

**Serum tau protein as a marker of disease activity in enterohemorrhagic
Escherichia coli O111-induced hemolytic uremic syndrome**

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Running Head: serum tau protein in EHEC O111-induced HUS

Abstract Tau protein levels in cerebrospinal fluid (CSF) and serum are elevated in patients with various central nervous system diseases. We investigated whether serum tau protein levels are useful for predicting and assessing disease activity of acute encephalopathy (AE) in enterohemorrhagic *Escherichia coli* (EHEC) O111-induced hemolytic uremic syndrome (HUS; EHEC encephalopathy). Serum samples were obtained from 14 patients with EHEC O111/HUS, 20 patients with non-EHEC-related AE, and 20 age- and sex-matched healthy controls. CSF samples were obtained from 2 patients with EHEC encephalopathy and 20 patients with non-EHEC-related AE. Tau protein levels and levels of several proinflammatory cytokines were quantified by enzyme-linked immunosorbent assays. Results were compared with the clinical features of EHEC encephalopathy, including magnetic resonance image (MRI) findings. Serum tau levels in patients with EHEC encephalopathy were significantly elevated compared with those in patients with EHEC O111/HUS without encephalopathy, patients with non-EHEC-related AE, and healthy controls. The ratio of CSF tau levels to serum tau levels was >1.0 in all patients with non-EHEC-related AE but <1.0 in 2 patients with EHEC encephalopathy. Serum tau protein levels increased rapidly and markedly in patients with severe EHEC O111/HUS and encephalopathy when HUS occurred, but were not elevated in mild patients, even in the HUS phase. Furthermore, changes in serum tau protein levels in patients with EHEC encephalopathy were consistent with abnormalities on brain MRI and were positively correlated with proinflammatory cytokine levels. Our results indicate that serum tau protein might be useful to predict and assess disease activity of EHEC encephalopathy.

Keywords: Tau protein, Enterohemorrhagic *Escherichia coli*, Hemolytic uremic syndrome, Encephalopathy

Introduction

Hemolytic uremic syndrome (HUS) is a multisystem disease characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia, and renal failure. HUS occurs after a prodrome of hemorrhagic colitis caused by Shiga toxin-producing *Escherichia coli*, also known as enterohemorrhagic *E. coli* (EHEC). Neurologic complications involving the central nervous system (CNS) occur in approximately 20% of patients with HUS [1]. Common clinical symptoms include seizures and alteration of consciousness, and morbidity and mortality are increased in affected patients [2]. The pathogenesis of CNS involvement remains unclear, although various proinflammatory cytokines have been implicated in the pathogenesis of neurological complications in HUS [3, 4].

The most prevalent serotype involved in HUS is EHEC O157. However, EHEC O111 can also cause HUS and neurological complications [5, 6]. An outbreak of EHEC O111 occurred in Toyama and other prefectures in Japan between late April and early May 2011. The course of the disease in some patients was extremely aggressive, and some cases were fatal [5]. Most of the severely affected HUS patients in this outbreak had neurological complications [5].

Tau protein is a microtubule-associated protein localized in both neurons and oligodendrocytes. It is found primarily in axons, at the branching points of cellular processes and at the ends of cellular extensions [7]. Tau protein is necessary for cytoskeletal structure and axonal transport [7]. Previous studies have reported that tau protein levels in the cerebrospinal fluid (CSF) and serum are elevated in patients with traumatic brain injury or CNS diseases, such as acute ischemic stroke [8-10] and acute encephalopathy (AE) [11]. Although little is known about the passage of tau from the brain into the blood, disruption of the blood brain barrier might lead to the leakage of tau from CSF to the blood.

In this study, we examine serum tau protein levels in EHEC O111-induced HUS

(EHEC O111/HUS) to determine whether serum tau levels are useful to predict and assess disease activity of AE in EHEC O111/HUS (EHEC encephalopathy).

Patients and methods

Patients and samples

An outbreak of EHEC O111 occurred in Toyama and other prefectures in Japan between late April and early May 2011. Serum and CSF samples were obtained from EHEC O111-infected patients during the outbreak. Serum samples were obtained from 14 patients during the HUS phase, and CSF samples were also obtained from 2 of these 14 patients (patient 2 and patient 7). Serum samples from 7 patients were obtained serially from the hemorrhagic colitis (HC) phase to the HUS phase. Serum samples from 3 patients were obtained in both the pre-HUS phase (1 day before the diagnosis of HUS) and the HUS phase.

The clinical characteristics of the patients with EHEC O111/HUS are shown in [Table 1](#). EHEC O111 infection was diagnosed based on the presence of bloody diarrhea, vomiting, and/or bowel cramps with microbiological identification of EHEC O111. HUS was defined by thrombocytopenia (platelet count of $<150,000/\text{mm}^3$), hemolytic anemia, and acute renal dysfunction. Acute renal dysfunction was defined as renal injury evidenced by hematuria, proteinuria, or elevated creatinine levels (≥ 1.0 mg/dl in children <13 years old and ≥ 1.5 mg/dl in patients ≥ 13 years old, or a $\geq 50\%$ increase over baseline) [12]. AE was defined by the presence of behavioral abnormalities or neurological symptoms with pathological magnetic resonance imaging (MRI) findings.

The severity of HUS was classified according to Gianantonio's criteria: mild, no anuria; moderate, <7 days of anuria; and severe: ≥ 7 days of anuria [13]. One patient (patient 1) did not have anuria but suffered from AE, acute lung injury, and acute pancreatitis. Therefore, this patient was classified as severe. Eleven patients were classified as severe, no patients were classified as moderate, and three patients were classified as mild ([Table 1](#)).

Eight patients presented with complications of AE. Two patients died of AE with diffuse brain edema. Some patients developed neurological sequelae despite aggressive treatment, including continuous renal replacement therapy, plasma exchange, and anti-inflammatory therapy with steroids and intravenous immunoglobulin.

Serum samples were also obtained from 20 patients with non-EHEC-related AE, including acute disseminated encephalomyelitis, biphasic seizures and late reduced diffusion, and AE associated with viral infections (such as influenza) and from 20 age- and sex-matched healthy controls. CSF samples were obtained from the same 20 patients with non-EHEC-related AE. Serum and CSF samples were separated, divided into aliquots, frozen, and stored at -80°C until analysis. This study was approved by the institutional review board of Kanazawa University, and all patients provided informed consent for the collection and analysis of specimens.

Quantification of tau protein and cytokines

Serum concentrations of tau, neopterin, interleukin (IL)-6, tumor necrosis factor (TNF)- α , soluble TNF receptor (sTNFR)I, and sTNFRII were evaluated using commercial enzyme-linked immunosorbent assays, according to the manufacturer's instructions (tau: Invitrogen, Camarillo, CA, USA; neopterin: IBL, Hamburg, Germany; IL-6, sTNFR I, and sTNFR II: R&D Systems, Inc., Minneapolis, MN, USA). CSF concentrations of tau were also evaluated using commercial enzyme-linked immunosorbent assays according to the manufacturer's instructions (Invitrogen, Camarillo, CA, USA).

Statistical analysis

Within-group comparisons were analyzed using the Mann–Whitney *U* test.

Correlations were expressed using the Spearman rank correlation coefficient. A p -value < 0.05 was considered statistically significant.

Results

Serum and CSF levels of tau protein in EHEC O111/HUS

We determined serum levels of tau in patients with EHEC O111/HUS (with and without encephalopathy) and compared them with the levels in patients with non-EHEC-related AE and in healthy controls. Serum tau levels in patients with EHEC encephalopathy were significantly elevated compared with those in patients with EHEC O111/HUS without encephalopathy, in patients with non-EHEC-related AE, and in healthy controls (Fig. 1A). Serum tau levels in patients with non-EHEC-related AE were significantly elevated compared with controls (Fig. 1A). CSF tau levels were determined in 2 patients with EHEC encephalopathy and 20 patients with non-EHEC-related AE. CSF tau levels were not elevated in two EHEC encephalopathy patients despite increased serum tau levels, whereas CSF tau levels were elevated in most of the patients with non-EHEC-related AE who had increased serum tau levels (Fig. 1B, C). The ratio of CSF tau levels to serum tau levels was >1.0 in all patients with non-EHEC-related AE (Fig. 1C); however, it was <1.0 in the 2 patients with EHEC encephalopathy (Fig. 1C).

Changes in serum tau protein levels in patients with severe or mild EHEC O111/HUS

To investigate whether serum tau protein levels reflect disease activity of EHEC O111/HUS, serum tau protein levels were serially monitored in 10 patients with EHEC O111/HUS (Fig. 2). Serum tau protein levels in patients with severe EHEC O111/HUS who developed encephalopathy were rapidly and markedly elevated when HUS developed, whereas levels in patients with mild EHEC O111/HUS were not elevated, even during the HUS phase. Furthermore, elevated serum tau protein levels in 2 patients with severe EHEC O111/HUS and encephalopathy were consistent with

abnormalities on brain MRI (Fig. 3).

Correlation between serum tau protein and proinflammatory cytokine levels in patients with EHEC O111/HUS

Serum tau protein levels were positively correlated with levels of the proinflammatory cytokines neopterin, IL-6, TNF- α , sTNFR1, and sTNFR2 (Fig. 4A-E).

Discussion

The presence of CNS dysfunction is an important predictive factor for morbidity and mortality in HUS [1]. In an outbreak of EHEC O104:H4 in Germany in May 2011, 30% of HUS patients showed signs and symptoms of encephalopathy, including delirium, stimulus-sensitive myoclonus, aphasia, and epileptic seizures requiring mechanical ventilation [14]. In the outbreak of EHEC O111 in Japan between late April and early May 2011 investigated in the present study, 169 people suffered from diarrhea, 30 (17.8%) developed HUS, and encephalopathy developed in 14 (47%) of the HUS patients [5]. Furthermore, 5 hospitalized patients died from neurological manifestations, including somnolence, coma, and convulsions [5]. In view of the outcomes of this outbreak in Japan, we identified the need to establish a monitoring system with useful clinical markers to predict severe clinical outcomes, in particular AE, in patients with EHEC O111 infection.

Tau is a microtubule stabilizing protein primarily localized in CNS neurons, but is also expressed at low levels in astrocytes and oligodendrocytes [15, 16]. Elevated levels of tau are seen in the CSF of patients with severe head injuries [8-10], AE [11], and neurodegenerative disease [17], suggesting its extracellular release during neuronal damage and supporting its role as a biomarker with specificity for brain injury. The potential movement of increased amounts of tau from the CSF across the blood–brain barrier in brain disease or injury raises the possibility that measurements of serum tau could provide a convenient peripheral window into the status of the brain [18].

In the present study, we demonstrated that serum tau levels in patients with EHEC encephalopathy are significantly elevated compared with those in patients with EHEC O111/HUS without encephalopathy. Serum tau levels in patients with severe EHEC O111/HUS who developed encephalopathy were rapidly and markedly elevated at the start of the HUS phase, whereas levels in patients with mild EHEC

0111/HUS were not elevated, even during the HUS phase. Furthermore, changes in serum tau protein levels in patients with severe EHEC 0111/HUS and encephalopathy were consistent with abnormalities on brain MRI. These results indicate that serum tau protein levels might be a useful marker to reflect disease activity of EHEC 0111/HUS.

The pathophysiology of EHEC-associated HUS remains obscure. Endothelial cell damage is the main histopathological feature of HUS. Increasing experimental evidence suggests that immune responses of the host to Shiga toxin and/or lipopolysaccharide (LPS) are involved in the pathophysiology of EHEC infections [19-21]. In the present study, we found that serum tau protein levels were positively correlated with proinflammatory cytokines including neopterin, IL-6, sTNFR1, and sTNFR2. These results indicate that these proinflammatory cytokines might be closely associated with the pathogenesis of brain injury in EHEC-associated HUS. These cytokines are useful to predict neurological complications in patients with HUS, as we previously reported [3, 4]. Combined monitoring of serum tau and these proinflammatory cytokines might be useful in the clinical management of EHEC-associated HUS.

Previous studies have shown that tau protein levels in CSF and serum are elevated in patients with various CNS diseases including AE [8-11, 17]. It is thought that increased levels of serum tau could be related to the leakage of tau from the CSF to the blood after disruption of the blood–brain barrier [18]. As shown in Fig. 1C, the ratio CSF tau levels to serum tau levels was >1.0 in all patients with non-EHEC-related AE. These results support this hypothesis.

However, in contrast to patients with non-EHEC-related AE, in 2 patients with EHEC encephalopathy, CSF tau levels were not elevated, despite elevated levels of serum tau. Therefore, the ratio of CSF tau level to serum tau level was <1.0 (Fig. 1C). This serum dominant pattern for tau levels observed in EHEC encephalopathy was unique. Endothelial cell damage is the main pathological feature

of HUS. In rabbits, Shiga toxin passes through the blood–brain barrier promptly after intravenous injection, and localizes on the endothelial cells of capillaries, ependymal cells, and myelin sheaths in 24–57 h [22]. Because deterioration of the blood–brain barrier occurs in the early stage of HUS, tau protein released from injured axons might leak rapidly into the vascular space, resulting in a serum-dominant increase in tau protein in EHEC encephalopathy.

A limitation of the present study was the small number of patients with EHEC O111/HUS. A study with a larger sample including other serotypes of EHEC infection would help to determine the true diagnostic value of serum tau protein. Despite this limitation, our results indicate that serum tau protein might be useful to predict disease activity and severity of neurological complications in EHEC O111/HUS.

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Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Siegler RL (1994) Spectrum of extrarenal involvement in postdiarrheal hemolytic-uremic syndrome. *J Pediatr* 125:511-518.
2. Gallo EG, Gianantonio CA (1995) Extrarenal involvement in diarrhoea-associated hemolytic-uraemic syndrome. *Pediatr Nephrol* 9:117-119.
3. Shiraishi M, Ichiyama T, Matsushige T, Iwaki T, Iyoda K, Fukuda K, et al (2008) Soluble tumour necrosis factor receptor 1 and tissue inhibitor of metalloproteinase-1 in hemolytic uremic syndrome with encephalopathy. *J Neuroimmunol* 196:147-152.
4. Shimizu M, Kuroda M, Sakashita N, Konishi M, Kaneda H, Igarashi N, et al (2012) Cytokine profiles of patients with enterohemorrhagic *Escherichia coli* O111-induced hemolytic-uremic syndrome. *Cytokine*. 60:694-700.
5. Matano S, Inamura K, Konishi M, Okumura T, Kawai H, Okamura T, et al (2012) Encephalopathy, disseminated intravascular coagulation, and hemolytic-uremic syndrome after infection with enterohemorrhagic *Escherichia coli* O111. *J Infect Chemother*. 18:558-564.
6. Piercefield EW, Bradley KK, Coffman RL, Mallonee SM (2010) Hemolytic uremic syndrome after an *Escherichia coli* O111 outbreak. *Arch Intern Med* 170:1656-1663.
7. Gorath M, Stahnke T, Mronga T, Goldbaum O, Richter-Landsberg C (2001) Developmental changes of tau protein and mRNA in cultured rat brain oligodendrocytes. *Glia* 36:89-101.
8. Bitsch A, Horn C, Kemmling Y, Seipelt M, Hellenbrand U, Stiefel M, et al (2002) Serum tau protein level as a marker of axonal damage in acute ischemic stroke. *Eur Neurol* 47:45-51.
9. Shaw GJ, Jauch EC, Zemlan FP (2002) Serum cleaved tau protein levels and clinical outcome in adult patients with closed head injury. *Ann Emerg Med* 39:254-257.

10. Bazarian JJ, Zemlan FP, Mookerjee S, Stigbrand T (2006) Serum S-100B and cleaved-tau are poor predictors of long-term outcome after mild traumatic brain injury. *Brain Inj* 20:759-765.
11. Tanuma N, Miyata R, Kumada S, Kubota M, Takanashi J, Okumura A, et al (2010) The axonal damage marker tau protein in the cerebrospinal fluid is increased in patients with acute encephalopathy with biphasic seizures and late reduced diffusion. *Brain Dev* 32:435-439.
12. Elliott EJ, Robins-Browne RM (2005) Hemolytic uremic syndrome. *Curr Probl Pediatr Adolesc Health Care* 35:305-344.
13. Gianantonio CA, Vitacco M, Mendilaharsu F, Gallo GE, Sojo ET (1973) The hemolyticuremic syndrome. *Nephron* 11:174-192.
14. Greinacher A, Friesecke S, Abel P, Dressel A, Stracke S, et al (2011) Treatment of severe neurological deficits with IgG depletion through immunoadsorption in patients with *Escherichia coli* O104:H4-associated haemolytic uraemic syndrome: a prospective trial. *Lancet* 378:1166-1173.
15. Avila J, Lucas JJ, Perez M, Hernandez F (2004) Role of tau protein in both physiological and pathological conditions. *Physiol Rev* 84:361-384.
16. Shin RW, Iwaki T, Kitamoto T, Tateishi J (1991) Hydrated autoclave pretreatment enhances tau immunoreactivity in formalin-fixed normal and Alzheimer's disease brain tissues. *Lab Invest* 64:693-702.
17. Kapaki E, Kilidireas K, Paraskevas GP, Michalopoulou M, Patsouris E (2001) Highly increased CSF tau protein and decreased β -amyloid (1-42) in sporadic CJD: a discrimination from Alzheimer's disease? *J Neurol Neurosurg Psychiatry* 71:401-403.
18. Morris M, Maeda S, Vossel K, Mucke L (2011) The many faces of tau. *Neuron* 70:410-426.
19. Cleary TG (2004) The role of Shiga-toxin-producing *Escherichia coli* in hemorrhagic colitis and hemolytic uremic syndrome. *Semin Pediatr Infect Dis*

15:260-265.

20. Banatvala N, Griffin PM, Greene KD, Barrett TJ, Bibb WF, Green JH, et al (2001) Hemolytic uremic syndrome study collaborators. The United States National prospective hemolytic uremic syndrome study: microbiologic, serologic, clinical, and epidemiologic findings. *J Infect Dis* 183:1063-1070.
21. Keepers TR, Psocka MA, Gross LK, Obrig TG (2006) A murine model of HUS: Shiga toxin with lipopolysaccharide mimics the renal damage and physiologic response of human disease. *J Am Soc Nephrol* 17:3404.
22. Fujii J, Kinoshita Y, Kita T, Iquire A, Takeda T, Tanaka N, Yoshida S (1996) Magnetic resonance imaging and histopathological study of brain lesions in rabbits given intravenous Verotoxin 2. *Infect Immun* 64:5053-5060.

Table 1 Clinical characteristics of patients with *Escherichia coli* O111-induced hemolytic uremic syndrome

case	age	sex	ARF	encephalopathy	severity	prognosis
1	13	M	—	+	severe	alive
2	7	F	+	+	severe	alive
3	17	F	+	+	severe	alive
4	14	M	+	+	severe	deceased
5	26	F	+	+	severe	alive
6	1	M	+	+	severe	alive
7	7	F	+	+	severe	alive
8	7	M	+	+	severe	deceased
9	16	F	+	+	severe	alive
10	14	F	+	+	severe	alive
11	16	F	+	—	severe	alive
12	16	M	—	—	mild	alive
13	6	F	—	—	mild	alive
14	8	F	—	—	mild	alive

M male patient, *F* female patient, *ARF* acute renal failure

Figures

Fig.1 Serum and cerebrospinal fluid (CSF) levels of tau protein

(A) Serum levels of tau protein at the time of diagnosis of hemolytic uremic syndrome (HUS) in patients with *Escherichia coli* O111-induced HUS (EHEC O111/HUS) with and without encephalopathy, in patients with non-EHEC-related acute encephalopathy (AE), and in healthy controls. Bars represent the median values. Statistically significant differences between groups are $p < 0.05$.

(B) CSF levels of tau protein at the time of diagnosis of HUS in 2 patients with severe

EHEC O111/HUS and AE and in patients with non-EHEC-related AE. Bars represent the median values.

(C) Plot of the serum and CSF levels of tau protein during the HUS phase in 2 patients with severe EHEC O111/HUS and AE and in patients with non-EHEC-related AE.

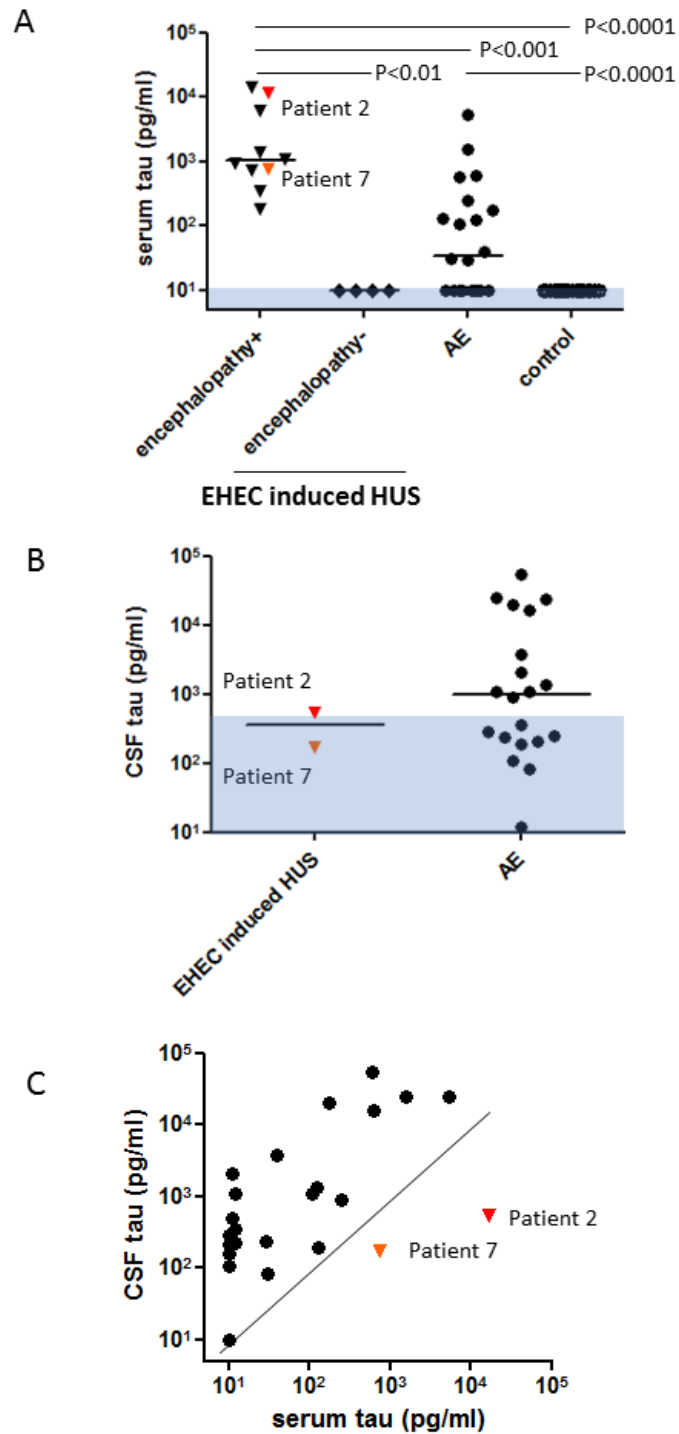


Fig. 2 Longitudinal changes of serum tau protein in patients with *Escherichia coli* O111-induced hemolytic uremic syndrome (EHEC O111/HUS)

(A) Serum tau protein levels in patients with severe EHEC O111/HUS and encephalopathy, (B) Serum tau protein levels in patients with mild EHEC O111/HUS.

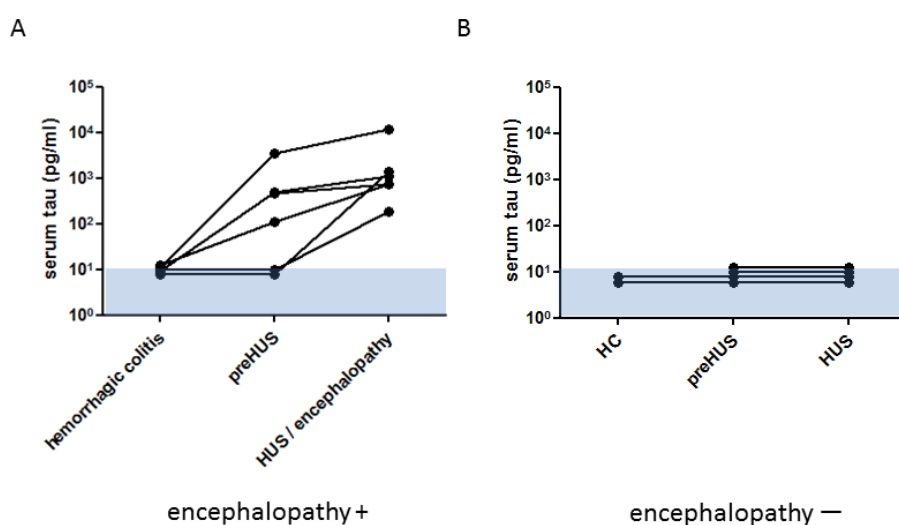


Fig. 3 Longitudinal assessment of serum tau protein in 2 patients with *Escherichia coli* O111-induced hemolytic uremic syndrome

(A) case 2, (B) case 6. Changes in serum tau protein levels (solid lines) are shown in the upper panels and the details of the therapeutic interventions are shown in the lower panels.

MRI magnetic resonance image, *IVIg* intravenous immunoglobulin, *mPSL* methylprednisolone, *RRT* renal replacement therapy

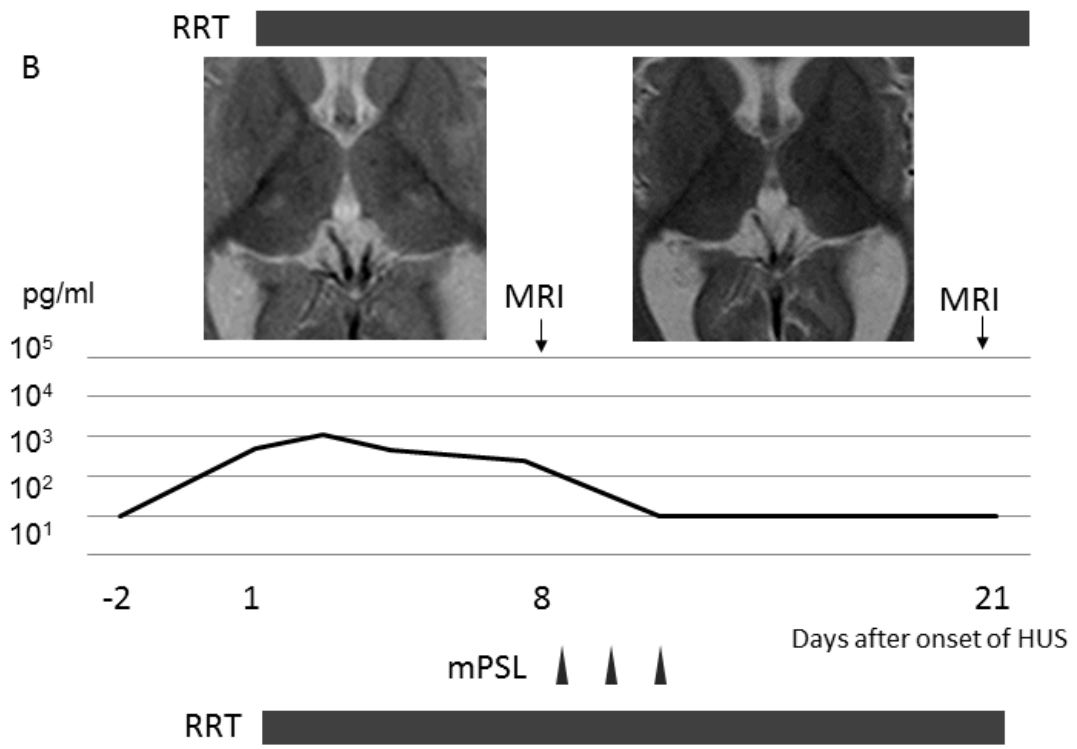
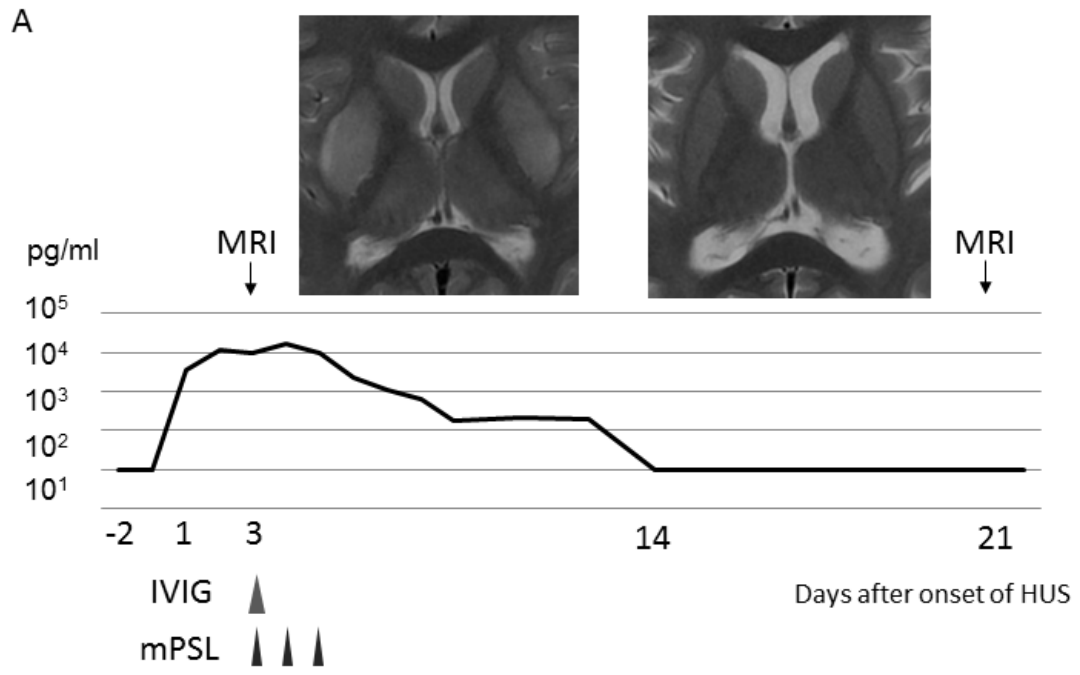


Fig. 4 Positive correlations between serum tau protein levels and proinflammatory cytokines at the time of diagnosis of hemolytic uremic syndrome (HUS) in patients with *Escherichia coli* O111-induced HUS

(A) neopterin, (B) IL-6, (C) TNF- α , (D) sTNFR I, and (E) sTNFR II.

IL, interleukin; TNF, tumor necrosis factor; sTNFR, soluble TNF receptor

