Table 3 Characteristics of patients with and without mPSL

Variable	mPSL (n = 12)	No mPSL (n = 9)	p Value
Age, y	14.3 ± 8.0	28.3 ± 24.3	NS
Sex, M	2 (16.7)	4 (44.4)	NS
Ingestion to enteritis, d	3.0 ± 1.0	3.0 ± 0.7	NS
Enteritis to HUS, d	3.8 ± 1.9	3.4 ± 0.7	NS
HUS to encephalopathy, d	2.3 ± 2.2	1.2 ± 1.2	NS
WBC, ×1,000/μL	40.0 ± 23.7	39.4 ± 9.9	NS
Hb, g/dL	6.2 ± 1.0	7.0 ± 2.3	NS
PLT, ×10,000/μL	1.7 ± 0.7	2.2 ± 2.1	NS
AST, IU/L	118.2 ± 53.0	179.6 ± 111.6	NS
Creatinine, mg/dL	3.4 ± 2.3	5.8 ± 3.3	NS
CRP, mg/dL	15.4 ± 11.2	14.0 ± 7.3	NS
Basal ganglia lesion	5 (41.7)	5 (55.6)	NS
Thalamus lesion	8 (66.7)	4 (44.4)	NS

Abbreviations: AST = aspartate aminotransferase; CRP = C-reactive protein; Hb = hemoglobin; HUS = hemolytic-uremic syndrome; mPSL = methylprednisolone; NS = not significant; PLT = platelets; WBC = white blood cells.
Data are presented as mean ± SD or n (%).

neuropathology in animal models injected with Stx2 shows lesions suggestive of ischemic damage and arteriolar necrosis due to thrombotic microangiopathy.[17,18] Stxs that injure endothelial cells may negatively affect the blood-brain barrier, and thereby infiltrating brain parenchyma,[14,19] where they can directly injure neurons and result in neuronal dysfunction.[20]

Proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) markedly increase the Gb3 content and Stx-binding to brain endothelial cells, resulting in increased cytotoxicity and upregulation of apoptotic cell death.[21–23] A rabbit model study in which animals were given IV Stx2 injections showed that in addition to neuronal apoptotic death, microglial activation and significant upregulation of TNF-α and IL-1β transcription occurs in the brain parenchyma.[18] Activated microglia are known to produce proinflammatory cytokines,[24] and TNF-α directly induces neurodegeneration through multiple pathways.[25–27] Proinflammatory cytokines are, therefore, closely related to the pathogenesis of STEC-encephalopathy. Gb3 is upregulated by proinflammatory cytokines, and these cytokines are, in turn, released through the interaction of Stxs with activated microglia.

The high fatality rate in the STEC O111 outbreak in Toyama resulted from progressive encephalopathy. MRI or CT of 4 patients who later died revealed acutely progressive cerebral edema and possible herniation on days 1 to 3 within 48 hours after previous imaging with no or little cerebral edema. These findings were confirmed by postmortem neuropathologic examination, which revealed severe noninflammatory cerebral edema and herniation in 3 patients so examined (patients 3, 6, and 8).[28] Previous reports of MRI findings in patients with neurologic complications associated with other STEC outbreaks, including the STEC O104 outbreak in Germany, did not describe acute and diffuse cerebral edema.[3,4,6,7] In addition, neither cerebral edema nor herniation was documented on postmortem examination in 5 fatal cases of STEC O104 in Germany.[7] Therefore, it is reasonable to consider that progressive encephalopathy leading to severe cerebral edema is characteristic of the STEC O111 infection in Toyama.

Clinical and neuroradiologic features and neuropathologic findings of diffuse noninflammatory cerebral edema are similar to those observed in Japanese children with infectious encephalopathy, especially cases associated with influenza.[29,30] Children with STEC O111-HUS developed encephalopathy (10/11) more frequently than adults (11/25), which has also been the case with influenza encephalopathy in Japan. During the acute stage of influenza encephalopathy, serum and CSF concentrations of inflammatory cytokines (i.e., TNF-α and IL-6) are abnormally high in many patients,[31,32] suggesting that cytokine storm has a major role in the pathogenesis. Vascular injury leading to brain edema has actually been ascribed to endothelial damage caused by cytokines.[29]

Corticosteroids suppress proinflammatory cytokine gene expression, and activate genes encoding inhibitors of inflammation.[33] mPSL, IVIg, and other therapies that suppress inflammatory cytokines have, therefore, been recommended for influenza encephalopathy.[9] mPSL therapy is effective for influenza encephalopathy caused by hypercytokinemia such as acute necrotizing encephalopathy, and improves neurologic outcomes.[9,34] Physicians in Toyama decided to treat patients with STEC O111-encephalopathy with mPSL and IVIg after May 1, 2011, based on clinical, radiologic, and pathologic similarity to influenza encephalopathy. We successfully showed that mPSL pulse therapy increased the probability of a good outcome. Indeed, no patient with STEC O111-encephalopathy died after mPSL therapy. Cytokine studies on affected patients in the STEC O111 outbreak in Toyama showed more severe hypercytokinemia in 11 patients with severe STEC O111-HUS (including 8 patients with encephalopathy) than in 3 with mild HUS without encephalopathy,[8] supporting the hypothesis that cytokine storm is important in the pathogenesis of STEC O111-encephalopathy. Although no specific therapy has been established for STEC-encephalopathy, plasma exchange, eculizumab, and immunoabsorption treatments have been proposed.[6] Corticosteroid therapy, especially mPSL pulse therapy, should be considered for the treatment of STEC-encephalopathy.

Progressive encephalopathy leading to severe cerebral edema and death is not observed in countries other than Japan. This may be because Japanese people are genetically more susceptible to infectious encephalopathy than people of other countries. Viral encephalopathy, most often secondary to influenza and human herpes virus 6, is the most prevalent type of encephalopathy in Japanese children.[29] Several syndromes, such as acute encephalopathy with biphasic seizures and late reduced diffusion, and acute necrotizing encephalopathy,[29,30,35] are by far more common in East Asia than in the rest of the world. The mechanisms underlying racial or regional differences are not fully understood; however, single nucleotide polymorphisms of several genes, such as those for the carnitine palmitoyltransferase II and adenosine A2a receptors, are reported to be risk factors for acute encephalopathy with biphasic seizures and late reduced diffusion.[36,37] Differences in such single nucleotide polymorphism frequencies between Japanese and other individuals may account for racial differences in neurologic symptoms associated with viral or STEC infections. It is also possible that the STEC O111 prevalent in Toyama was more toxic than the previous STEC, but bacteriologic studies to date have not elucidated the mechanism by which this specific strain caused many cases with severe complications.[38]

Renal function during the course of infection in patients with a poor outcome was worse than in individuals with a good outcome. Because uremia per se can cause brain dysfunction, and neurologic symptoms occur at the peak of renal dysfunction,[7] it is possible that more severe uremia caused severe neurologic symptoms resulting in accompanying poor outcomes. Neither hemodialysis nor plasma exchange affected the neurologic symptoms or outcome, which were compatible with a previous study.[6] In addition, some patients with STEC infection showed neurologic symptoms in the absence of renal dysfunction,[7,39] and 9% to 15% of patients with STEC-encephalopathy showed cerebral dysfunction before the onset of HUS.[40] These findings suggest that mechanisms other than uremia, such as the direct effects of Stxs and inflammatory responses in the CNS, may have major roles in the pathogenesis of STEC-encephalopathy.

Symmetrical lesions that we observed in our patients with STEC O111-encephalopathy in the lateral thalamus, basal ganglia, external capsule, and dorsal brainstem or cerebellum are similar to those reported previously in patients with STEC-encephalopathy.[3,4,6,7] This characteristic distribution may provide a radiologic clue for early diagnosis because, although it takes time for microbiologic identification of STEC, STEC-encephalopathy can be observed on the same day as HUS. Early diagnosis by radiologic identification of STEC-encephalopathy could be a useful tool promoting prevention of encephalopathy progression through use of the suggested treatments described herein.

Of interest, the ADC value revealed different patterns in the thalamus with reduced diffusion compared with the putamen and external capsule with increased diffusion in the acute stage of STEC O111-encephalopathy, suggesting that the former reflects cytotoxic edema, and the latter vasogenic edema, probably due to breakdown of the blood-brain barrier. Neuropathologic examination of 3 patients (patients 3, 6, and 8) revealed severe edema without inflammatory cells in both the thalamus and basal ganglia,[28] which could not explain the ADC difference. A neuropathologic study involving patients with STEC O104-encephalopathy revealed that astrogliosis and microgliosis were prominent in the thalamus and pons,[7] which were compatible with prominent cytotoxic edema in these regions. We know that Gb3 is highly expressed in neurons of all brain regions in patients with STEC O104 infection,[7] suggesting no correlation between Gb3 distribution and MRI lesions. We remain uncertain as to what determined the topographical pathology distribution seen on MRI.

Because we had to treat severely ill patients immediately without any evidence-based protocol at the beginning of this outbreak, the timing or combination of therapies for encephalopathy was not uniform. We did not perform multivariate statistics to confirm the effectiveness of mPSL because of the small number of patients. Definite treatment recommendations cannot, therefore, be drawn directly from the study.

AUTHOR CONTRIBUTIONS

J. Takanashi contributed to the design and conceptualization of the study, data collection, data analysis, data interpretation, statistical analysis, writing, literature search, and figures. H. Taneichi, T. Misaki, and Y. Yahata contributed to the data collection, data analysis, data interpretation, and manuscript revision. A. Okumura contributed to the design of the study, data analysis, data interpretation, and manuscript revision. Y. Ishida and T. Miyawaki contributed to the data collection and manuscript revision. N. Okabe and T. Sata contributed to the data collection, data analysis, data interpretation, and manuscript revision. M. Mizuguchi contributed to the design and conceptualization of the study, data collection, data analysis, data interpretation, and writing.

ACKNOWLEDGMENT

The authors thank Dr. Kazumasa Ogura, Department of Pediatrics, Fukui Red Cross Hospital, Fukui; Drs. Keiko Takada, Katsuhisa Inamura, and Toshiyuki Okamura, Department of Gastroenterology, Tonami General Hospital, Tonami; Dr. Michio Konishi, Department of Pediatrics, Tonami General Hospital, Tonami; Dr. Masaru Nakagawa, Department of Nephrology, Kanazawa Medical University, Kahoku-gun; Dr. Hisashi Kaneda, Department of Pediatrics, Toyama City Hospital, Toyama; Dr. Hideki Mizuno, Department of Medicine, Toyama City Hospital, Toyama; Dr. Satoshi Hiraide, Department of Nephrology and Hypertension, Seirei Yokohama Hospital, Yokohama; Dr. Teiichi Terasaki, Department of Medicine, Saiseikai Takaoka Hospital, Takaoka; Dr. Kazuya Inoki, Department of Gastroenterology, Yodogawa Christian Hospital,

Osaka; Dr. Shigeru Azuma, Department of Medicine, Koseiren Takaoka Hospital, Takaoka; Dr. Mondo Kuroda, Department of Pediatrics, Kanazawa University, Kanazawa; Dr. Kiyoki Kitagawa, Department of Nephrology, Kanazawa University, Kanazawa; Dr. Takata Hiroyuki, Toyama Red Cross Hospital, Toyama; Dr. Hiroki Misawa, Department of Medicine, Takaoka City Hospital, Takaoka; Dr. Mika Ito, Department of Obstetrics and Gynecology, University of Toyama; and Dr. Tomomi Tanaka, Department of Pediatrics, University of Toyama, Japan, for the clinical support and for collecting the data for this study.

STUDY FUNDING

No targeted funding reported.

DISCLOSURE

J. Takanashi was funded by a Grant-in-Aid for Research on Measures for Intractable Diseases (H25-Nanji-Ippan-009) from the Ministry of Health, Labor and Welfare, Japan; and a Grant-in-Aid for Scientific Research (B24390258) from Japan Society for the Promotion of Science; and is an editorial board member of *Brain and Development*. H. Taneichi was funded by a Grant for Research on Emerging and Re-emerging Diseases (H24-Shinko-Ippan-012) from the Ministry of Health, Labor and Welfare, Japan. T. Misaki and Y. Yahata report no disclosures. A. Okumura was funded by a Grant-in-Aid for Research on Measures for Intractable Diseases (H25-Nanji-Ippan-009) from the Ministry of Health, Labor and Welfare, Japan. Y. Ishida reports no disclosures. T. Miyawaki was funded by a Health Labour Sciences Research Grant (H23-TOKUBETU-SHITEI-004) from the Ministry of Health, Labor and Welfare, Japan. N. Okabe was funded by a Health Labour Sciences Research Grant (H23-TOKUBETU-SHITEI-004) from the Ministry of Health, Labor and Welfare, Japan. T. Sata was funded by a Health Labour Sciences Research Grant (H23-TOKUBETU-SHITEI-004) from the Ministry of Health, Labor and Welfare, Japan. M. Mizuguchi was funded by a Grant-in-Aid for Research on Measures for Intractable Diseases (H25-Nanji-Ippan-009) and a Grant for Research on Emerging and Re-emerging Diseases (H24-Shinko-Ippan-012), both from the Ministry of Health, Labor and Welfare, Japan; a Grant-in-Aid for Scientific Research (B24390258) from Japan Society for the Promotion of Science; and is an editor-in-chief of *Brain and Development*. Go to Neurology.org for full disclosures.

Received April 11, 2013. Accepted in final form November 12, 2013.

REFERENCES

1. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing Escherichia coli and haemolytic uraemic syndrome. Lancet 2005;365:1073–1086.
2. Pennington H. Escherichia coli O157. Lancet 2010;376: 1428–1435.
3. Nathanson S, Kwon T, Elmaleh M, et al. Acute neurological involvement in diarrhea-associated hemolytic uremic syndrome. Clin J Am Soc Nephrol 2010;5:1218–1228.
4. Donnerstag F, Ding X, Pape L, et al. Patterns in early diffusion-weighted MRI in children with haemolytic uremic syndrome and CNS involvement. Eur Radiol 2012; 22:506–513.
5. Piercefield EW, Bradley KK, Coffman RL, Mallonee SM. Hemolytic uremic syndrome after an Escherichia coli O111 outbreak. Arch Intern Med 2010;170:1656–1663.
6. Weissenborn K, Donnerstag F, Kielstein JT, et al. Neurologic manifestations of E coli infection-induced hemolytic-uremic syndrome in adults. Neurology 2012;79:1466–1473.
7. Magnus T, Röther J, Simova O, et al. The neurological syndrome in adults during the 2011 northern German E. coli serotype O104:H4 outbreak. Brain 2012;135:1850–1859.
8. Shimizu M, Kuroda M, Sakashita N, et al. Cytokine profiles of patients with enterohemorrhagic Escherichia coli O111-induced hemolytic-uremic syndrome. Cytokine 2012;60:694–700.
9. The Research Committee on the Clarification of the Etiology and on the Establishment of Therapeutic and Preventive Measures for Influenza Encephalopathy. Guidelines for Influenza Encephalopathy. Japan: Ministry of Health, Labor and Welfare; 2009:23–32.
10. Matano S, Inamura K, Konishi M, et al. Encephalopathy, disseminated intravascular coagulation, and hemolytic-uremic syndrome after infection with enterohemorrhagic Escherichia coli O111. J Infect Chemother 2012;18:558–564.
11. Gould LH, Demma L, Jones TF, et al. Hemolytic uremic syndrome and death in persons with Escherichia coli O157:H7 infection, Foodborne Diseases Active Surveillance Network sites, 2000–2006. Clin Infect Dis 2009; 49:1480–1485.
12. Loos S, Ahlenstiel T, Kranz B, et al. An outbreak of Shiga toxin-producing Escherichia coli O104:H4 hemolytic uremic syndrome in Germany: presentation and short-term outcome in children. Clin Infect Dis 2012; 55:753–759.
13. Frank C, Werber D, Cramer JP, et al. Epidemic profile of Shiga-toxin-producing Escherichia coli O104:H4 outbreak in Germany. N Eng J Med 2011;365:1171–1180.
14. Obata F, Tohyama K, Bonev AD, et al. Shiga toxin 2 affects the central nervous system through receptor globotriaosylceramide localized to neurons. J Infect Dis 2008; 198:1398–1406.
15. Jones NL, Islur A, Haq R, et al. Escherichia coli Shiga toxins induce apoptosis in epithelial cells that is regulated by the bcl-2 family. Am J Physiol Gastrointest Liver Physiol 2000;278:G811–G819.
16. Erwert RD, Eiting KT, Tupper JC, Winn RK, Harlan JM, Bannerman DD. Shiga toxin induces decreased expression of the antiapoptotic protein Mcl-1 concomitant with the onset of endothelial apoptosis. Microb Pathog 2003;35:87–93.
17. Fujii J, Kinoshita Y, Kita T, et al. Magnetic resonance imaging and histopathological study of brain lesions in rabbits given intravenous verotoxin 2. Infect Immun 1996;64: 5053–5060.
18. Takahashi K, Funata N, Ikuta F, Sato S. Neuronal apoptosis and inflammatory responses in the central nervous system of a rabbit treated with Shiga toxin-2. J Neuroinflammation 2008;5:11.
19. Goldstein J, Loidl CF, Creydt VP, Boccoli J, Ibarra C. Intracerebroventricular administration of Shiga toxin type 2 induces striatal neuronal death and glial alterations: an ultrastructural study. Brain Res 2007;1161:106–115.
20. Tironi-Farinati C, Loidl CF, Boccoli J, Parma Y, Fernandez-Miyakawa ME, Goldstein J. Intracerebroventricular Shiga toxin 2 increases the expression of its receptor globotriaosylceramide and causes dendritic abnormalities. J Neuroimmun 2010;222:48–61.
21. Ramegowda B, Samuel JE, Tesh VL. Interaction of Shiga toxins with human brain microvascular endothelial cells: cytokines as sensitizing agents. J Infect Dis 1999;180: 1205–1213.
22. Eisenhauer PB, Chaturvedi P, Fine RE, et al. Tumor necrosis factor alpha increases human cerebral endothelial cell Gb3 and sensitivity to Shiga toxin. Infect Immun 2001;69:1889–1894.
23. Stricklett PK, Hughes AK, Ergonul Z, Kohan DE. Molecular basis for up-regulation by inflammatory cytokines of Shiga toxin 1 cytotoxicity and globotriaosylceramide expression. J Infect Dis 2002;186:976–982.

24. Aloisi F. Immune function of microglia. Glia 2001;36: 165–179.
25. Yang L, Lindholm K, Konishi Y, Li R, Shen Y. Target depletion of distinct tumor necrosis factor receptor subtypes reveals hippocampal neuron death and survival through different signal transduction pathways. J Neurosci 2002;22: 3025–3032.
26. Akassoglou K, Bauer J, Kassiotis G, et al. Oligodendrocyte apoptosis and primary demyelination induced by local TNF/p55TNF receptor signaling in the central nervous system of transgenic mice: models for multiple sclerosis with primary oligodendrogliopathy. Am J Pathol 1998; 153:801–813.
27. Zhao X, Bausano B, Pike BR, et al. TNF-α stimulates caspase-3 activation and apoptotic cell death in primary septo-hippocampal cultures. J Neurosci Res 2001;64: 121–131.
28. Nishida N, Hata Y, Sasahara M, Ishii Y, Hamashima T, Shin J. Postmortem examination in patients with O111 infection [in Japanese]. Sata T, editor. Annual Report on Epidemiology, Microbiologic Features and Clinical Manifestations in EHEC/O111 Outbreak. Special Research (H23-TOKUBETU-SHITEI-004). Japan: Ministry of Health, Labor and Welfare of Japan; 2012:179–182.
29. Mizuguchi M, Yamanouchi H, Ichiyama T, Shiomi M. Acute encephalopathy associated with influenza and other viral infections. Acta Neurol Scand 2007;115:45–56.
30. Takanashi J. Two newly proposed encephalitis/encephalopathy syndromes. Brain Dev 2009;31:521–528.
31. Ichiyama T, Isumi H, Ozawa H, Matsubara T, Moroshima T, Furukawa S. Cerebrospinal fluid and serum levels of cytokines and soluble tumor necrosis factor receptor in influenza virus-associated encephalopathy. Scand J Infect Dis 2003;35:59–61.
32. Ichiyama T, Endo S, Kaneko M, Ishumi H, Matsubara T, Furukawa S. Serum cytokine concentrations of influenza-associated acute necrotizing encephalopathy. Pediatr Int 2003;45:734–736.
33. Flammer JR, Rogatsky I. Glucocorticoids in autoimmunity: unexpected targets and mechanisms. Mol Endocrinol 2011;25:1075–1086.
34. Okumura A, Mizuguchi M, Kidokoro H, et al. Outcome of acute necrotizing encephalopathy in relation to treatment with corticosteroids and gammaglobulin. Brain Dev 2009;31:221–227.
35. Takanashi J, Oba H, Barkovich AJ, et al. Diffusion MRI abnormalities after prolonged febrile seizures with encephalopathy. Neurology 2006;66:1304–1309.
36. Shinohara M, Saitoh M, Takanashi JI, et al. Carnitine palmitoyl transferase II polymorphism is associated with multiple syndromes of acute encephalopathy with various infectious diseases. Brain Dev 2011;33:512–517.
37. Shinohara M, Saitoh M, Nishizawa D, et al. ADOR2A polymorphism predisposes children to encephalopathy with febrile status epilepticus. Neurology 2013;80:1–6.
38. Watahiki M, Ohnishi M, Sekizuka T. Summary of microbiologic research (in Japanese). Sata T, editor. Annual Report on Epidemiology, Microbiologic Features and Clinical Manifestations in EHEC/O111 Outbreak. Special Research (H23-TOKUBETU-SHITEI-004). Japan: Ministry of Health, Labor and Welfare of Japan; 2012;99–103.
39. Siegler RL. Spectrum of extrarenal involvement in post-diarrheal hemolytic-uremic syndrome. J Pediatr 1994;125: 511–518.
40. Ahrens F, Ludwig K, Terstegge K, Querfeld U. Encephalopathy and exposure to Shiga toxin without evidence of haemolytic uraemic syndrome. Eur J Pediatr 2002;16: 462–463.