Table 3. Results of Multiple Logistic Regression Analyses for Prognostic Factors in BM

Variables	Regression coefficient	Standard error	p value	Odds ratio <95%CI>
(2) Age	0.087	0.050	0.080	1.091 <0.990-1.202>
(4) GCS score at the initiation of antibiotics	-0.638	0.273	0.020*	0.528 <0.309-0.902>
(6) Thrombocyte count	-0.221	0.106	0.037*	0.802 <0.652-0.987>
(14) Positive blood culture	3.031	1.733	0.080	20.718 <0.694-618.446>

Immunocompromised status, presence of antibiotic-resistant bacteria and CSF glucose were excluded from this logistic model. CI = confidence interval; GCS = Glasgow Coma Scale; * = statistically significant ($p<0.05$).

otics. Patients with BM caused by penicillin-sensitive *Streptococcus pneumoniae*/methicillin-sensitive *Staphylococcus aureus* were treated with a third-generation cephalosporin and ampicillin.

The results of the multiple logistic regression analyses in relation to the clinical outcome are summarized in Table 3. A low GCS score at the initiation of antibiotic therapy (p= 0.020, odds ratio=0.528, 95%CI=0.309-0.902) and a low thrombocyte count (p=0.037, odds ratio=0.802, 95%CI= 0.652-0.987) were strongly associated with unfavorable outcome. However, the presence of antibiotic-resistant bacteria, as well as other variables, was not statistically significantly associated with outcome.

Discussion

Many prognostic factors have been reported in adult patients with BM, including: age (3, 7, 8, 10-12), duration from onset to the initiation of antibiotic therapy (6, 8), impairment of consciousness on admission (3, 4, 6-11), bacteremia (3, 7, 11), septic shock (11), immunocompromised status (3, 11, 12), focal neurological deficits on admission (3, 10), seizures (6, 7, 10, 12), CSF cell count (3, 7, 12), high CSF protein level (12), low CSF glucose level (12), low blood pressure (4, 6), need for mechanical ventilation (7, 9, 12), Acute Physiology and Chronic Health Evaluation (APACHE II) score $\geq$13 (10), *Streptococcus pneumoniae* (4), *Staphylococcus aureus* (8), penicillin-resistant bacteria (7), and the presence of pneumonia (3, 12). In almost all previous studies, a between-groups comparative analysis was used. In the report by Van de Beek et al (5), the results of the first large scale multiple logistic analyses involving 696 adult patients with community-acquired BM in the Netherlands were presented. They identified several factors that were associated with an unfavorable outcome: a low admission GCS score, a low CSF cell count, advanced age, presence of otitis or sinusitis, pneumonia, immunocompromised status, and absence of a rash. However, in the Netherlands, the rate of antibiotic resistance to penicillin was very low: 0.57% (2 of 351 patients) in patients with pneumococcal meningitis, and 1.56% (4 of 256 patients) in patients with meningococcal meningitis. In several northern European countries, the rate of penicillin-resistant isolates was also less than 5% for pneumococci, less than 15% for *Haemophilus influenzae*, and less than 1% for meningococci (14, 15), whereas penicillin-susceptible bacteria were responsible for 95% of BM according to surveillance data (16). The relationship between antibiotic-resistant bacteria and the outcome of meningitis remains to be elucidated in countries with a high resistance rate, such as Japan and the United States. This is the first study using multiple logistic analyses to report prognostic factors in adults with acute community-acquired BM in Tokyo, Japan, which is an area with a high antibiotic resistance rate. In our country, the incidence of community-acquired meningitis caused by penicillin-resistant *Streptococcus pneumoniae* has increased, accounting for 45% of children with BM and 27% of adults with BM during the period from 1999 to 2002 (17). In the USA, the penicillin resistance (intermediate/resistant) rate for *Streptococcus pneumoniae* increased to 33.9% (15.5%/ 18.4%) in 2002, and the ceftriaxone resistance rate increased to 3.8% (2.1%/1.7%) (18). Therefore, the recommendations for the initial choice of antibiotics in adult patients without identified pathogens differ among countries according to the rate of antibiotic resistance. In the Netherlands, penicillin alone is recommended for adult meningitis in patients aged less than 50 or 60 years without particular risk factors (16, 19), whereas in the UK, a third-generation cephalosporin is preferred (20, 21). In the USA, which has a high rate of antibiotic resistance, the Infectious Disease Society of America recommends a combination of a third-generation cephalosporin and vancomycin for adults under 50 years of age without risk factors (22).

According to the data of a nationwide survey in Japan (23), about 80% of BM pathogens are pneumococci in patients from the ages of 6 to 49 years, followed by *Haemophilus influenzae* and streptococci. Few cases of BM are caused by *Neisseria meningitidis* in Japan. In adults older than 50 years of age, the pathogens included *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, enterococci (*Enterococcus faecalis*, *Enterococcus faecium*), and intraoral streptococci. In our hospitals, the frequency of antibiotic-resistant bacteria, including PRSP, MRSA and beta-lactamase-negative, ampicillin-resistant (BLNAR) *Haemophilus influenzae*, increased significantly from 27.3% to 66.7% during the period from 1983-1998 to 1999-2004 (24). Ubukata et al (17) analyzed 219 isolates of *Streptococcus pneumoniae* from patients with meningitis and described the MIC_{90}s of the seven antibiotics against PRSP. In order of concentration, the MIC_{90}s of panipenem-betamipron (PAPM/ BP) (0.125 μg/mL) were excellent, followed by meropenem

(MEPM) and vancomycin (VCM) (0.5 μg/mL for both), cefotaxime (CTX) and ceftriaxone (CTRX) (1 μg/mL for both), and ampicillin (AMP) and penicillin (PC) (4 μg/mL for both). In the Japanese Clinical Guideline for Bacterial Meningitis published in 2004 (23), a combination of third-generation cephalosporin (CTX or CTRX) and VCM, or carbapenem (PAPM/BP or MEPM) alone is recommended, accompanied by corticosteroids at the same time or before antibiotic administration, until the causative pathogen and its sensitivity are identified. In the present study, therapy with these antibiotics was initiated as soon as BM was suspected. Then, when the causative pathogen and its sensitivity were identified, the treatment was changed accordingly. In 65% of the present patients, they were given both antibiotics and corticosteroids.

In the present study, a low GCS score at the initiation of antibiotic therapy and a low thrombocyte count were identified as unfavorable prognostic factors. These factors were also included among the predictors reported in the study from the Netherlands in which a low rate of antibiotic resistance was observed (5). Impairment of consciousness, such as a low GCS score, has been reported to be related to complications such as cerebrovascular events, seizures, brain edema, hydrocephalus, and abscesses (3, 25).

BM is often associated with septic shock and disseminated intravascular coagulation (DIC) (3, 25, 26). At least 11 of our 44 patients in whom laboratory tests for DIC were performed showed overt DIC. Kowalik et al (26) reported that mortality in 118 adult patients with purulent meningitis correlated significantly with acute thrombocytopenia and DIC. Thrombocytopenia, usually defined as a thrombocyte count of less than $100\times10^3/\mu L$, is one of the markers of DIC (27). The International Society of Thrombosis and Hemostasis overt and non-overt DIC criteria include thrombocytopenia (28). These criteria include generally available laboratory tests (thrombocyte count, elevated fibrin related markers or fibrin degradation products, prolonged prothrombin time, fibrinogen level) plus at least one test for activated clotting that reflects the process of blood coagulation in the systemic circulation, such as thrombin-antithrombin III complexes (TAT) and fibrin monomers. However, thrombocytopenia is a late phase marker of overt DIC, whereas activation of the inflammatory cascade is an early phase marker of non-overt DIC (29), representing activated coagulation proteins and pro-inflammatory cytokines. Thus, monitoring of the markers of the early phase, such as TAT and fibrin monomers, in addition to the thrombocyte count, may be required in adult patients with BM to detect DIC, thereby leading to its treatment during the non-overt phase.

The presence of antibiotic-resistant bacteria was not identified as a significant predictor of unfavorable outcome in the present study. This suggests that, when the appropriate antibiotics are administered, antibiotic-resistant bacteria may not be a significant factor related to an unfavorable outcome, even in areas where the rate of antibiotic resistance is high, as in Japan.

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Prognostic value of cerebrospinal fluid cytokine changes in herpes simplex virus encephalitis

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ABSTRACT

A recent trial suggested that corticosteroid was beneficial in herpes simplex virus encephalitis (HSVE), but that precise role remains unclear. We assessed the differences of cerebrospinal fluid (CSF) cytokine changes between different outcomes and between patients with and without corticosteroid administration at the acute stage of HSVE. Interleukin (IL)-1β, IL-2, IL-6, IL-10, interferon (IFN)-γ, and tumor necrosis factor-α were measured in 56 serial CSFs taken from 20 adult HSVE patients. Their outcomes were poor in 7 and good in 13 patients, and corticosteroid was administered in 10. The differences in the initial and maximum cytokine values were assessed among the different outcomes. The decline rate of cytokine values between the initial and second CSF samples was also assessed between patients with and without corticosteroid. The initial IFN-γ and maximum IL-6 with a poor outcome were higher than those with a good outcome ($p = 0.019$ for IFN-γ and $p = 0.013$ for IL-6). The decline rate of IL-6 in patients with corticosteroid was higher than that without corticosteroid ($p = 0.034$). The initial IFN-γ and maximum IL-6 CSF values represented prognostic biomarkers in HSVE. One pharmacological mechanism related to corticosteroid in HSVE is apparently inhibition of pro-inflammatory cytokines such as IL-6.

1. Introduction

Herpes simplex virus encephalitis (HSVE) is associated with a significant morbidity and mortality, even when appropriate antiviral therapy is administered at the acute stage of the illness [1–3]. Antiviral therapy is highly effective in reducing the rate of mortality from HSVE. However, only less than one-half of HSVE patients are able to return to normal [1–3]. This finding indicates a need to develop further improved therapeutic regimens for HSVE. We recently reported that combination therapy with corticosteroid and aciclovir appeared to give a better outcome in adult patients with HSVE [4]. The pharmacological mechanism of corticosteroid in HSVE, except for improvement of brain edema, remains unclear. In a recent clinical guide [5] and reiterated in a clinical review [6] concerning HSVE, it has also been noted that "A recent trial suggests that steroid may be beneficial even in patients without marked swelling. Their role in HSVE merits further study".

Only a few previous clinical investigations have described the intrathecal cytokine and chemokine changes occurring in patients with HSVE [7–10]. These earlier studies indicated that several cerebrospinal fluid (CSF) cytokines including IFN-γ and IL-6 were elevated at the acute stage of HSVE. Moreover, it was also found that the values for monocyte chemotactic protein (MCP)-1 in the CSF were reciprocally correlated with the Modified Barthel Index at the time of CSF sampling [9]. However, the differences in serial intrathecal cytokine changes between different clinical outcomes and between patients with and without additional administration of corticosteroid under aciclovir treatment at the acute stage of HSVE remain unclear.

Based on the above-mentioned background, the present investigation provides a first assessment of the differences in serial CSF cytokine changes among different clinical outcomes, and also an assessment of the differences between treatment with and without corticosteroid administration under aciclovir at the acute stage in adult patients with HSVE.

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2. Methods

2.1. Patients and materials

The evaluated serial patients consisted of 20 individuals with HSVE among 98 acute encephalitis patients who were initially suspected of having HSVE at four different hospitals (Nihon University Itabashi Hospital, Tohoku University Hospital, and two affiliated hospitals) during the period from January 2000 to May 2007. These patients were diagnosed and treated according to the previously reported protocol [4]. The etiological diagnosis of HSVE was based on positive results being obtained in the following three laboratory tests: the nested PCR, chemiluminescence assay, and specific intrathecal HSV antibody synthesis. All patients were started on the treatment with intravenous aciclovir (30 mg/kg body weight/day) for 14 days at the time of admission. The therapeutic protocol permitted the use of corticosteroid at the discretion of the treating physician, but the protocol did not specify the dose or duration of corticosteroid treatment. When used, corticosteroid was started at the initiation of aciclovir treatment. The materials examined consisted of 56 CSF samples from the 20 enrolled patients with HSVE, which were taken at their admission up to 20 days after admission. The initial CSF samples of all patients were taken at the time of their admission, the second CSF samples of all patients except 1 were taken within 1 week, and the subsequent CSF samples were taken within 2 weeks. Further CSF samples at after 2 weeks from admission were taken in only 3 patients. Following collection of the serial CSF samples from each of the patients, the materials obtained were immediately transferred to different laboratories to be measured by chemiluminescence assay (S. Kamei, Division of Neurology, Department of Medicine, Nihon University School of Medicine, Tokyo) and by the nested PCR (T. Morishima, Department of Pediatrics, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama), and also to be determined for their cytokine concentrations at another independent laboratory institution (SRL Co., Tokyo, Japan). Each CSF sample was immediately aliquoted and frozen at -70°C, and then transferred to these three independent institutes. Within 48 h after taking the CSF samples, each sample was measured at these institutes. Samples were thus thawed only once. The results of the PCR and chemiluminescence assays for each sample were immediately informed to the sender of the sample; however the cytokine data for each CSF sample were accumulated at the determining laboratory institution. The setup for the measurement of CSF cytokines was organized so as to be independent of the hospitals into which the registered patients were admitted, in order to insure reliability of the present study. Thus, no information was available concerning the serological data for each patient at the time of measurement at these laboratories. All of the patients and/or their families gave informed consent to participate in the present study according to a protocol approved by the Ethics Committees for Human Studies at Nihon University Itabashi Hospital and Tohoku University Hospital.

2.2. Clinical data

The following clinical characteristics of the 20 enrolled patients with HSVE were checked from the patients' medical records: (1) sex, (2) age, (3) days after onset at initiation of aciclovir, (4) Glasgow Coma Scale (GCS) score at initiation of aciclovir, (5) initial leukocyte cell count in the CSF, (6) initial CSF protein concentration, (7) evidence of focal lesions detected by initial cranial computed tomography (CT), and (8) evidence of focal lesions detected by initial magnetic resonance imaging (MRI). The initial CT examinations in all subjects were performed within 24 h after admission and the MRI examinations were performed within 2 days after admission. Furthermore, (9) data on the detailed conditions of treatment with corticosteroid at the acute stage were also collected from the patients' medical records. These conditions included: given or not given corticosteroid administration at the acute stage; and the type of corticosteroid, initial dosage of corticosteroid, and duration of their administration in the administered patients. The morbidity was classified into five groups, as reported previously [11]: normal; mild sequelae for patients with minor neuropsychological deficits; moderate sequelae for patients having limitations due to motor, speech, memory, or seizure disorders; severe sequelae for patients requiring supportive care; and death.

2.3. Measurement of cytokine concentrations in serial CSFs

The concentrations of cytokines in the CSF, including IL-1β, IL-2, IL-6, IL-10, IFN-γ, and tumor necrosis factor (TNF)-α, were determined according to a previously reported study [7]. The measurements of IL-1β, IL-2, IL-10, and IFN-γ in the CSF employed a sandwich-type enzyme amplified sensitivity immunoassay (EASIA) kit (BioSource Europe, S.A., Belgium), those of IL-6 were made with a chemiluminescent enzyme immunoassay (CLEIA) kit (Fujirebio Inc., Japan), and those of TNF-α with an ELISA kit (Jimro Inc., Japan). Since the initial CSF samples of the 20 patients in the present study were taken on admission, all of the initial CSF samples were obtained just before initiation of the treatments that included aciclovir and corticosteroid. The minimum detection limits of the above-mentioned assays were 10 pg/ml for IL-1β, 0.8 U/ml for IL-2, 0.2 pg/ml for IL-6, 2 pg/ml for IL-10, 0.1 IU/ml for IFN-γ and 5 pg/ml for TNF-α. The accumulated results for the determined cytokine concentrations in the serial CSFs were sent for analysis to the biostatistical analyst at another institute (K. Hirayanagi, Department of Hygiene and Public Health, Nihon University of Physical Education, Tokyo) at the end of July 2007.

2.4. Statistical analysis

SPSS statistical software Version 12.0 (SPSS Inc., Chicago, Illinois) was employed. At the end of July 2007, the biostatistical analyst at the other institute collected the data for the clinical parameters from two neurologists (N. Taira, Division of Neurology, Department of Medicine, Nihon University School of Medicine, Tokyo, and T. Sekizawa, Yamagata Prefectural University of Health Science, Yamagata) and the data on cytokine concentrations from the independent laboratory institution. The clinical outcomes of the patients were divided into two groups: a poor outcome consisting of moderate sequelae to death, and a good outcome consisting of normal and mild sequelae.

The statistical differences in each baseline clinical characteristic between the poor outcome and good outcome groups, and between the patients who were treated with and without corticosteroid were assessed by Fisher's exact probability test or the Mann–Whitney *U* test. The differences in each cytokine concentration in the initial CSF samples on admission and the maximum values in the serial CSF samples were assessed between the two different clinical outcomes by the Mann–Whitney *U* test. Moreover, the differences in the decline rates at the acute stage were also assessed between the patients who were treated with and without corticosteroid administration. The decline rate at the acute stage was defined as follows: the difference in cytokine values divided by the duration in days between the initial and second CSF samples. The level of significance for this study was defined as 0.05.

Table 1
Clinical characteristics of each patient with herpes simplex virus encephalitis.

Patient No.	(1) Sex	(2) Age (years)	(3) Days after onset at initiation of aciclovir	(4) GCS score at initiation of aciclovir	CSF examinations		Neuroradiological findings		(9) Corticosteroids			Outcome at three months after completion of acyclovir treatment
					(5) Initial leukocyte cell count (/ml)	(6) Initial CSF protein (mg/dl)	(7) Detection of lesion by initial CT	(8) Detection of lesion by initial MRI	Administration	Drug and initial dosage	Duration	
1	f	57	7	3	377	133	Yes	Yes	Not given	–	–	Poor (death)
2	m	74	2	6	52	33	Yes	Yes	Given	Dexamethasone, 6 mg/day	6 days	Poor (moderate sequelae)
3	m	19	10	7	149	120	Yes	Yes	Given	Dexamethasone, 12 mg/day	3 days	Good (complete recovery)
4	f	24	4	12	362	40	No	No	Not given	–	–	Good (complete recovery)
5	m	17	4	7	139	104	Yes	Yes	Given	Dexamethasone, 10 mg/day	3 days	Good (mild sequelae)
6	m	57	7	3	944	410	Yes	Yes	Not given	–	–	Poor (death)
7	m	20	10	10	22	92	No	Yes	Not given	–	–	Good (complete recovery)
8	f	53	1	3	45	72	Yes	Yes	Given	Dexamethasone, 12 mg/day	3 days	Good (mild sequelae)
9	m	58	11	7	132	103	Yes	Yes	Not given	–	–	Poor (severe sequelae)
10	m	29	6	6	1376	240	Yes	Yes	Given	Dexamethasone, 8 mg/day	3 days	Good (complete recovery)
11	m	56	2	8	503	37	No	Yes	Given	Dexamethasone, 8 mg/day	8 days	Good (mild sequelae)
12	m	77	1	12	571	580	No	Yes	Given	Dexamethasone, 8 mg/day	8 days	Good (mild sequelae)
13	m	66	5	3	56	85	Yes	Yes	Not given	–	–	Poor (severe sequelae)
14	m	59	2	3	5	40	No	Yes	Not given	–	–	Poor (death)
15	f	32	2	10	36	65	No	Yes	Not given	–	–	Poor (moderate sequelae)
16	f	21	1	6	22	92	No	No	Not given	–	–	Good (complete recovery)
17	f	77	3	10	7	88	No	Yes	Given	Methylprednisolone 1000 mg/day	3 days	Good (mild sequelae)
18	m	30	5	10	16	88	No	Yes	Not given	–	–	Good (complete recovery)
19	m	54	9	3	9	38	No	Yes	Given	Methylprednisolone 1000 mg/day	3 days	Good (mild sequelae)
20	m	32	5	3	253	171	No	Yes	Given	Methylprednisolone 1000 mg/day	3 days	Good (mild sequelae)

GCS, Glasgow Coma Scale; CSF, cerebrospinal fluid.

3. Results

The clinical characteristics of the 20 patients with HSVE enrolled in the present study are listed in Table 1. The numbers of good and poor outcomes were 13 and 7. The numbers of patients with and without corticosteroid administration amounted to 10 in each group. The baseline clinical characteristics of the groups with different outcomes and of the patients with and without corticosteroid administration are summarized in Table 2. The age of the patients with a poor outcome was greater than that of the patients with a good outcome ($p = 0.027$, Mann–Whitney U test). All factors among the clinical characteristics of the patients with and without corticosteroid administration showed no significant differences. Among the measurements of the CSF cytokine values in the present 20 patients with HSVE, IL-6 was elevated in all patients. Also, IL-10 and IFN-γ were elevated in 17 out of the 20 patients. Other cytokines revealed less than the minimum detection limit in all the CSF samples at the acute stage of HSVE. The initial value of IL-6 represented the maximum value among the serial CSFs in 17 out of the 20 patients. The IL-6 values in the subsequent CSF samples declined in these 17 patients. However, in the remaining 3 patients, the value of IL-6 in the second CSF sample was higher than that in the initial CSF sample. The initial values of IL-10 and IFN-γ represented the maximum values among the serial CSF samples in all 17 patients who revealed detectable values, and the values in the subsequent CSF samples declined to the range of less than the minimum detection limit. The results for the CSF cytokines including the statistical differences among different clinical outcomes are illustrated in Fig. 1. The mean, median, and rang of initial IFN-γ values in the 13 patients with a good outcome revealed 6, 3, and 0–23 IU/ml. Those of initial IFN-γ values in the 7 patients with a poor outcome revealed 25, 24, and 0–53 IU/ml. The mean, median, and rang of maximum IL-6 values in the 13 patients with a good outcome revealed 346, 221, and 45–1285 pg/ml. Those of maximum IL-6 values in the 7 patients with a poor outcome revealed 2617, 2390, and 134–8340 pg/ml. The mean values of initial IFN-γ and the maximum IL-6 of in the patients with a poor outcome were significant higher than those in the patients with a good outcome ($p = 0.019$ for the initial value of INF-γ, and $p = 0.013$ for the maximum value of IL-6; Mann–Whitney U test). The initial values of IL-6 and IL-10 among the different clinical outcomes were not significantly different.

Another notable finding of our study was that the rate of decline of IL-6 in patients with corticosteroid administration at the acute stage of HSVE was significant higher than that without corticosteroid administration ($p = 0.034$, Mann–Whitney U test). The results for the changes in IFN-γ, IL-6, and IL-10 values of the serial CSF samples at the acute stage in each HSVE patient with and without corticosteroid administration are illustrated in Fig. 2. The IL-6 CSF values in the patients with corticosteroid administration declined rapidly to less than the minimum detection limit as compared to those in the patients without corticosteroid administration. The mean, median, and range of the IL-6 decline rate in the patients with corticosteroid administration revealed 173, 73, and 29–637 pg/ml/day. On the other, those values of the IL-6 decline rate in the patients without corticosteroid administration revealed −996 (increase), 11, and −8293 (increase) to 459 pg/ml/day. The differences in decline rates of IFN-γ and IL-10 between the patients with and without administration of corticosteroids were not significant. The differences in decline rates of IL-6, IFN-γ, and IL-10 between the different outcomes were also not significant.

4. Discussion

There have been only four previous detailed clinical studies on serial changes in intrathecal cytokines and chemokines in patients with HSVE [7–10]. One of these earlier studies described the serial intrathecal cytokine changes in 9 patients with HSVE, demonstrating that IFN-γ and IL-6 were first increased during the first week of illness, and TNF-α, IL-2, and soluble CD8 antigen then became elevated at 2–6 weeks, but IL-1β could not be detected [8]. Another previous studies [7,10] have also noted that the elevation of intrathecal IL-6 values in 6 patients with HSVE was greater than that in patients with limbic encephalitis and that the CSF concentrations of IFN-γ and soluble tumor necrosis factor receptor 1 in HSVE was higher than those with non-herpetic acute limbic encephalitis. The results obtained for the elevation of intrathecal IFN-γ and IL-6 values at the acute stage of HSVE in the present study confirmed these earlier findings.

There have been several previous fundamental and basic investigations of serial changes in cytokines employing experimental animal HSVE models [12–15]. In these experimental studies, the acute neuroinflammatory reaction in HSVE was found to comprise a rapid secretion of IFN-γ, pro-inflammatory cytokines including IL-6, and anti-inflammatory cytokines such as IL-10, followed by a specific synthesis of chemokines in order to attack inflammatory cells [12–14]. Moreover, a recent investigation employing an experimental HSVE mouse model has indicated that IL-6 acted as a potent mediator of neuronal injury at the acute stage of HSVE [15]. Furthermore, it has been suggested that a host-sided immune response associated with HSVE, such as that involving cytokines, could play some role in the outcome of HSVE based on the findings of an in vitro experimental study on infection with HSV [16]. The results obtained in the present study appeared to represent appropriate clinical findings for the acute stage of HSVE based on the descriptions given in recent experimental studies [12,15,16]; namely, the initial CSF value of IFN-g and the maximum CSF value

Table 2
Baseline clinical characteristics of the patient groups.

	Outcome			Corticosteroid treatment under acyclovir therapy		
	Good* ($N = 13$)	Poor* ($N = 7$)	Difference between the 2 groups**	Administration ($N = 10$)	No treatment ($N = 10$)	Difference between the 2 groups**
(1) Male (%)	69	71	NS	20	40	NS
(2) Age at onset (M ± SD; years)	39.2 ± 21.6	57.6 ± 12.9	0.027*	48.8 ± 23.3	42.4 ± 18.4	
(3) Days after onset at initiation of acyclovir (M ± SD)	4.7 ± 3.3	5.1 ± 3.4	NS	4.3 ± 3.2	5.4 ± 3.4	
(4) GCS score at initiation of acyclovir (M ± SD)	7.5 ± 3.2	5.0 ± 2.8		6.5 ± 3.0	6.7 ± 3.6	
(5) Number of cells in initial CSF (M ± SD; /ml)	267 ± 386	229 ± 339		197 ± 298	310 ± 424	
(6) Concentration of protein in initial CSF (M ± SD; mg/dl)	143 ± 143	124 ± 130		115 ± 107	158 ± 163	
(7) Detection of lesion by initial CT (%)	31	71		60	50	
(8) Detection of lesion by initial MRI (%)	85	100		80	100	

M, mean; SD, standard deviation; GCS, Glasgow coma scale; CSF, cerebrospinal fluid; CT, cranial computed tomography; MRI, magnetic resonance imaging; NS, not significant ($p > 0.05$).
* Significant difference ($P < 0.05$).
** Statistical differences among the two groups evaluated by Fisher's exact probability test or the Mann–Whitney U test.

of IL-6 in our patients with a poor outcome were significant higher than those in patients with a good outcome. Moreover, a recent investigation of the levels of CSF cytokines and chemokines in patients with Japanese encephalitis has yielded similar findings in which the IFN-α, IL-6, and IL-8 values of non-survivors were higher than those of survivors [17]. Based on the notion that IFN-γ is an inhibitor of viral replication [18,19] and IL-6 is one of the pro-inflammatory cytokines, the results of the present study indicate that the initial IFN-γ and maximum IL-6 values at the acute stage of HSVE could be appropriate for use as prognostic biomarkers in adult patients with HSVE.

Another notable finding of the present study was that the rate of decline in CSF IL-6 values at the acute stage of HSVE among the patients receiving corticosteroid administration was significantly higher than that without corticosteroid. Some recent reports have examined the effects of corticosteroid administration with aciclovir treatment in animal models of HSVE [20,21]. The data obtained indicated that the HSV viral load of the brain tissue in such animals, which were treated with both aciclovir and corticosteroid, was similar to that of the brain tissue in animals which were treated with aciclovir alone. These studies also revealed that the corticosteroid did not inhibit the antiviral action of aciclovir and might decrease the extent of HSVE infection [20,21]. Seven out of 9 patients in the previous detailed clinical report on serial cytokine values in HSVE [8] were administered corticosteroid with aciclovir, so that the above-mentioned observations made it difficult to assess the differences in intrathecal cytokine changes between the patients with and without corticosteroid administration. In the Discussion section of this clinical study [8], it was speculated that a decline of intrathecal cytokines might accompany the therapeutic use of corticosteroid. The marked decline rate of IL-6 observed in the adult HSVE patients with therapeutic use of corticosteroid in the present study could provide the first supportive finding for this earlier speculation [8]. This finding thus suggests that the pharmacological mechanism of corticosteroid treatment in the acute stage of adult HSVE patients may involve not only improvement of brain edema, but also inhibition of the pro-inflammatory cytokine cascade based on the host-sided immune response associated with the acute stage of HSVE.

Disclosures

The authors report no financial conflicts of interest.

Ethics approval

This study was approved by the Ethics Committees for Human Studies at Nihon University Itabashi Hospital and Tohoku University Hospital.

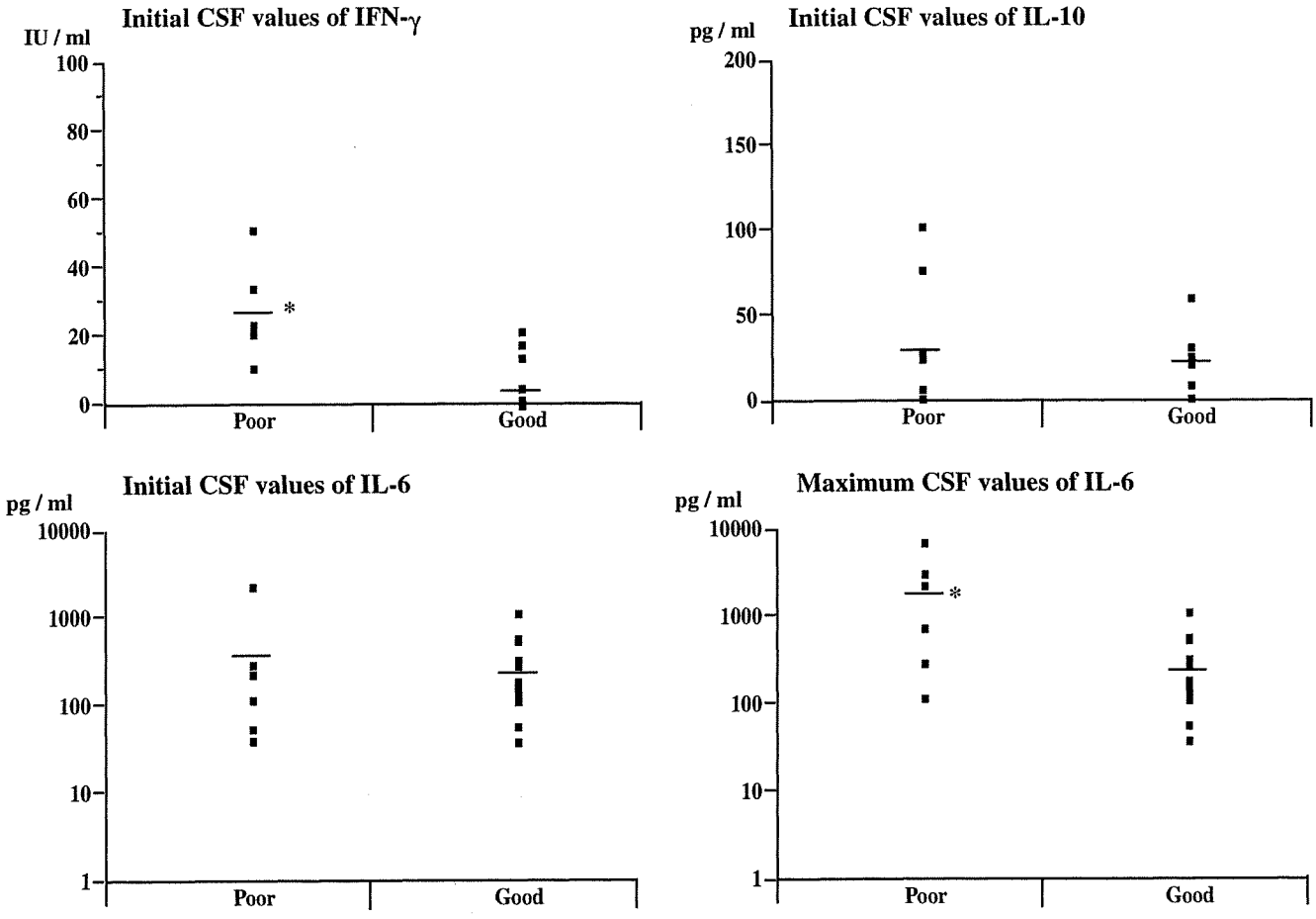

Fig. 1. Results for intrathecal cytokine concentrations among different outcomes. CSF, cerebrospinal fluid; and IU, international units. In the figure, a horizontal line is placed at the median. Symbols: * indicates a significant difference among different outcomes ($p < 0.05$). The differences of the initial values of INF-γ (left upper graph) and the maximum values of IL-6 (right lower graph) among different outcomes were significant (Mann–Whitney *U* test). Those of the initial values of IL-10 and IL-6 among different outcomes were not significant. The initial CSF values of INF-γ and IL-10 in all patients represented the maximum values of the serial CSFs. Since all initial CSF samples at admission were taken at the time just before treatment, a comparison of CSF cytokine concentrations between the patients with corticosteroid and those without corticosteroid was not performed.

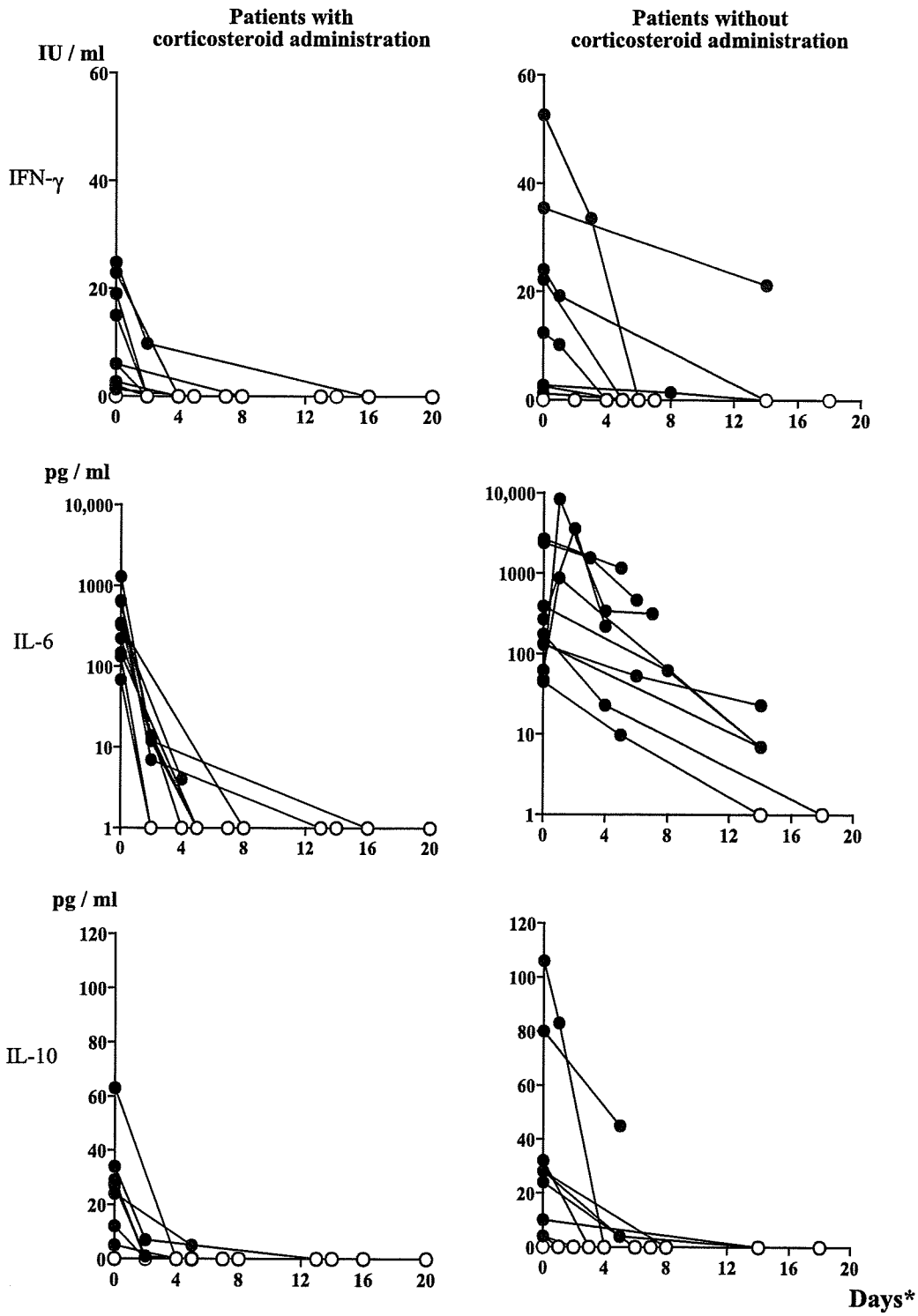

Fig. 2. Serial changes of intrathecal cytokine concentrations in each patient with and without corticosteroid administration. IU, international units. Symbols: ○ indicates less than the minimum detection limit; Days* indicates days after initiation of treatment. The data for the IFN-γ (upper graphs) and IL-10 (lower graphs) serial concentrations between the patients with corticosteroid administration and those without corticosteroid administration indicated that the rates of decline of both cytokines in the patients with corticosteroid administration were higher than those in the patients without corticosteroid, but such differences in decline rates among the different treatments were not significant. The IL-6 concentrations (middle graphs) in the initial CSF samples, which were taken just before treatment, demonstrated high values in all patients with HSVE. The initial CSF values for the IL-6 concentration revealed no significant difference between the patients with corticosteroid administration (middle left graph) and those without corticosteroid administration (middle right graph). The serial changes of IL-6 CSF values in 9 out of the 10 patients with corticosteroid administration revealed a marked decline to less than the minimum detection limit in the interval from the 2nd to 16th day after initiation of the treatment with aciclovir and corticosteroid. However, the ongoing IL-6 values in the CSF of only 2 out of the 10 patients without corticosteroid administration declined to less than the minimum detection limit by the 20th day. All of the 3 patients who exhibited a transient increase in IL-6 values at the second CSF sample, did not receive corticosteroid.

Acknowledgments

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Nationwide Survey of Acute Juvenile Female Non-Herpetic Encephalitis in Japan: Relationship to Anti-N-Methyl-D-Aspartate Receptor Encephalitis

Satoshi Kamei[1], Shigeki Kuzuhara[2], Masaki Ishihara[1], Akihiko Morita[1], Naoto Taira[1], Masaki Togo[1], Makoto Matsui[3], Masafumi Ogawa[2], Kinya Hisanaga[4], Tomohiko Mizutani[1] and Sadako Kuno[2]

Abstract

Objective To study the incidence and clinical features of acute juvenile female non-herpetic encephalitis (AJFNHE) in Japan.

Methods A nationwide questionnaire on patients with severe non-herpetic encephalitis of unknown etiology with a prolonged clinical course or death was sent to the departments of Internal Medicine, Neurology, Pediatrics, and Emergency and Critical Care at all hospitals with 200 beds or more in Japan.

Results The recovery rate was 25% (1,279 out of 5,030 departments) and 90 patients were enrolled in this study. The annual incidence was $0.33/10^6$ population. 85% of patients were female. The means and standard deviations of age at onset and hospital stay were 26±10 years and 180±228 days. As first symptoms, fever and psychosis were presented in 90%. Among the neurological symptoms, disturbance of consciousness was presented in 92%, convulsions in 65%, and involuntary movements in 55%. Respiratory failure during hospitalization was observed in 71% and required care with mechanical ventilation. The detection rate of anti-GluR ε2 and/or δ1 antibodies was 67% of patients. Anti-N-methyl-D-aspartate receptor NR1/NR2 antibody was detected in all four examined patients with anti-GluR ε2 antibody, and also detected in both of the two examined patients without anti-GluR ε2 antibody. As for outcome, 46% returned to work and 37% returned home, but 7% died. Associated tumors were demonstrated in 39%. All reported patients had ovarian tumors, among which teratoma was the most frequent.

Conclusion A nationwide survey provided data for the annual incidence and clinical features of AJFNHE in Japan.

Key words: incidence, clinical features, acute juvenile female non-herpetic encephalitis (AJFNHE), anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis

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Introduction

In 1997, Nishimura et al (1) first reported five young adult female patients with acute non-herpetic encephalitis who presented with severe prolonged coma and status epilepticus, but achieved a good recovery. Following this report, the results of a clinical analysis on 89 serial patients with encephalitides indicated that 11 such patients presented specific and different clinical features, including the frequent

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Table 1. Regional Differences in Annual Incidence of the Registered Patients

Regions	Annual incidence (/ 10^6 population)	Regional differences
Hokkaido	0.39	
Tohoku	0.43	
Kanto	0.25	
Chubu-Hokuriku	0.20	non-significant
Kinki-Chugoku	0.50	
Shikoku	0.36	
Kyushu-Okinawa	0.32	

detection of anti-glutamate receptor (GluR) antibody as compared with other etiologies of encephalitis. The specific clinical features of such patients demonstrated severe encephalitis with a prolonged clinical course and the negative result of herpes simplex virus infection, but these patients achieved a relatively good recovery. Since all of their 11 patients were young adult women, we designated these patients as "acute juvenile female non-herpetic encephalitis (AJFNHE)" (2).

In 2007, Dalmau et al (3) reported anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis associated with ovarian teratoma. Based on the similarity of clinical features in both kinds of patients, Iizuka T et al (4) recently described the detection of anti-NMDAR antibody in four patients who were clinically diagnosed as having AJFNHE; moreover, in three of these four patients, ovarian teratoma was confirmed.

Here, we describe the results of a nationwide survey on AJFNHE in Japan, including the relationships between AJFNHE and anti-NMDAR encephalitis.

Methods

A nationwide questionnaire on the incidence and clinical features in patients with severe encephalitis of unknown etiology with a prolonged clinical course or death, who were also confirmed to have a negative result for herpes simplex virus infection, was sent for completion to the 5,030 departments of Internal Medicine, Neurology, Pediatrics, and Emergency and Critical Care at all hospitals with 200 beds or more in Japan during a period from April 2004 to March 2007. A prolonged clinical course was defined as no improvement of their neurological symptoms during the period of more than one month after onset. The criteria of AJFNHE in the present study did not thus include the sex of patients, age of onset, nor the MRI finding. The questionnaire on clinical features in such patients was also sent to all members of the Research Group for Comprehensive Clinical Studies on the Diagnosis, Treatment and Prevention of Neurological Disorders (Chairman: Sadako Kuno, M.D., National Center Hospital of Neurology and Psychiatry) during the period from April 1987 to March 2007. The overlap of the registered patients in the present study was excluded based on detailed history of their admission and transfer.

After confirmation of the above-mentioned criteria in each patient, we evaluated the following factors: annual incidence and regional differences in incidence, sex, age of onset, duration of hospitalization, clinical symptoms, respiratory failure, laboratory data, treatment, outcome, and associated tumors. In addition, we analyzed the data of GluR antibodies and anti-NMDAR antibody.

The annual incidence per 10^6 population was estimated on the basis of the number of registered patients and the total population of Japan in 2005, and regional differences in incidence were analyzed by the Ryan test (5). Differences in clinical features between female and male patients were assessed by Fisher's exact probability test or the Mann-Whitney U test. SPSS statistical software Version 12.0 (SPSS Inc., Chicago, Illinois) was employed for the statistical analysis. The level of significance for this study was defined as 0.05.

Results

The questionnaire recovery rate was 25% (1,279 out of 5,030 departments), and the questionnaires of 129 patients returned for this nationwide survey. 90 out of these 129 patients were enrolled in the present study based on the above-mentioned criteria. The excluded 39 patients consisted of 12 patients with the encephalopathy associated with influenza virus infection, 5 patients with human herpes virus 6 encephalitis, 2 patients with acute disseminated encephalomyelitis, 10 patients without data confirming negative result of herpes simplex virus infection, and 10 patients without the prolonged clinical course of more than one month after onset. A total of 44 of 90 patients were encountered during the period from April 2004 to March 2007. Therefore, the annual incidence and regional differences in incidence were estimated based on the data of 44 patients, the other analyses were estimated based on the data of 90 patients.

Estimated annual incidence and regional differences

The estimated annual incidence in Japan was $0.33/10^6$ population. The annual incidences in each region ranged from 0.20 to 0.50 per 10^6 population and the regional differences was found to be non-significant (Table 1).

Gender

Among the enrolled patients, 85% were female. This form of encephalitis thus predominantly affected females, although it also occurred in male patients.

Age of onset and duration of hospitalization

The mean and standard deviation (SD) of age of onset

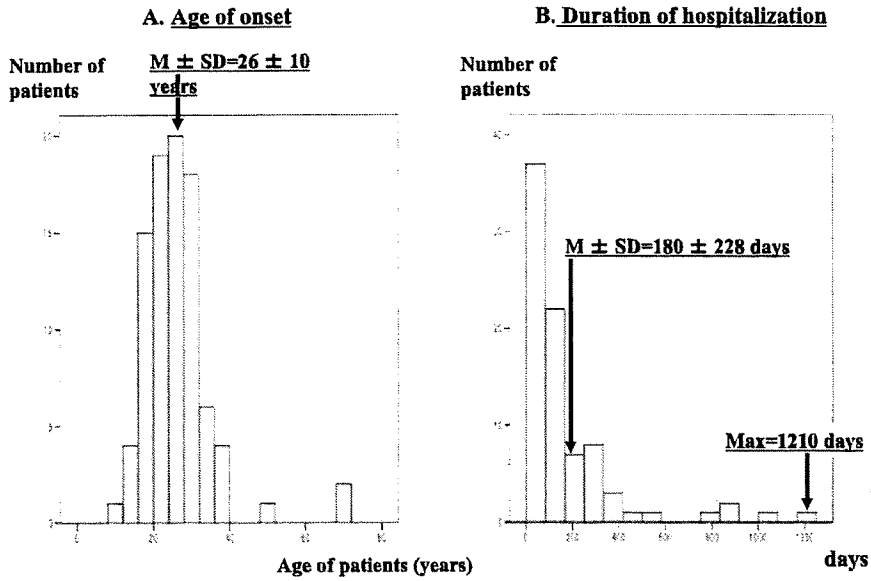

Figure 1. **Distribution of age of onset (A) and duration of hospitalization (B). M: mean value, Max: maximum value, SD: standard deviation**

was 26±10 years. The mean and SD of length of hospital stay was 180±228 days, and the maximum was 1,210 days (Fig. 1).

Clinical symptoms

A prodrome of cold-like symptoms was observed in 90% of enrolled patients. As first symptoms, fever and psychosis were presented in 90% of patients. Concerning the vital signs on admission, the mean and SD of body temperature was 37.7±0.9℃, that of systolic blood pressure was 123±18 mmHg, and that of respiration was 20±6.7 per min. Among the neurological symptoms observed during the entire clinical course, disturbance of consciousness was demonstrated in 92% of patients, fluctuations of blood pressure in 78%, convulsions in 65%, and involuntary movements in 55%. The distribution of the duration from onset to initial improvement of the neurological findings was ranged from 31 to 730 days, and the mean and SD value was 149±160 days (Fig. 2).

Respiratory failure

Respiratory failure on admission was observed in 30% of patients, but care with mechanical ventilation during hospitalization was required in 78% of patients. Among the patients with care with mechanical ventilation, 91% suffered central respiratory failure without respiratory infection and the remaining 9% received mechanical ventilation because of general anesthesia due to control seizures. Central hypoventilation during the entire of clinical course was demonstrated in 71% of patients. The mean and SD of the duration of care with mechanical ventilation was 102±153 days and the maximum value was 933 days (Fig. 3).

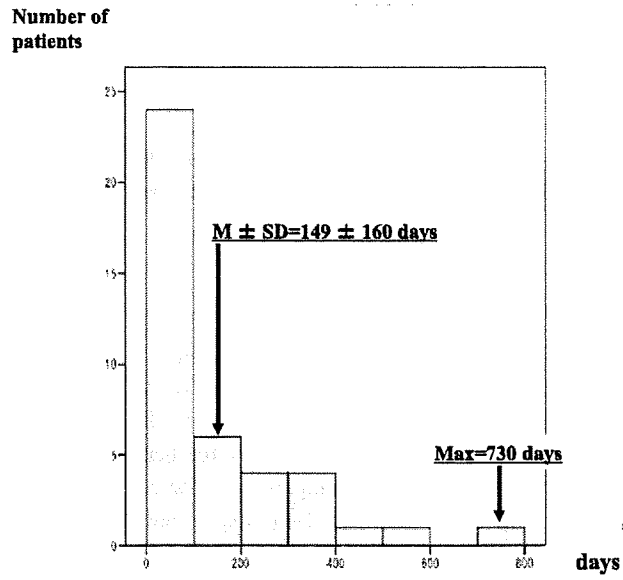

Figure 2. **Distribution of duration from onset to initial improvement of neurological findings. M: mean value, Max: maximum value, SD: standard deviation**

Laboratory data

The mean value and SD of the cell count in the initial cerebrospinal fluid (CSF) on admission was 71±112/μL, and that of the protein concentration was 50±54 mg/dL. The EEG findings revealed that a diffuse slow and/or paroxysmal wave was present in 89% of patients. Concerning the findings of cranial MRI, 74% of patients demonstrated normal findings and the remaining 26% revealed medial temporal lobe lesions.

Regarding anti-GluR antibody, the anti-NMDAR GluR ε2 and δ1 antibodies, which were measured by Dr. Yukitoshi

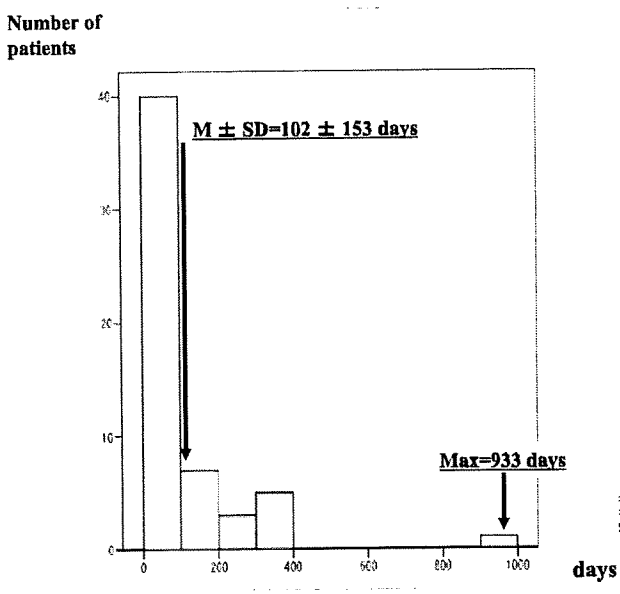

Figure 3. **Distribution of duration of care with mechanical ventilation. M: mean value, Max: maximum value, SD: standard deviation**

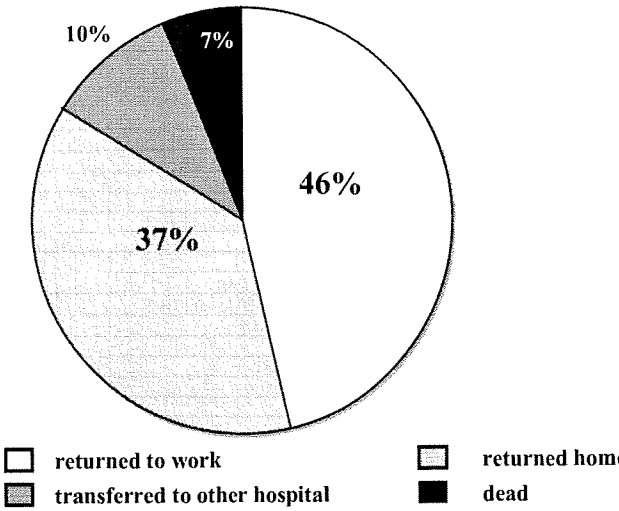

Figure 4. **Outcome of registered patients with AJFNHE. AJFNHE: acute juvenile female non-herpetic encephalitis**

Table 2. **Detailed Positive Rates of Anti-GluR Antibodies which Have a Linear Epitope to a Single Subunit of NMDAR**

Sample	Anti-GluR ε2 antibody				Anti-GluR δ1 antibody			
	Serum		CSF		Serum		CSF	
	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
Numbers of examined patients	15	17	20	19	14	14	19	19
Numbers of patients with positive result	3	8	7	4	4	5	5	5
Positive rate (%)	20	47	35	21	29	36	26	26

CSF= cerebrospinal fluid; GluR= glutamate receptor; NMDAR= N-methyl-D-aspartate receptor.

Takahashi (6), their data were available in 24 patients, and the rate of detection of either of these antibodies in the serum, CSF or both samples was 67% of patients (16 out of the 24 patients examined). The detailed results for the positive rates of GluR ε2 and δ1 antibodies (Table 2) showed that the rate of GluR ε2 IgM antibody in the serum was 47%, and that of GluR ε2 IgG in the CSF was 35%. On the other hand, anti-NMDAR NR1/NR2 heteromer antibody, which was measured by Dr. Josep Dalmau (3), was detected in all out of the four examined patients who had also revealed anti-NMDAR GluR ε2 antibody. Moreover, anti-NMDAR NR1/NR2 heteromer antibody was further detected in both of the two examined patients who presented negative results for anti-NMDAR GluR ε2 antibody.

Outcome and treatment

Data on outcome demonstrated that 46% of patients returned to work, and 37% returned home, but 7% of patients died (Fig. 4). The patients with the present form of encephalitis had a severe and prolonged clinical course, but their outcome in the long term was relatively good. The rate of patients with sequelae was 52% of the survived patients. The observed sequelae consisted of cognitive dysfunction in 39% of patients with sequelae, and psychosis and epilepsy in 23% each.

The treatments included anti-viral drugs in 89% of patients, corticosteroids in 83%, intravenous immunoglobulin in 32%, and plasma exchange in 3%. Moreover, anti-epileptic drugs were administered in 94% of patients, and intravenous anesthetics in 49%. Overall, half of the patients required intravenous administration of anesthetics in order to control their convulsions.

Associated tumors

Associated tumors were demonstrated in 23 (39%) of the 59 examined patients, ovarian tumors were found in all 23 patients. The types of associated tumor in the 23 patients consisted of teratoma in 17 patients, ovarian cyst in two patients, ovarian cancer in one patient, a history of ovarian tumor in two patients, and not described in one patient. Tera-

Table 3. Differences in Clinical Features between Female and Male Patients

	Females (68 patients*)	Males (12 patients*)	Difference* of clinical features between female and male patients
Age of onset (years) [mean ± SD]	24.5 ± 9.0	31.3 ± 13.4	p=0.026*
Duration of hospitalization (days) [mean ± SD]	179 ± 222	143 ± 238	NS
Prodromes	91%	82%	NS
First symptoms			
Fever	90%	83%	NS
Psychosis	93%	73%	NS
Vital signs on admission			
Body temperature (°C) [mean ±SD]	37.6 ± 0.8	37.7 ± 0.8	NS
Systolic blood pressure (mmHg) [mean ± SD]	125.1 ± 17.9	130.7 ± 16.1	NS
Respiration (/min) [mean ± SD]	20.0 ± 7.5	19.8 ± 5.2	NS
Neurological symptoms			
Disturbance of consciousness	91%	92%	NS
Convulsions	67%	67%	NS
Involuntary movements	54%	50%	NS
Mechanical ventilation	78%	75%	NS
Diffuse slow and/or paroxysmal wave on EEG	90%	92%	NS
MRI findings normal	71%	67%	NS
abnormal (site; medial temporal lobe)	29%	33%	
Outcome	improved 95.6% dead 4.4%	improved 75% dead 25%	p=0.041*
Positive rate of anti-GluR antibody in serum and/or CSF samples (positive numbers of patients / total numbers of examined patients)	68% (15/22)	50% (1/2)	NS
Associated tumor (numbers of patients with associated tumor / total numbers of examined patients)	49% (20/41)**	0% (0/10)	p=0.004*

*The differences were assessed by Fisher's exact probability test or Mann-Whitney *U* test.
* Data on sex were not given for 10 patients in the present survey.
** All patients demonstrated ovarian tumor.
SD= standard deviation, NS= not significant.

toma was thus the most frequent.

Moreover, we attempted to contact the 14 registered patients including 11 previously reported patients (2) in the present study who had been treated at the Division of Neurology, Department of Medicine, Nihon University School of Medicine, to undergo further examinations for ovarian teratoma. Three patients could not be contacted because they moved to another town. Ten (91%) out of the remaining 11 patients demonstrated ovarian teratoma by MRI or had a history of a removal operation for ovarian tumor. Among the 11 previously reported patients (2), the eight (89%) out of nine patients except for two patients, who were impossible to have contact, demonstrated ovarian teratoma or had a history of a removal operation for ovarian tumor.

Differences in clinical features between female and male patients

The clinical features in male patients with the present encephalitis were almost identical to those in female patients, except for slightly older age of onset and somewhat higher mortality rate, and the absence of associated tumor including teratoma (Table 3).

Discussion

A comparison of clinical features between the enrolled patients with AJFNHE in the present study and the reported patients with anti-NMDAR encephalitis (3) (Table 4) indicated that both patient groups showed almost identical clinical features. Most of the patients were female, and young adults were predominantly affected. A prodrome was presented in over 80% of patients. The first neurological symptom was psychosis in over 90% of patients. Convulsions, disturbance of consciousness, and involuntary movements were presented as the main neurological symptoms during the entire clinical course. Mechanical ventilation was required in about 80% of patients. Regarding outcome, 93% of patients in this survey were improved, whereas Dalmau et al (3) reported that 75% of patients were improved in spite of marked severe condition at acute stage. Concerning the MRI findings, the frequency of medial temporal lesion was about one-fourth of patients.

The detection of GluR ε2 or δ1 antibodies in this survey was 67% of examined patients, whereas Dalmau et al (3) reported the NMDAR NR1 and NR2 heteromer antidody was detected in all 12 patients. The GluR ε2 antibody, which was measured by Yukitoshi Takahashi (6), and the NMDAR NR1/NR2 heteromer antibody, which was measured by Josep Dalmau (3), are both antibodies to NMDA type glutamate receptor. The GluR ε2 antibody has a linear epitope which recognizes N-terminal regions in the NR2B subunit. The binding region of such an antibody is thus a single subunit. On the other hand, the NMDAR NR1/NR2 antibody has a conformational epitope which recognizes the NR 1 and NR2 heteromers. This difference in epitopes might be reflected in the difference of frequency of positive antibodies in patients with such encephalitis.

Dalmau et al (3) reported that ovarian teratoma was demonstrated in 11 out of 12 patients, and mediastinal teratoma in one patient, whereas associated tumors were demonstrated in 23 (39%) of the 59 examined patients in this survey; ovarian tumors were found in all 23 patients. The period of this nationwide survey just preceded the publication of Dalmau's report (3), and an association with ovarian teratoma

Table 4. Comparison of Clinical Features between the National Survey of AJFNHE in the Present Study and the Reported Patients with Anti-NMDAR Encephalitis

	National survey of AJFNHE in the present study 90 patients	Anti-NMDAR encephalitis 12 patients (Dalmau J et al: 2007)	Difference* of clinical features between the two studies
Females	85%	100%	NS
Age of onset (years: mean ± SD)	26 ± 10	28 ± 9	NS
Prodrome	90%	83%	NS
First neurological symptom	psychosis (90%)	psychosis (75%) memory loss → psychosis (25%)	NS
Convulsions	65%	92%	NS
Disturbance of consciousness	92%	100%	NS
Involuntary movements	55%	67%	NS
Mechanical ventilation	78%	83%	NS
Outcome	improved 93%, dead 7%	improved 75%, dead 25%	NS
Frequency of medial temporal lesion using MRI	26%	25%	NS
Antibody (positive %)	GluR ε2 or δ1 (67%)	NMDAR NR1/NR2 heteromer (100%)	p=0.03
Associated tumor	Ovarian tumors were found in all 23 patients with associated tumor.	Ovarian teratoma: 11 patients Mediastinal teratoma: 1 patient	NS (Frequency of ovarian tumor)

*The differences were assessed by Fisher's exact probability test or Mann-Whitney *U* test.
AJFNHE=acute juvenile female non-herpetic encephalitis; GluR=glutamate receptor; MRI=magnetic resonance imaging; NMDAR= N-methyl-D-aspartate receptor, NS= not significant

in such encephalitis thus could not be well recognized in Japan. However, the results for associated tumors of AJFNHE in the present study indicated that ovarian teratoma was the most frequent. Moreover, it was also able to confirm the presence of ovarian teratoma using MRI or a history of a removal operation for ovarian tumor in 91% of patients including Kamei's previously reported patients (2) in the present study. Therefore, AJFNHE and anti-NMDAR encephalitis were inferred to be almost identical condition. AJFNHE represented a clinical concept based on the specific clinical features, and anti-NMDAR encephalitis represented a clinical entity based on the neuro-oncological findings including the NMDAR NR1 and NR2 heteromer antibody.

Their clinical features in this nationwide survey on AJFNHE were uniform and also in concordance with those previously reported as AJFNHE (2). The present nationwide survey of AJFNHE undertaken in Japan revealed the following: the annual incidence was $0.33/10^6$ population; there were no regional differences in the incidence; respiratory failure was observed in about 70% of patients and required care with mechanical ventilation; associated tumors were demonstrated in about 40% of patients, and ovarian teratoma was the most frequent; and male patients with such encephalitis were also registered and their clinical features were very similar to those of female patients except for the absence of associated tumor.

There has been a short description of male patients with anti-NMDAR NR1/NR2 encephalitis as unpublished data in a recently reported review (7). Such encephalitis was therefore considered to affect not only female patients with related ovarian teratoma, but also male patients. Based on a consideration of the association with teratoma, mediastinal teratoma and seminoma might be examined in male patients with such encephalitis.

However, several problems still remain to be unresolved including the etiology of patients without anti-NMDAR antibody, the etiology of male patients, the lack of detectable ovarian teratomas in some patients, the reason why predominantly young adults are affected, the reason why many Japanese patients are reported (4, 8-10), and the reason why there is a good outcome in the long term without tumor removal.

Conclusion

The patients collected in our nationwide survey of AJFNHE were predominantly young adult women. Their clinical features were uniform and also in concordance with those previously reported as AJFNHE except for the presence of small number of male patients. The survey data revealed that the annual incidence was $0.33/10^6$ population without regional differences, respiratory failure was observed in about 70% and required care with mechanical ventilation, associated tumors were demonstrated in about 40%, ovarian teratoma was the most frequent, and the clinical feature of male patients was very similar to that of female patients except for the absence of associated tumor.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Relationships between Quantitative Electroencephalographic Alterations and the Severity of Hepatitis C Based on Liver Biopsy in Interferon-α Treated Patients

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Abstract

Objective We have observed alterations of quantitative (q)-EEG findings occurring in interferon (IFN)-α treated chronic hepatitis C (CH-C) patients, and found patient's age to be one factor influencing such EEG alterations. In the present study we evaluated the correlation between q-EEG alterations during IFN-α treatment and the severity of hepatitis based on liver biopsies.

Methods A total of 102 CH-C patients underwent blind, prospective and serial q-EEG examinations. The IFN-α was administered under the same therapeutic regimen to all patients. Serial EEGs were obtained before, at 2 and 4 weeks, and at 2-3 days after the conclusion of treatment. The absolute powers of each frequency band in different periods were determined by q-EEG. Staging (of fibrosis) and grading (of inflammatory cell infiltration) were scaled according to Desmet's classification. We evaluated the relationship between q-EEG and scales of staging or grading.

Results Age distributions did not differ significantly among stages or grades. As the stage or grade increased, the alterations of EEG during IFN-α treatment became more pronounced, and significant (repeated-measures analysis of variances; both, $p<0.0001$).

Conclusion Alterations of the EEG occurring during IFN-α treatment became pronounced with more severe pathological findings for CH-C. Alterations in the EEGs during IFN-α treatment should be carefully monitored in CH-C patients with severe pathological findings.

Key words: interferon-α, quantitative-EEG, chronic hepatitis C, staging, grading

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Introduction

Alterations of brain waves on electroencephalograms (EEGs) during treatment with interferon (IFN)-α have been described previously in several case reports (1-3). We have confirmed a diffuse slowing based on an analysis of blind, prospective and serial quantitative-EEG (q-EEG) examina-

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Table 1. Mean Values and Standard Deviations of Age for Each Stage and Grade Based on Liver Biopsies in 102 Subjects

Findings of liver biopsy	Number of patients	Mean ± standard deviation of patients' age (years old)	Difference in mean values between different stages or grades (Mann-Whitney U test)
Stage (intrahepatic fibrosis)			
Mild	49	48.4 ± 6.1	
Moderate	38	51.9 ± 7.4	NS
Severe	15	46.9 ± 8.9	
Grade (inflammatory cell infiltration)			
Minimal	43	48.2 ± 6.0	
Mild	30	50.6 ± 7.1	NS
Moderate	14	51.3 ± 6.8	
Severe	15	47.3 ± 8.8	

NS = not significant.

tions undertaken in many patients with IFN-α treated chronic hepatitis C (4). We speculated that such diffuse slowing on the EEGs could reflect a mild encephalopathy due to the IFN-α. We recently reported that the alteration of the q-EEG could be estimated clinically by the change in score on Mini-Mental State Examinations (5). We have also reported that the age of the patients was one of the factors affecting such alterations on the q-EEG (6). However, no other such factors have been reported. The present study was the first to evaluate the relationship between the alterations in q-EEG findings that occur during IFN-α treatment and the severity of hepatitis as estimated according to scales of staging and grading based on liver biopsies.

Methods

Patients

A total of 168 serial patients with chronic hepatitis C patients underwent our blind, prospective and serial q-EEG examinations, during the period from August 1997 to May 2007. These patients were independently registered at three different hospitals, viz. Nihon University Itabashi Hospital, Nihon University Surugadai Hospital, and Itabashi Medical Association Hospital, during the above period. All patients were investigated and treated under the same clinical regimen and conditions, including diagnostic criteria, q-EEG examinations, and IFN-α treatment, as reported previously (4). The clinical diagnosis of chronic hepatitis C was confirmed by serological findings of serum antibody for hepatitis C virus, histopathological findings obtained by liver biopsy, detection of the viral genome sequence for hepatitis C virus by the reverse transcriptase-polymerase chain reaction (RT-PCR), serum liver function tests, and the clinical course of the patients. Staging (of intrahepatic fibrosis) and grading (of inflammatory cell infiltration) of the chronic hepatitis based on liver biopsies was scored according to Desmet's classification (7). Based on grading of histopathological findings according to the Desmet's classification (7), the patients with liver cirrhosis (LC) were excluded from the present study. A total of 102 patients ranging in age from 40 to 59 years were included in this study. All patients were alert during IFN-α treatment as graded according to the Glasgow Coma Scale. The mean values and standard deviations of patient age at each stage and grade of chronic hepatitis are listed in Table 1. There were no significant differences in mean age among stages or grades (Mann-Whitney U test). IFN-α was administered intramuscularly at a dose of 9×10^6 IU daily for the first 4 weeks and then administered 3 times/week for the following 20 weeks, according to the same regimen of IFN-α treatment. Informed consent to perform the present study was obtained from all patients. The serological hepatic function parameters of the 102 patients improved during the IFN-α treatment. The means and standard deviations for the values of AST (GOT) (normal: 8-38 IU/L) were 126.2±75.1 before the treatment, 50.7±23.6 at 2 weeks of treatment, 43.2±24.1 at 4 weeks of treatment, and 40.4±23.1 IU/L after the treatment. The values for ALT (GPT) (normal: 4-44 IU/L) were 164.1±93.2 before the treatment, 60.4±33.2 at 2 weeks of treatment, 55.0±29.1 at 4 weeks of treatment, and 52.4±27.1 IU/L after the treatment. No patients exhibited elevation of serological hepatic function parameters during IFN-α treatment. There were also no patients with significant elevation of serum ammonia concentration during this treatment. All of the patients gave informed consent to participate in the present study according to a protocol approved by the Ethics Committee for Human Studies at Nihon University.

Q-EEG analysis

The EEG recordings and q-EEG analysis employed in the present study were as described previously (4). Briefly, serial EEGs were obtained before the IFN treatment, at 2 and 4 weeks of treatment and at 2-3 days after the conclusion of treatment. The serial EEGs at 2 and 4 weeks of treatment were obtained during the period from 1 to 6 hours after the injection of IFN-α. The EEGs in each subject were recorded on a magnetic optical disk from 16 electrode locations according to the 10-20 international system using a digital EEG instrument (Neurofax EEG-4518, Nihon Kohden, To-

kyo, Japan). The EEGs were referenced to the ipsilateral earlobes. Sixty seconds of q-EEG data were selected visually from each subject and digitized at 128 Hz with a time constant of 0.3, employing a high frequency filter of 60 Hz. Thirty epochs with a duration of 2 seconds each were collected from the subsequent resting period with eyes closed for analysis of the q-EEGs. The procedure used for analysis involved the application of fast Fourier transformation of the collected EEG signals by Rhythm, version 10.0 (Stellate Systems Inc, Montreal, Quebec, Canada). The frequency ranges were divided into 6 bands, as follows: delta (1.17-3.91 Hz), theta 1 (4.30-5.86 Hz), theta 2 (6.25-7.81 Hz), alpha 1 (8.20-10.16 Hz), alpha 2 (10.55-12.89 Hz), and beta (13.28-30.86 Hz). The absolute powers of each frequency band were calculated at each electrode location in all of the subjects. Each power value was obtained by integrating the appropriate part of the spectrum. The present quantitative analysis was carried out blindly during routine EEG work involving many other disease states, including epilepsy, cerebrovascular disease, encephalitis, meningitis, metabolic encephalopathy, and brain tumor, as well as in normal controls. The only knowledge that the EEG analyst (S. Kamei) possessed regarding each patient was the latter's identification number, and he had no other information regarding any other information concerning any of the studied subjects such as their clinical diagnosis, date of treatment, or type of treatment.

Statistical analysis

In September 2007, a statistical analyst (K. Hirayanagi) at another independent institute collected the analyzed q-EEG data, the data on patient ages, and that on histopathological findings on liver biopsy based on the Desmet's classification (7) for the 102 patients. Using Desmet's classification (7), the stage of intrahepatic fibrosis in each sample was classified as mild, moderate, or severe. The grade of inflammatory cell infiltration in each sample was classified as follows: minimal, mild, moderate, or severe. The distributions of the power values at each frequency band for each electrode location were evaluated in terms of their skewness and kurtosis. Based on findings regarding skewness and kurtosis, repeated measure analysis of variances (rANOVAs) was applied to the alterations in power values as the main factor among 4 different periods: before the IFN-α treatment, at 2 and 4 weeks of treatment, and after the treatment, with the frequency bands, electrode locations, and staging and grading on the hepatitis classifications as co-factors. SPSS statistical software Version 12.0 (SPSS Inc., Chicago, IL) was employed for statistical analysis. Relationships between q-EEG variables and stages or grades were evaluated by post hoc ANOVAs (Scheffe's test). The level of significance for this study was 0.05.

Results

There were no patients with IFN-α induced irreversible encephalopathy in the present study. Stages of the chronic hepatitis based on liver biopsies in the 102 subjects were distributed over the range from mild to severe fibrosis. Similarly, grades were also distributed from minimal to severe inflammatory cell infiltration. The results of serial q-EEG studies at each selected frequency of EEG during the IFN-α treatment for each staging and grading scale (Figs. 1, 2) revealed that increased slow waves (delta, theta 1 and 2) and decreased alpha 2 and beta waves were evident during the IFN-α treatment at all stages and grades. These EEG alterations during IFN-α treatment in the present study confirmed our previously reported observations (4). Moreover, the alterations in power values during the IFN-α treatment became more pronounced as the stage or grade of hepatitis increased. Statistical results obtained by rANOVAs (Table 2) for the interactions between the q-EEG alterations during the IFN-α treatment and differences of staging scale or grading scale were significant (both, $p<0.0001$). Results of post hoc ANOVAs results (Table 3) also indicated significant differences in the alterations of absolute power values during the IFN-α treatment for all comparisons with increasing staging or grading scale in the case of the delta, theta 1, and beta waves with the exception of several comparisons involving differences of only one grade or stage. There were no significant differences in the alterations of power values during the IFN-α treatment in the case of the alpha 1 and total power values. We also examined the correlations at each electrode location between severity based on liver biopsy findings and alteration of qEEG during the administration of IFN-α. These correlations were significant for all electrode locations (frontal pole location $p=0.03$ and $p=0.004$ for stage and grade, respectively; frontal location $p<0.0001$ for both stage and grade; temporal location $p<0.0001$ for both; central location $p=0.005$ and $p=0.002$; parietal location $p=0.002$ and $p=0.01$; occipital location $p=0.005$ and $p=0.01$).

There were only two patients with mild pyrexia (37.3 and 37.4℃) at the time of q-EEG examination after 2 weeks of IFN-α administration, and no patient with pyrexia at the time of examination at 4 weeks. The two patients with mild pyrexia had findings of mild severity on liver biopsy. No significant effects of pyrexia on q-EEG were found.

Discussion

Although numerous patients have undergone IFN-α treatment, detailed assessments of the adverse effects of IFN-α on central nervous system function have not yet been presented. Evaluations of alterations in brain function have been presented in only three previous reports based on data from small numbers of patients who underwent EEG examinations (1-3). We recently confirmed a significant, diffuse slowing on q-EEGs that occurred in chronic hepatitis C patients during IFN-α treatment at a relatively low dosage (4). With such a low dosage of IFN-α administration to chronic hepatitis C patients, the diffuse slowing of the EEG is re-