was related to ribavirin and a febrile reaction to IVIG, respectively.

Among the three patients who received supportive management only, one patient (33.3%) showed partial recovery at discharge, with the mRS score improving from 5 to 3, while the other two patients (67.7%) improved neither clinically nor radiologically.

While the number of patients was too small to confirm statistical significance, in terms of mRS scores, poor initial neurological status (mRS score=5) resulted in partial recovery in two patients (33.3%) with combination therapy and in one patient (33.3%) with supportive management only. In contrast, two patients with initial neurological statuses of mRS scores of 4 and 3 showed partial and complete recoveries to mRS scores of 3 and 0, respectively (100% of those patients).

DISCUSSION

JE is a medically challenging disease for which no definite antiviral treatment is currently available, despite its mortality and morbidity causing a devastating decline in the functional status of patients even if they survive.[2,3] In the current study, combination treatment of immunoglobulin, ribavirin, and interferon-α2b was well tolerated and had few side effects. Five of the eight patients (62.5%) treated with the combination therapy in our study showed complete or partial recovery: four of these patients showed an improved mRS score at discharge, and the other patient experienced improvement in terms of the neurological evaluation of awareness. The other three patients (37.5%) treated with the combination therapy showed no recovery, as did two of the three patients (66.7%) who received supportive management only. Several studies have suggested high mortality rates of up to 30% for JE,[3] and approximately half of patients with JE did not recover to their functional status prior to the disease.[3] However, there were no patient deaths in our study, which is relatively favorable considering the high mortality rate of JE.

JEV is neurotropic and invades the central nervous system (CNS). Even though the mechanisms by which JEV causes encephalitis are not completely understood, possible models of the immune response to viral infection have been suggested for the damage caused to the CNS and for the disease progression.[11] The best known effects of immunoglobulin are immunomodulation in general via blocking the Fc receptors of macrophages and complement activation, reducing inflammatory cytokines, and suppressing T-cell responses, and it has been applied to several inflammatory autoimmune diseases and infectious conditions.[11] Several factors including antibody replacement, enhanced neutralization, and an augmented opsonocytophagic function have been suggested to contribute to the antimicrobial efficacy of immunoglobulin.[12] IVIG has also been considered for treating cases of infection with the West Nile virus, which shares the characteristic of being a positive-sense, single-stranded RNA virus with JEV in the Flaviviridae family (*Flavivirus* genus).[13,14]

Table 1. Clinical features, outcomes, and treatment histories of patients with Japanese encephalitis

No.	Sex/age (years)	Initial symptoms	Onset to treatment (days)	MRI lesions	CSF	Recovery	Initial GCS grade	Initial mRS score	mRS score at discharge	Hospital stay (days)	Adverse event
Combination treatment of IVIG, oral ribavirin, and subcutaneous interferon-α2b											
1	M/61	Confusion	44	Bilateral thalami and bilateral cerebral peduncles	WBC 60 Ptn 276.7	No	E4VtM1	5	5	55	
2	F/73	Confusion	12	Bilateral medial temporal lobes and medial thalamus	WBC 630 Ptn 131	Partial	E4V3M5	4	3	48	
3	F/50	Fever, confusion, altered mentality	10	Bilateral medial temporal lobes and thalami	WBC 38 Ptn 43	No	E4V3M5	5	5	108	
4	M/59	Fever, headache, myalgia	6	Bilateral BG, thalami, hippocampi, and midbrain	WBC 475 Ptn 143	Partial	E1VtM4	5	4	24	Hemolytic anemia (ribavirin)
5	M/47	Confusion	32	Bilateral medial thalami, left hippocampus, and left SN	WBC 70 Ptn 48.7	No	E1VtM1	5	5	27	
6	M/47	Diplopia, altered mentality	26	Bilateral hemisphere, medial temporal lobe, medial thalamus, and tegmentum	WBC 393 Ptn 150.5	Partial	E1VtM1	5	5	22	
7	F/68	Confusion	10	Normal	WBC 125 Ptn 56	Partial	E4V4M4	5	3	52	
8	M/53	Fever, headache	7	Left medial temporal lobe with edema	WBC 413 Ptn 134.4	Complete	E3V2M5	3	0	13	Febrile reaction (IVIG)
Supportive management only											
9	F/82	Fever		Bilateral cerebral white matter, BG, thalami, pons, and cerebellar peduncles	WBC 25 Ptn 64	Partial	E1V1M2	5	3	181	
10	F/87	Fever, confusion		Bilateral thalamus, medial temporal lobe, and tegmentum	WBC 255 Ptn 134.2	No	E4V1M4	5	5	18	
11	F/63	Confusion, altered mentality		N/A	N/A	No	E1VtM1	5	5	14	

BG: basal ganglia, CSF: cerebrospinal fluid, F: female, GCS: Glasgow Coma Scale, IVIG: intravenous immunoglobulin, M: male, MRI: magnetic resonance imaging, mRS: modified Rankin Scale, Ptn: protein (mg/dL), SN: substantia nigra, WBC: white blood cells (/μL)
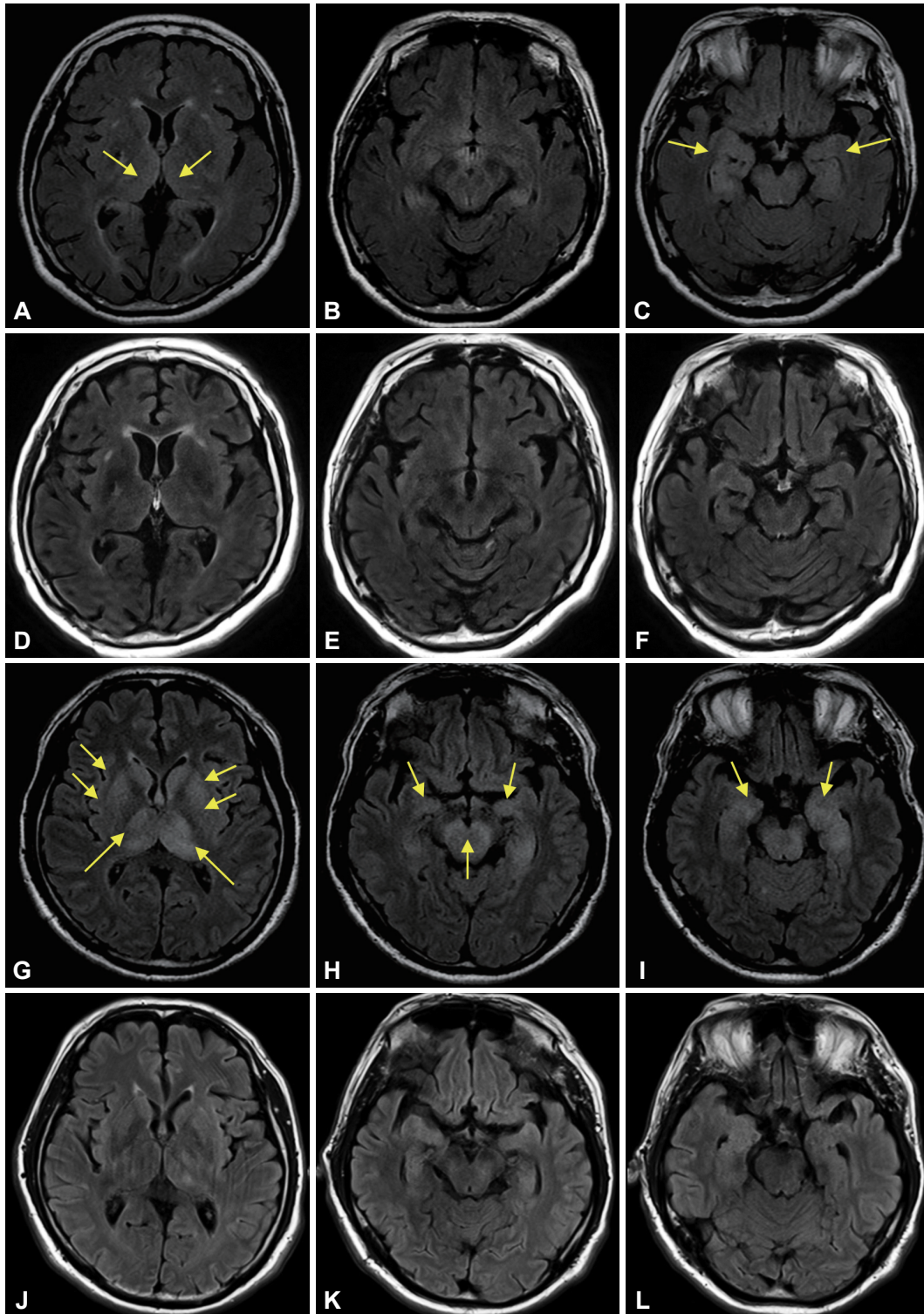
Fig. 1. Initial and follow-up magnetic resonance imaging (MRI) studies were performed in two patients (Patient 2 and 5) at the initial hospital admission (A, B, C, G, H, and I) and at 2 weeks after symptom onset (D, E, F, J, K, and L), respectively. Relatively symmetric high signal intensities (yellow arrows) in the bilateral medial thalamus and medial temporal lobes (A, B, and C) of Patient 2 and in the bilateral basal ganglia, thalamus, hippocampus, medial temporal lobe, and midbrain (G, H, and I) of Patient 5 were evident in T2-weighted fluid-attenuated inversion recovery (FLAIR) images. Lesions in the initial MRI were decreased on the follow-up FLAIR images (D, E, F, J, K, and L).

Interferon-α2b, which is known to have antiviral activity that is not directly related to interfering with viral replication or the viral life cycle, plays an important role in the innate immune response to virus infection mediated by interferon-stimulated genes (ISGs).[15,16] The guanosine analog ribavirin possesses antiviral activity against the life cycle of several RNA and DNA viruses, such as respiratory syncytial virus and HCV. Its phosphorylation activity interferes in the life cycle of viruses via early chain termination and the inhibition of viral replication.[15] Synergistic effects during combination treatment of HCV infection using ribavirin and interferon have been suggested by several studies, in which ribavirin enhances the interferon-α signaling pathway by inducing the expression of ISGs, although the exact mechanism is unknown.[17]

Most of the above-mentioned studies were conducted at the molecular or cellular level, and not in vivo or in clinical settings, and unexpected consequences in the clinical course of the disease remain. Moreover, several side effects of each treatment are known, including hemolytic anemia for ribavirin and bone marrow suppression (especially decreased numbers of granulocytes and thrombocytes), flu-like symptoms (fever, chills, headache, arthralgia, and myalgia), neuropsychiatric side effects, and autoimmune phenomena for interferon.[18] In our study, one case of hemolytic anemia (Patient 4) resulting from ribavirin and one case of febrile reaction to IVIG were observed. Hemolytic anemia of mild to moderate severity was corrected by transfusion with a single pack of 400 mL of red blood cell, and it did not prolong the hospital stay. The febrile reaction subsided after IVIG treatment without further intervention.

This study had several limitations. It had an uncontrolled, observational design with a small number of patients who were not randomly distributed or compared with historical controls adjusted for sex or age. The baseline characteristics of the patients were heterogeneous, and variations in treatment other than the combination therapy were not considered. Other than evaluating functional outcomes and performing neurological examinations, there is no consensus about objective assessment tools for evaluating the disease burden or any changes correlated with the clinical course of JE, such as neither the viral burden nor the follow-up JEV-specific antibody titer. It is considered that the viral load of JEV is not correlated with disease activity, which makes it difficult to assess the effectiveness of treatment.[11]

With only a small spectrum of treatments to choose from for JE, and with no effective antiviral treatment being available, combination treatment of immunoglobulin, ribavirin, and interferon-α2b can be considered due to its relative safety regarding adverse drug reactions. Further prospective con-

trolled studies with larger numbers of patients are warranted to explore the therapeutic potential of the combination treatment of immunoglobulin, ribavirin, and interferon-α2b in JE.

Author Contributions

Conceptualization: Soon-Tae Lee, Kon Chu, Sang Kun Lee. Methodology: Soon-Tae Lee, Kon Chu. Investigation: Hyoshin Son, Jun-Sang Sunwoo. Data curation: Hyoshin Son, Jun-Sang Sunwoo. Writing—original draft: Hyoshin Son. Writing—review & editing: all authors.

ORCID iDs

Hyoshin Son	https://orcid.org/0000-0001-6643-3712
Jun-Sang Sunwoo	https://orcid.org/0000-0001-8834-0568
Sang Kun Lee	https://orcid.org/0000-0003-1908-0699
Kon Chu	https://orcid.org/0000-0001-5863-0302
Soon-Tae Lee	https://orcid.org/0000-0003-4767-7564

Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

REFERENCES

1. Sikka V, Chattu VK, Popli RK, Galwankar SC, Kelkar D, Sawicki SG, et al. The emergence of Zika virus as a global health security threat: a review and a consensus statement of the INDUSEM Joint Working Group (JWG). *J Glob Infect Dis* 2016;8:3-15.
2. Tsai TF. New initiatives for the control of Japanese encephalitis by vaccination: minutes of a WHO/CVI meeting, Bangkok, Thailand, 13-15 October 1998. *Vaccine* 2000;18 Suppl 2:1-25.
3. Solomon T, Dung NM, Wills B, Kneen R, Gainsborough M, Diet TV, et al. Interferon alfa-2a in Japanese encephalitis: a randomised double-blind placebo-controlled trial. *Lancet* 2003;361:821-826.
4. Hoke CH Jr, Vaughn DW, Nisalak A, Intralawan P, Poolsuppasit S, Jongsawas V, et al. Effect of high-dose dexamethasone on the outcome of acute encephalitis due to Japanese encephalitis virus. *J Infect Dis* 1992;165:631-637.
5. Sunwoo JS, Jung KH, Lee ST, Lee SK, Chu K. Reemergence of Japanese encephalitis in South Korea, 2010-2015. *Emerg Infect Dis* 2016;22:1841-1843.
6. Anderson JF, Rahal JJ. Efficacy of interferon α-2b and ribavirin against West Nile virus in vitro. *Emerg Infect Dis* 2002;8:107-108.
7. Ben-Nathan D, Lustig S, Tam G, Robinzon S, Segal S, Rager-Zisman B. Prophylactic and therapeutic efficacy of human intravenous immunoglobulin in treating West Nile virus infection in mice. *J Infect Dis* 2003;188:5-12.
8. Falzarano D, de Wit E, Rasmussen AL, Feldmann F, Okumura A, Scott DP, et al. Treatment with interferon-α2b and ribavirin improves outcome in MERS-CoV-infected rhesus macaques. *Nat Med* 2013;19:1313-1317.
9. Omrani AS, Saad MM, Baig K, Bahloul A, Abdul-Matin M, Alaidaroos AY, et al. Ribavirin and interferon alfa-2a for severe Middle East respiratory syndrome coronavirus infection: a retrospective cohort study. *Lancet Infect Dis* 2014;14:1090-1095.
10. National Cancer Institute (U.S.). Common terminology criteria for adverse events (CTCAE). Rev. ed. Bethesda, MD: U.S. Dept. of Health and Human Services, National Institutes of Health, National Cancer Institute; 2009.
11. Růžek D, Dobler G, Niller HH. May early intervention with high dose

intravenous immunoglobulin pose a potentially successful treatment for severe cases of tick-borne encephalitis? *BMC Infect Dis* 2013;13:306.

12. Sullivan KM, Kopecky KJ, Jocom J, Fisher L, Buckner CD, Meyers JD, et al. Immunomodulatory and antimicrobial efficacy of intravenous immunoglobulin in bone marrow transplantation. *N Engl J Med* 1990;323:705-712.

13. Walid MS, Mahmoud FA. Successful treatment with intravenous immunoglobulin of acute flaccid paralysis caused by West Nile virus. *Perm J* 2009;13:43-46.

14. Shimoni Z, Niven MJ, Pitlick S, Bulvik S. Treatment of West Nile virus encephalitis with intravenous immunoglobulin. *Emerg Infect Dis* 2001;7:759.

15. Feld JJ, Hoofnagle JH. Mechanism of action of interferon and ribavirin in treatment of hepatitis C. *Nature* 2005;436:967-972.

16. Jiang D, Guo H, Xu C, Chang J, Gu B, Wang L, et al. Identification of three interferon-inducible cellular enzymes that inhibit the replication of hepatitis C virus. *J Virol* 2008;82:1665-1678.

17. Thomas E, Feld JJ, Li Q, Hu Z, Fried MW, Liang TJ. Ribavirin potentiates interferon action by augmenting interferon-stimulated gene induction in hepatitis C virus cell culture models. *Hepatology* 2011;53:32-41.

18. Manns MP, Wedemeyer H, Cornberg M. Treating viral hepatitis C: efficacy, side effects, and complications. *Gut* 2006;55:1350-1359.