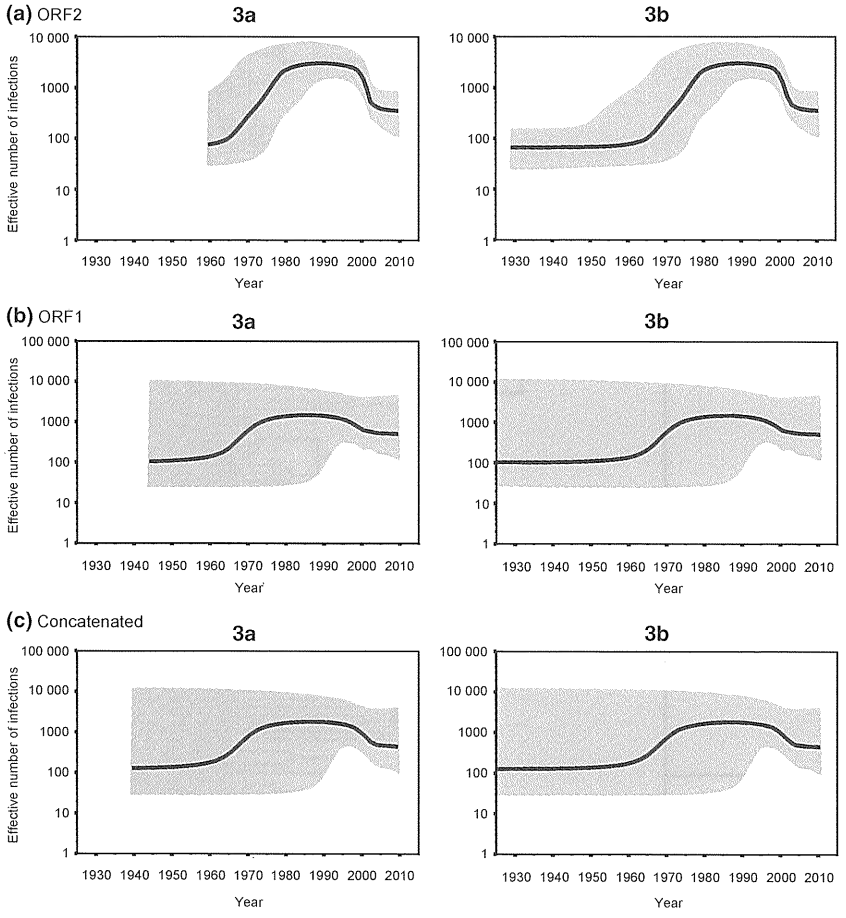

Fig. 4. Bayesian Skyline plots estimated from the Japan-indigenous 3a and 3b clusters of the ORF2 dataset (a), ORF1 dataset (b) and concatenated dataset (c). The thick black line represents the estimated effective population size through time. The grey area represents the 95% highest posterior density confidence intervals for these estimates.

The relationships among the three major lineages and strains from various countries shown in Figs 1 and 2 suggest that epidemic history may differ among the three lineages, and their histories should be studied separately. Other minor lineages, such as the undetermined clusters in Figs 1 and 3, may also be indigenous. Consideration of the different epidemic histories of the three lineages led to the detailed population dynamics of Japan-indigenous genotype 3 HEV infection, which could not be observed in the study of Tanaka *et al.* (29).

The 3b cluster consists mostly of Japanese strains, with the highest number of isolated strains among the three major Japanese genotype 3 lineages. The 3b cluster is probably the major type of Japan-indigenous genotype 3 HEV. However, its origin or specific relationship with strains from other countries is not distinguished by the structure of the trees constructed by the short ORF2 or ORF1 region. In the 3a cluster, many groups of Japanese strains are intricately intertwined with many groups of strains from New World countries. However, no individual Japanese strain demonstrated a direct close relationship with strains from individual New World countries. The presence of multiple old phylogenetic nodes that connect Japanese clusters and lineages from New World countries (Figs 1 and 2) suggests that historical inflow of 3a strains occurred several times, or several 3a lineages disseminated at some time in the past. The direction or origin of the inflow or dissemination is not clear from the short region trees. The relatively minor lineage, Japanese cluster 3e, is clearly nested within the European 3e cluster, which consists mainly of UK strains. This observation strongly suggests that Japanese 3e lineage originated in Europe, most likely in the UK.

Recent molecular and serological data have led to the consensus that hepatitis E arising from genotype 3 and 4 strains involves zoonosis with a reservoir in pigs and possibly a range of other mammals (10, 12, 14, 43). Many of the HEV infections in Japan are most likely zoonotic in origin (8). Particularly, swine-borne infection is a major route of HEV infection in Japan (10, 44, 45). Although genotype 3 HEV infections in wild boars or other mammals have been reported in Japan (12, 14,

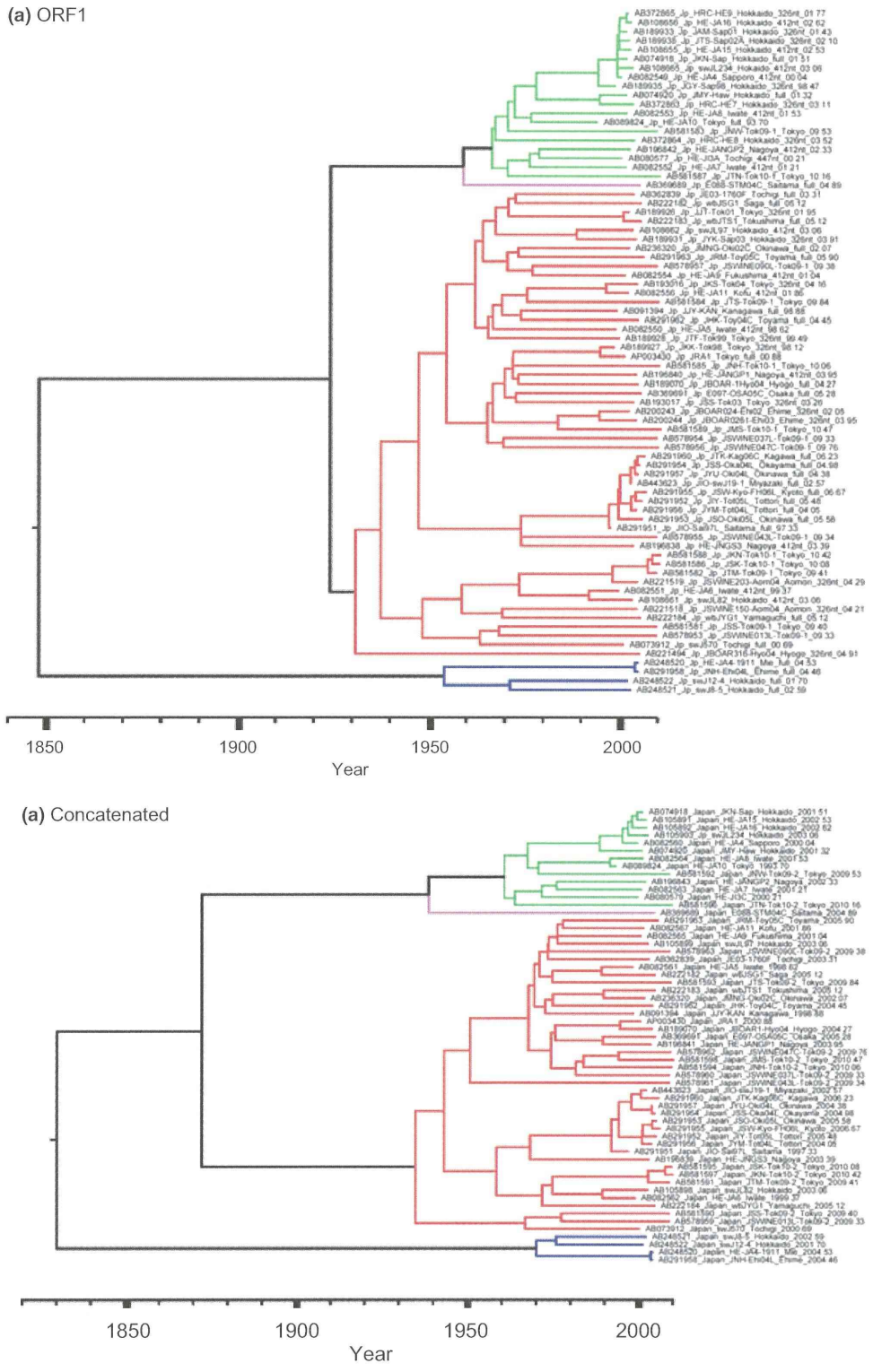

Fig. 5. Maximum clade support phylogeny for the dated Japanese sequence set of the ORF1 region (326 nt) (a) and concatenated dataset (b). The lineages are coloured as in the ORF2 tree (Fig. 1). The branch lengths and node heights are in units of years.

46), their impact on the total number of genotype 3 infections may be limited.

Until the modern age, eating pork was traditionally inhibited by Buddhism in Japan. Japanese governments then changed their policy to ensure that the people (especially soldiers) gained strength by eating meat, and have encouraged the pig industry since the 1870s. The history of the Japanese pig industry has been recorded in detail in Japanese (http://www.jppa.biz/book.html). The Japanese government began importing pedigree pigs in 1869 to improve the pig industry. The Japanese government imported 270 pigs (Berkshire, Yorkshire, and others) between 1899 and 1936, mostly from the UK. Some private companies also imported 137 pigs between 1923 and 1938 from the UK and USA. Other government branches also imported Berkshire, Yorkshire and other breeds of pigs from the UK, USA and Canada until the beginning of World War II.

This period is almost concordant with our TMRCA results for cluster 3b (Table 3). However, small-scale breeding, with 1 or 2 pigs per pig breeder, as conducted until 1960, could not influence a drastic population change of swine HEV infections, and may then represent an endemic constant phase of infections until 1960 in spite of frequent importation of many pigs. It seems that one limited lineage, 3b, which entered Japan from Western countries before World War II, established the major lineage in Japan. Most pigs were imported from the UK, but cluster 3b was nested within the 3j cluster, which included Canadian strains in the ORF2 phylogeny. Although Tanaka *et al.* previously suggested that Japan-indigenous genotype 3 was imported from the UK by quoting a report indicating a close phylogenetic relationship between two UK genotype 3 swine strains and some Japanese swine strains (29, 47), the UK and Japanese strains belonged to subtype 3e, and not 3b. The precise origin of the major lineage of Japan-indigenous genotype 3 strains, cluster 3b, will remain unclear until more reliable phylogentic analyses using a sufficient number of longer or even whole-genome nucleotide sequences from other countries' genotype 3 strains are performed.

According to the historical records, the Japanese pig industry developed gradually until World War II (1939–1945), but faced extinction because of difficulty in obtaining feedstuff during and after the War. Our results of coalescent analyses show two new findings after 1960: the appearance of two other lineages, 3a and 3e, and similar radical population growth of the lineages until 1980. These two big changes can also be explained by two improvements that occurred in the modern Japanese pig industry after 1960. One of these improvements is the beginning of the breeding of large pigs. After 1960, Japanese pig breeders imported large-race pigs to increase economic efficiency. Several types of large pigs, i.e. Landrace, large Yorkshire, Hampshire and Duroc, were imported from the UK, the Netherlands, Sweden, Denmark and the USA. Importation of large pigs from several countries may be a possible reason for the introduction of the 3a and 3e HEV strains and the multiplicity of the 3a lineages.

The other improvement is the increase in the number of pigs at individual pig farms after approximately 1960. Large-scale pig breeding probably increased the opportunity for HEV infections through contact exposure compared with small-scale breeding. Indeed, we reported that almost all 5- or 6-month-old pigs from 25 swine herds throughout Japan had detectable anti-HEV (25). The total number of pigs in Japan was the lowest in 1946, at only 88 000, and began to increase because of large-scale pig breeding from around 1960, reaching approximately 10 million pigs in 1981. Subsequently, the number has remained almost constant to the present. Most of the observations in our coalescent analyses, the endemic phase of cluster 3b, appearance of 3a and 3e lineages, epidemic phase of clusters 3b and 3a from 1960 to 1980 and constant phase after 1980, are concordant with the history of the Japanese pig industry as described above.

The authenticity of the observed decrease of HEV infections in the BSPs of clusters 3b and 3a after 2000 remains unclear. Our previous study conducted between 2000 and 2002 showed that almost all pigs in 25 swine herds throughout Japan had IgG class anti-HEV antibody (25), suggesting that almost all Japanese pigs bred around 2000 were infected by HEV. Since 2002, HEV infection in Japanese pigs based on IgG class antibody has not been studied. As large-scale pig breeding has become common, current pig breeders have come to pay more attention to pig health and sanitation to prevent swine-specific diseases including toxoplasmosis, dysentery, Aujeszky's disease, mycoplasmal pneumonia and atrophic rhinitis. These procedures may also have decreased the chance of pigs getting infected with HEV.

Another possibility is that the decrease observed in the BSPs is an artefact of the sequences used for the analyses. We carefully removed closely related sequences within single outbreaks of hepatitis E from the same origin, leaving just one sequence as the representative. However, identical or very similar sequences whose epidemiological linkage was not certified remained in the datasets. As a result, some identical or very similar sequences were included in our coalescent analyses. Such similar sequences may have had an unknown epidemiological linkage. In other words, close relatedness of sequences may be evidence of close epidemiological linkage. When identical or very similar sequences were removed, and the same analyses were repeated, the decrease in the BSPs after 2000 diminished considerably and was not statistically significant (data available upon request). Therefore, the decrease may be an artefact related to non-random sampling of analysed sequences. It cannot be determined whether the decrease in HEV infection observed in the BSPs after 2000 is true or artefactual until epidemiological reinvestigation of HEV infections in swine herds is conducted throughout Japan.

Recently, Purdy and Khudyakov reported the epidemic history of all genotypes of HEV strains using the same method, but using a different genomic region from that used in the present study (33). In their analyses, they divided genotype 3 HEV into two lineages. One of them (tentatively assigned as lineage 3.1) consisted mainly of Japanese 3b, Japanese 3a and several 3c strains from Germany. The other lineage (tentatively assigned as lineage 3.2) consisted of a few 3e and 3f strains. They observed an endemic phase, epidemic phase, constant phase and decrease to the present of HEV infections in their BSP of lineage 3.1. The shape is similar to our BSP of Japan-indigenous 3b strains analysed from a different genomic region, which supports the reliability of both studies to some extent.

In the present study, we focused on the epidemic history of Japan-indigenous genotype 3 HEV. Our BSP of clusters 3b and 3a indicated an epidemic phase between 1960 and 1980, whereas the BSP of clade 3.1 from the previous study demonstrated an epidemic phase between 1940 and 1960. Increase of HEV infections in this period in Japan is not plausible because the number of pigs serving as an HEV reservoir was the lowest in Japan in those days during and just after World War II. Their analyses are precise and sophisticated, but concerning Japan-indigenous lineages, we analysed many more strains and obtained results supported by historical records. It is possible that sampling error, a smaller number of dated sequences and ambiguous sampling dates may underlie the deviation in timing of the epidemic phase in their analyses.

We attempted to stabilize the molecular clock by adding more information, that is, by including 14 newly determined sequences from 2009 and 2010. The selected molecular clock was not a strict clock model, but a relaxed clock model. The relaxed clock on the evolution of HEV seems realistic. The relaxation may be an innate characteristic of HEV infection, which does not persist chronically in one individual except in particular cases under conditions of immune suppression (48). HEV maintains its population in the community by hopping from host to host. On some occasions, such as under the conditions of swine herds, HEV may infect frequently and simultaneously, followed by rapid evolution. In other situations, as in environments such as sewage, HEV may stop replication and await the opportunity to infect the next host (49, 50), followed by sluggish evolution.

We observed new findings on the epidemic history and population dynamics of Japan-indigenous genotype 3 HEV infections and confirmed the strong relationship between the infection and pig farming. In particular, importation of large-race pigs and the transition to large-scale pig breeding may have considerably influenced the population dynamics of HEV infection. Infection control on pig farms should be the primary effective method for prevention of future endemic or epidemic HEV infection of humans in Japan and other developed countries.

Acknowledgements

This study was supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan and from the Ministry of Health, Labour and Welfare of Japan.

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J Gastroenterol
DOI 10.1007/s00535-011-0420-z

Novel classification of acute liver failure through clustering using a self-organizing map: usefulness for prediction of the outcome

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Received: 21 February 2011 / Accepted: 14 April 2011

Abstract
Background Patients with acute liver failure are classified according to the interval between the onset of hepatitis symptoms and the development of hepatic encephalopathy. We examined the validity of such classifications.
Methods The subjects were 1,022 patients enrolled in a nationwide survey in Japan. The intervals between the onset of the hepatitis symptoms and the development of encephalopathy were 10 days or less in 472 patients (group-A), between 11 and 56 days in 468 patients (group-B), and longer than 56 days in 82 patients (group-C). Data on a total of 104 items collected from the patients were subjected to clustering using a self-organizing map.
Results The patients were classified into three clusters. The first cluster consisted of 411 patients (group-A: 57%, group-B: 39%, group-C: 4%). Their incidence of complications was low; 34% underwent liver transplantation (LT), and their survival rate was 90%, while 94% of those treated without transplant were rescued. The second cluster consisted of 320 patients (21, 65, and 14% groups A, B, and C, respectively), who showed a high incidence of complications; the survival rate was 7% in the patients treated conservatively without LT. Sixteen percent underwent LT and survival rate of these patients was 52%. There was a third cluster, of 291 patients (59, 34, and 7% groups A, B, and C, respectively). Without LT, 81% of the patients died. Seven percent were treated by LT and their survival rate was 60%.
Conclusions Clustering revealed that patients with acute liver failure could be classified into three clusters independent of the interval between the onset of disease symptoms and the development of encephalopathy. This technique may be useful, since the outcomes of the patients differed markedly among the clusters.

Keywords Hepatic encephalopathy · Fulminant hepatitis · Data-mining · Artificial neural network · Liver transplantation

Abbreviations

LOHF	Late-onset hepatic failure
LT	Liver transplantation
DIC	Disseminated intravascular coagulation
SOM	Self-organizing map
HBV	Hepatitis B virus
HAV	Hepatitis A virus

Introduction

In Japan, fulminant hepatitis is diagnosed when grade II or more severe hepatic encephalopathy develops with a prothrombin time of <40% of the standardized value. In Japan,

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Published online: 21 May 2011

as well as in the United States and Europe, patients with acute liver failure are classified according to the interval between the onset of hepatitis symptoms and the development of hepatic encephalopathy [1–5]. In Japan, fulminant hepatitis is classified into two types; an acute type, in which the encephalopathy develops within 10 days after the onset of the symptoms of hepatitis, and a subacute type, in which the encephalopathy develops between 11 days and 8 weeks after the onset of the hepatitis symptoms [1–3]. Also, patients showing a prothrombin time of <40% of the standardized values in whom hepatic encephalopathy develops between 8 and 24 weeks of the onset of the hepatitis symptoms are diagnosed as having late-onset hepatic failure (LOHF) [1, 3]. Thus, fulminant hepatitis and LOHF, respectively, are almost synonymous with the usage of the terms acute liver failure and LOHF, respectively, in the United States and Europe [4, 5], except that patients without histological evidence of hepatitis, such as those with toxic and ischemic liver injuries, are excluded from both disease conditions.

Although the definition and classifications of acute liver failure in Japan differ from those in Europe and the United States, the Japanese classifications are considered to be useful for prediction of the prognosis of the patients [1–3]. Thus, the indications for liver transplantation (LT) in patients with acute liver failure are currently determined according to the guideline published by the Acute Liver Failure Study Group of Japan in 1996, which includes the interval between the onset of the hepatitis symptoms and the development of hepatic encephalopathy as one of the parameters in the criteria [6]. Recently, however, a decrease in the predictive accuracy of the guideline was shown when it was adopted for patients seen later than 1998 [7]. Also, in the American Association for the Study of Liver Diseases (AASLD) position paper [4], regarding the interval between the onset of the hepatitis symptoms and the development of hepatic encephalopathy, the following statement was made: "terms used signifying length of illness such as hyperacute (<7 days), acute (7–21 days) and subacute (>21 days and <26 weeks) are not particularly helpful since they do not have prognostic significance distinct from the cause of the illness".

Several factors other than the disease type, depending on the interval from the onset of the hepatitis symptoms to the development of encephalopathy, may influence the outcome of patients with hepatitis, including the age of the patient, the etiology of hepatitis, liver function tests such as the prothrombin time and serum bilirubin concentration at the onset of hepatic encephalopathy, and the presence of complications such as bacterial infections, renal and cardiac failure, gastrointestinal bleeding, and disseminated intravascular coagulation (DIC) [3, 6]. These data prompted us to reevaluate, by clustering analysis, the validity of the disease type for determination of the outcome in patients with acute liver failure. We applied a self-organizing map (SOM), one of the data-mining methods introduced by Kohonen [8] as an artificial neural network, which has been shown to be suitable for analyses of complex multidimensional relationships in various medical science fields [9–15].

Patients and methods

Patients

The subjects of this study were 1,022 patients with acute liver failure who were enrolled in the nationwide survey performed by the Intractable Liver Diseases Study Group of Japan between 1999 and 2008. All of the patients showed grade II or more severe hepatic encephalopathy and a prothrombin time of <40% of the standardized value and were admitted to 610 hospitals of Japan specializing in hepatology between 1998 and 2007. The interval between the onset of the hepatitis symptoms and the development of encephalopathy was 10 days or less in 472 patients (group-A), between 11 and 56 days in 468 patients (group-B), and more than 56 days in 82 patients (group-C). Group-A, group-B, and group-C patients were diagnosed as having acute-type fulminant hepatitis, subacute-type fulminant hepatitis, and LOHF, respectively, based on the classification used in Japan (Tables 1, 2). All patients were followed up until they either died in hospital or were discharged. They were classified as having survived if they left hospital alive. The patients with hepatitis B virus (HBV) infection were classified into three subgroups according to the serum markers of HBV: (1) transient HBV infection; hepatitis B surface antigen (HBsAg)-positive before the onset of acute liver failure (ALF) or IgM-hepatitis B core antibody (HBcAb)-positive and low titer of IgG-HBcAb, (2) acute exacerbation in HBV carriers; HBsAg-negative before the onset of ALF or IgM-HBcAb-negative or high titer of IgG-HBcAb, and (3) undetermined [neither (1) nor (2)].

Self-organizing map for classification of patients with acute liver failure

Data on a total of 104 items, including the demographic and clinical features, laboratory and imaging data, and the therapies received, were collected from the patients, both before and after the development of grade II or more severe encephalopathy (Table 3). Items such as age, body weight, and biochemical data were analyzed as continuous variables, while those such as gender, outcomes, and complications were analyzed as nominal variables. Clustering analysis was performed through the SOM technique using the IBM Intelligent Miner software (IBM, Tokyo, Japan), as reported in a previous paper [15]. An SOM is a type of

Table 1 Demographic features and outcome and etiology of acute liver failure patients in Japan between 1998 and 2007

	Total (n = 1,022)	Group-A[a] (n = 472)	Group-B (n = 468)	Group-C (n = 82)
Male:female (:unknown)	498:522 (:1)	249:222 (:1)	216:252	33:49
Age (years)	48.3 ± 16.7[b]	46.3 ± 16.3	49.4 ± 17.1[†]	53.8 ± 14.7[†]
HBV carrier [% (n)]	13.5 (127/940)	11.8 (49/417)	16.2 (72/444)*	6.3% (5/79)[#]
Underlying diseases[c] [% (n)]	40.2 (404/1,005)	35.0 (162/463)	43.5 (200/460)*	51.2 (42/82)*,[#]
Previous medication [% (n)]	47.7 (466/977)	41.5 (187/451)	52.5 (234/446)*	56.3 (45/80)*
Etiology [% (n)]				
Viral infection	47.7 (487)	69.3 (327)	31.2 (146)*	17.1 (14)*
HAV	5.4 (55)	10.2 (48)	1.3 (6)*	1.2 (1)*
HBV	39.5 (404)	56.6 (267)	26.9 (126)*	13.4 (11)*
Transient infection	22.8 (233)	40.7 (192)	8.1 (38)*	3.7 (3)*
Carrier	13.1 (134)	10.2 (48)	17.1 (80)*	7.3 (6)[#]
Undetermined	3.6 (37)	5.7 (27)	1.7 (8)*	2.4 (2)
HCV	1.3 (13)	1.3 (6)	1.3 (6)	1.2 (1)
HEV	0.7 (7)	0.4 (2)	1.1 (5)	0 (0)
Other virus	0.8 (8)	0.8 (4)	0.6 (3)	1.2 (1)
Autoimmune hepatitis	7.8 (80)	2.1 (10)	12.0 (56)*	17.1 (14)*
Drug allergy-induced	11.0 (112)	8.1 (38)	13.0 (61)*	15.9 (13)*
Indeterminate	31.2 (319)	18.2 (86)	41.5 (194)*	47.6 (39)*
Insufficient examinations[d]	2.3 (24)	2.3 (11)	2.4 (11)	2.4 (2)

HBV hepatitis B virus, *HAV* hepatitis A virus, *HCV* hepatitis C virus, *HEV* hepatitis E virus

[†] $p < 0.05$ versus group-A by one-way analysis of variance (ANOVA) and multiple comparisons

* $p < 0.05$ versus group-A; [#]$p < 0.05$ versus group-B by χ^2 test and analysis of residuals in cross tabulation

[a] The interval between the onset of the hepatitis symptoms and the development of grade II or more severe hepatic encephalopathy was 10 days or less (group-A), between 11 and 56 days (group-B), and more than 56 days (group-C)

[b] Mean ± SD

[c] Diseases such as metabolic syndrome, malignancy, and psychiatric disorders

[d] The etiology was unknown because of insufficient examinations

Table 2 Outcome of acute liver failure patients in three groups

	% (Number of patients)			
	Total (n = 1,022)	Group-A[a] (n = 472)	Group-B (n = 468)	Group-C (n = 82)
Survival rates in all patients	46.3 (473/1,022)	56.4 (266/472)	39.7* (186/468)	25.6* (21/82)
Treated without liver transplantation	79.4 (811)	86.0 (406)	72.4 (339)	80.5 (66)
Survival rates with medical treatment alone in all patients	30.1 (308/1,022)	46.0 (217/47 2)	17.7* (83/468)	9.8* (8/82)
Survival rates in patients receiving medical therapies alone	38.0 (308/811)	53.4 (217/406)	24.5* (83/339)	12.1* (8/66)
Treated by liver transplantation	20.6 (211)	14.0 (66)	27.6 (129)	19.5 (16)
Survival rates	78.1 (165/211)	74.2 (49/66)	79.7 (103/129)	81.3 (13/16)

* $p < 0.05$ versus cluster 1 and versus cluster 2 by χ^2 test and analysis of residuals in cross tabulation

[a] The interval between the onset of the hepatitis symptoms and the development of grade II or more severe hepatic encephalopathy was 10 days or less (group-A), between 11 and 56 days (group-B), and more than 56 days (group-C)

artificial neural network and topology preserving mappings, consisting of two layers, an input layer and a competitive layer. It provides a low-dimensional representation of multi-dimensional datasets, an array of nodes, called a map. The most important feature of SOM is its preservation of the topological relations among high-dimensional input data, imposed on the output map, usually one or two dimensions, and its technique can be applied to clustering. SOM operates in two phases: training and mapping phases. Training constructs the map using input data, which is a competitive process called vector quantization. A new input vector is classified automatically during the mapping

Table 3 Item characteristics of acute liver failure patients used in the self-organizing map analysis

The types of hepatitis: acute and subacute types of fulminant hepatitis and LOHF
Outcomes: survived and dead among patients treated conservatively without liver transplantation and the patients who underwent transplantation
Gender: male and female
Age (years, continuous variable)
Complications preceding acute liver failure: diseases different from liver diseases such as metabolic syndrome, psychiatric diseases, and malignancies
HBV carrier
Past medical history: operations, blood infusions, alcohol intake, and medications
Family history: liver diseases
Etiology of hepatitis: viral infection, autoimmune hepatitis, drug-induced, indeterminate, and unknown due to insufficient examinations
Serum markers: anti-HAV (IgM), HBs antigen and antibodies, anti-HBc (IgM and IgG), HBV-DNA, anti-HCV, HCV-RNA, anti-HEV (IgM and IgG), HEV-RNA, anti-HDV, HGV-RNA, TTV-DNA, anti-nuclear antibodies, and serum IgG concentration
Interval between the onset of the hepatitis symptoms and the subsequent events (days, continuous variables): development of jaundice and grade II or more severe hepatic encephalopathy, recovery of consciousness, death, and/or transplantation
Interval between the onset of jaundice and the subsequent events (days, continuous variables): development of hepatic encephalopathy of grade II or more, recovery of consciousness, death, and/or liver transplantation
Interval between the onset of grade II or more severe hepatic encephalopathy and the subsequent events (days, continuous variables): recovery of consciousness, death, and/or transplantation
Maximal grade of hepatic encephalopathy (II–V, continuous variable)
Cause of death: liver failure, infection, and other complications
Symptoms at the onset of grade II or more severe hepatic encephalopathy: fever, jaundice, ascites, edema, flapping tremor, halitosis, loss of liver dullness, convulsion, tachycardia, and hyperventilation
Laboratory data at the onset of grade II or more severe hepatic encephalopathy (continuous variables): the grading of the encephalopathy; peripheral counts of WBC and platelets; prothrombin time; hepaplastin test; plasma concentrations of antithrombin III and ammonia; serum concentrations of AST, ALT, total albumin, total bilirubin, direct bilirubin, AFP, and HGF; the serum concentration ratios of direct-to-total bilirubin and the BCAA-to-tyrosine and Fischer ratio
Symptoms and laboratory data 5 days after the onset of encephalopathy (continuous variables): the grading of the encephalopathy, prothrombin time
Atrophy of the liver at the onset of grade II or more severe hepatic encephalopathy, 5 days after the onset of encephalopathy and during the whole course of the disease
Complications of acute liver failure at the onset of grade II or more severe hepatic encephalopathy, 5 days after the onset of encephalopathy and during the whole course of the disease: bacterial and fungal infections, gastrointestinal bleeding, renal failure, cardiac failure, disseminated intravascular coagulation, other complications
Number of complications at the onset of grade II or more severe hepatic encephalopathy, 5 days after the onset of encephalopathy, and during the whole course of the disease (continuous variables)
Hospitals providing medical care and liver transplantation: plasma exchange, hemodiafiltration, glucocorticoids, glucagon and insulin, prostaglandin E1, interferon, lamivudine or entecavir, cyclosporine-A, anticoagulants, fresh frozen plasma, and liver transplantation

LOHF late-onset hepatic failure, *HAV* hepatitis A virus, *HBV* hepatitis B virus, *HDV* hepatitis D virus, *HGV* hepatitis G virus, *TTV* torque teno virus, *WBC* white blood cell count, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *AFP* alpha-fetoprotein, *HGF* hepatocyte growth factor, *BCAA* branched-chain amino acids, *HBs* hepatitis B surface, *HBc* hepatitis B core

phase [16]. The training phase utilizes competitive learning. Unsupervised learning of SOM processes was carried out as follows. First, an input vector was selected randomly from among the 104 items. $\boldsymbol{D}$ was defined as the Euclidean distance between the input vector $\boldsymbol{X}$ and the reference vector $\boldsymbol{W}$. An individual $\boldsymbol{D}$ value was applied to every neuron j.

$$\boldsymbol{D}_j = \sqrt{\sum_{i=1}^{N} \left(\boldsymbol{X}_i - \boldsymbol{W}_{ij}\right)^2} \quad \text{for} \quad j \in L$$

where i is the individual item, N the number of items, j the individual neuron, and L is the number of neurons.

During the mapping phase, a neuron with the minimum $\boldsymbol{D}$ value was determined as the winning neuron c. Reference vectors of the winning and neighbor neurons $N_c(t)$ were modified according to the following formula:

$$\boldsymbol{W}_j(t+1) = \boldsymbol{W}_j(t) + \alpha(t)\left(\boldsymbol{X}(t) - \boldsymbol{W}_j(t)\right) \quad \text{for} \quad j \in N_c(t)$$

where α is the learning rate, initially close to 1 and diminished at each iteration ($0 < \alpha \leq 1$).

SOM processes were terminated when the change in $\boldsymbol{D}$ was less than a certain level. Calculation of cluster aggregation levels on the map resulted in the identification of three major clusters.

Statistical analysis

Statistical testing was performed using SPSS version 15.0J (SPSS, Tokyo, Japan). Results were presented as means ± SD. Continuous variables were compared using one-way factorial analysis of variance (ANOVA) and multiple comparisons with Tukey and Dunnett's T3 methods. Categorical data were compared using the χ^2 test, and further, we carried out an analysis of residuals in cross tabulation.

Results

Demographic and clinical features and outcome of patients with acute liver failure

As shown in Table 1, of the total study population, 40.2% had underlying diseases such as metabolic syndrome, and most of such patients were on daily medications. The etiology of fulminant hepatitis was viral infection in 69.3, 31.2, and 17.1% of the patients in group-A, group-B, and group-C, respectively. In most cases, the causative virus was HBV; transient infection was predominant in group-A, whereas asymptomatic carriers showing acute exacerbation of hepatitis predominated in group-B. The survival rates of the 811 patients who received medical treatment alone (without LT) were 53.4% in group-A, 24.5% in group-B, and 12.1% in group-C (Table 2).

Clustering analysis of the patients with acute liver failure

The results of analysis by the SOM technique revealed that the acute liver failure patients could be classified into three clusters, consisting of 411, 320, and 291 patients, respectively: cluster-1 (40.2%), cluster-2 (31.3%), and cluster-3 (28.5%). The demographic and clinical features of the patients in each cluster are shown in Tables 4, 5, 6, and 7 and Fig. 1.

As shown in Table 4, the interval between the onset of the hepatitis symptoms and the development of hepatic encephalopathy differed among the three clusters; the percentages of patients belonging to group-A, group-B, and group-C were 56.7, 39.2, and 4.1%, respectively, in cluster-1, and 58.8, 34.0, and 7.2%, respectively, in cluster-3. However, in cluster-2, the percentage of patients belonging to group-A was smaller, and those of patients belonging to group-B and group-C were greater (21.3, 65.0, and 13.8%, respectively) than in either cluster-1 or cluster-3.

Also, the demographic features of the patients differed among the three clusters (Table 5). There were a greater number of females in both cluster-1 and cluster-2 than in cluster-3, while the number of males was higher than that of females in cluster-3. The age (years: mean ± SD) of the patients was significantly higher in cluster-2 (54.9 ± 15.6) than in cluster-1 (41.0 ± 16.2) or cluster-3 (51.5 ± 14.2), and the age in cluster-3 was also significantly higher than that in cluster-1. The age distribution of the patients in each cluster is shown in Fig. 1. The percentage of HBV carriers was especially high in patients in cluster-3 (23.0%) compared with those in cluster-1 (7.9%) and cluster-2 (12.7%). In contrast, the underlying diseases preceding acute liver failure, such as metabolic syndrome, malignancies, and psychiatric disorders, were found more frequently in patients in cluster-2 (54.0%) and cluster-3 (49.3%) than in the patients in cluster-1 (23.3%). Such a tendency was also noted for the percentages of patients receiving previous medications (34.4, 60.1, and 53.7%, respectively, in cluster-1, cluster-2, and cluster-3).

Table 5 shows the relation between the etiology of the liver disease and the classification depending on the SOM analysis in the patients with acute liver failure. Viral infection was the most prevalent etiology in the patients in cluster-1 (53.0%) and cluster-3 (63.6%), but the causative virus differed between these clusters. Although the percentage of patients with transient HBV infection was similar in cluster-1 and cluster-3 (29.4 and 31.6%, respectively), the percentage of HBV carriers with hepatitis exacerbation was higher in cluster-3 (20.6%) as compared

Table 4 Intervals between the onset of hepatitis symptoms and grade II or more severe hepatic encephalopathy in acute liver failure patients and their clusters according to the classification by the self-organizing map

Groups[a]	% (Number of patients)			
	Total (n = 1,022)	Cluster-1 (n = 411)	Cluster-2 (n = 320)	Cluster-3 (n = 291)
Group-A	46.2 (472)	56.7 (233)*	21.3 (68)	58.8 (171)*
Group-B	45.8 (468)	39.2 (161)*	65.0 (208)	34.0 (99)*
Group-C	8.0 (82)	4.1 (17)*,#	13.8 (44)	7.2 (21)*

* $p < 0.05$ versus cluster 2; #$p < 0.05$ versus cluster 3 by χ^2 test and analysis of residuals in cross tabulation

[a] The interval between the onset of the hepatitis symptoms and the development of grade II or more severe hepatic encephalopathy was 10 days or less (group-A), between 11 and 56 days (group-B), and more than 56 days (group C)

Table 5 Demographic features and etiology of acute liver failure patients and their clusters according to the classification by the self-organizing map

	Total (n = 1,022)	Cluster-1 (n = 411)	Cluster-2 (n = 320)	Cluster-3 (n = 291)
Male:female (:unknown)	498:523 (:1)	187:224	122:197 (:1)	189:102*,#
Age (years)	48.3 ± 16.7[a]	41.0 ± 16.2	54.9 ± 15.6†	51.5 ± 14.2†,‡
HBV carrier [% (n)]	13.4 (126/940)	7.9 (31/390)	12.7 (39/306)*	23.0 (56/244)*,#
Underlying diseases[b] [% (n)]	40.2 (404/1,005)	23.3 (95/408)	54.0 (169/313)*	49.3 (140/284)*
Previous medication [% (n)]	47.7 (466/977)	34.4 (139/404)	60.1 (182/303)*	53.7 (145/270)*,#
Etiology [% (n)]				
Viral infection	47.7 (487)	53.0 (218)	26.3 (84)*	63.6 (185)#
HAV	5.4 (55)	9.0 (37)	1.6 (5)*	4.5 (13)*,#
HBV	39.5 (404)	40.1 (165)	22.2 (71)*	57.7 (168)*,#
Transient infection	22.8 (233)	29.4 (121)	6.3 (20)*	31.6 (92)#
Carrier	13.1 (134)	7.5 (31)	13.4 (43)*	20.6 (60)*,#
Undetermined	3.6 (37)	3.2 (13)	2.5 (8)	5.5 (16)*,#
HCV	1.3 (13)	1.7 (7)	1.9 (6)	0.0 (0)*,#
HEV	0.7 (7)	1.2 (5)	0.0 (0)	0.7 (2)
Other virus	0.8 (8)	1.2 (5)	0.3 (1)	0.7 (2)
Autoimmune hepatitis	7.8 (80)	5.4 (22)	15.3 (49)*	3.1 (9)#
Drug allergy-induced	11.0 (112)	9.2 (38)	15.9 (51)*	7.9 (23)*,#
Indeterminate	31.2 (319)	30.2 (124)	40.9 (131)*	22.0 (64)*,#
Insufficient examinations[c]	2.3 (24)	1.9 (8)	1.9 (6)	3.4 (10)

HBV hepatitis B virus, *HAV* hepatitis A virus, *HCV* hepatitis C virus, *HEV* hepatitis E virus

† $p < 0.05$ versus cluster 1; ‡$p < 0.05$ versus cluster 2 by one-way ANOVA and multiple comparisons

* $p < 0.05$ versus cluster 1; #$p < 0.05$ versus cluster 2 by χ^2 test and analysis of residuals in cross tabulation

[a] Mean ± SD

[b] Diseases such as metabolic syndrome, malignancy, and psychiatric disorders

[c] The etiology was unknown because of insufficient examinations

with that in cluster-1 (7.5%). In contrast, the percentages of patients with drug-induced liver injury, autoimmune hepatitis, and those with indeterminate etiology was higher in cluster-2 (15.9, 15.3, and 40.9%, respectively) than in cluster-1 (9.2, 5.4, and 30.2%, respectively) or cluster-3 (7.9, 3.1, and 22.0%, respectively).

As shown in Table 6, the frequencies of complications, such as bacterial infection, gastrointestinal bleeding, renal and cardiac failure, DIC, and cerebral edema, differed among the three clusters. There were no complications in 56.9% of the patients in cluster-1, while the percentages of patients with no complications were as low as 11.9 and 1.4% in cluster-2 and cluster-3, respectively. The percentage of patients with 2 or more complications was 11.9% in cluster-1, which was markedly lower than the values in cluster-2 (54.4%) and cluster-3 (90.4%).

Consequently, the outcome of the patients differed markedly among the three clusters (Table 7). LT was performed in 34.3% of the patients in cluster-1, which was a significantly greater percentage than the corresponding percentages in cluster-2 (15.6%) and cluster-3 (6.9%), and the survival rates of these patients with LT were 90.1, 52.0, and 60.0% in cluster-1, cluster-2, and cluster-3, respectively. Also, the survival rate in the patients not treated by LT was significantly higher in cluster-1 (94.4%) than in cluster-2 (6.7%) or cluster-3 (12.9%). The survival rate among patients receiving medical treatment alone was still significantly higher in cluster-1 (62.0%) than in cluster-2 (5.6%) or cluster-3 (12.0%), even when it was expressed as the ratio of number of survivors to the total number of patients including those receiving liver transplantation.

Discussion

In this study, the validity of the classification of acute liver failure according to the interval between the onset of the hepatitis symptoms and the development of hepatic encephalopathy was evaluated. Previous classifications were established based on decision analyses with a linear mode, such as multivariate regression analysis. Such analysis, however, is not suitable for modeling complex multidimensional relationships, which may also affect the prognosis of acute liver failure patients in a complex

Table 6 Numbers of complications in acute liver failure patients during the whole course of the disease and their clusters according to the classification by the self-organizing map

Number of complications	% (Number of patients)			
	Total (n = 1,022)	Cluster-1 (n = 411)	Cluster-2 (n = 320)	Cluster-3 (n = 291)
0	27.0 (276)	56.9 (234)	11.9 (38)*	1.4 (4)*
1	25.4 (260)	31.1 (128)	33.8 (108)	8.2 (24)*,#
2	20.3 (207)	10.2 (42)	28.8 (92)*	25.1 (73)*
3	14.2 (145)	1.7 (7)	16.6 (53)*	29.2 (85)*,#
4	7.9 (81)	0.0 (0)	6.6 (21)*	20.6 (60)*,#
5	3.7 (38)	0.0 (0)	2.5 (8)*	10.3 (30)*,#
6	0.9 (9)	0.0 (0)	0.0 (0)	3.1 (9)*,#

* $p < 0.05$ versus cluster 1; #$p < 0.05$ versus cluster 2 by χ^2 test and analysis of residuals in cross tabulation

Table 7 Outcome of acute liver failure patients and their clusters according to the classification by the self-organizing map

	% (Number of patients)			
	Total (n = 1,022)	Cluster-1 (n = 411)	Cluster-2 (n = 320)	Cluster-3 (n = 291)
Survival rates in all patients	46.2 (473/1,022)	92.9 (382/411)	13.8* (44/320)	16.2* (47/291)
Treated without liver transplantation	79.4 (811)	65.7 (270)	84.4 (270)	93.1 (271)
Survival rates with medical treatment alone in all patients	30.1 (308/1,022)	62.0 (255/411)	5.6* (18/320)	12.0* (35/291)
Survival rates in patients receiving medical therapies alone	38.0 (308/881)	94.4 (255/270)	6.7* (18/270)	12.9* (35/271)
Treated by liver transplantation	20.6 (211)	34.3 (141)	15.6 (50)	6.9 (20)
Survival rates	78.1 (165/211)	90.1 (127/141)	52.0* (26/50)	60.0* (12/20)

* $p < 0.05$ versus cluster 1 by χ^2 test and analysis of residuals in cross tabulation

manner. Thus, we evaluated the validity of the classification using artificial neural networks, one of the decision analyses with a non-linear mode.

The SOM is a neural network methodology that has been used for the categorization and interpretation of multidimensional data sets with a large scale [8]. The categorization can be achieved through transformation of an n-dimensional input vector into an r-dimensional separated map, in which r is smaller than n and is usually 2. Thus, the input vectors are preserved on the same area of the map, and these signal processing units are called "neurons". Each neuron shows an association through an n-dimensional reference vector that determines the linkage between the maps of the input and output vectors. The input vector is transformed onto a particular neuron through the calculation in which the n-dimensional distance between the input and reference vectors of the neuron is the minimal distance. Based on such calculations, the reference vectors of the neurons are updated repeatedly, and finally the best-matching neurons are established. As a result, the SOM can accomplish clustering of the input data sets without the experience of specialists. Such a process is called unsupervised learning.

In the present study, the results of the clustering analysis using the SOM revealed that the patients with acute liver failure could be classified into three clusters different from the disease groups, depending on the interval between the onset of the hepatitis symptoms and the development of hepatic encephalopathy as nominal variables. Also, similar results were obtained in analysis when such intervals were assessed as continuous variables (data not shown). As shown in Table 4, 404 of the 472 patients (85.6%) in group-A belonged to either cluster-1 or cluster-3. Also, while 208 of the 468 patients (44.4%) in group B and 44 of the 82 patients (53.7%) in group-C were classified into cluster-2, a distinct accumulation of the patients in these three clusters, as was the case in group-A, was not found in the other two groups. Moreover, it should be noted that the demographic and clinical features and the outcome differed between the patients in cluster-1 and those in cluster-3 (Tables 5, 6, and 7; Fig. 1), despite the percentages of patients belonging to groups A, B, and C being almost identical in these two clusters (Table 4). These observations strongly suggest that patients with acute liver failure in Japan may be classified into disease types different from those that are exclusively dependent on the interval between the onset of the hepatitis symptoms and the development of hepatic encephalopathy.

The novel classification through the SOM analysis may be clinically important, since the outcome of the patients

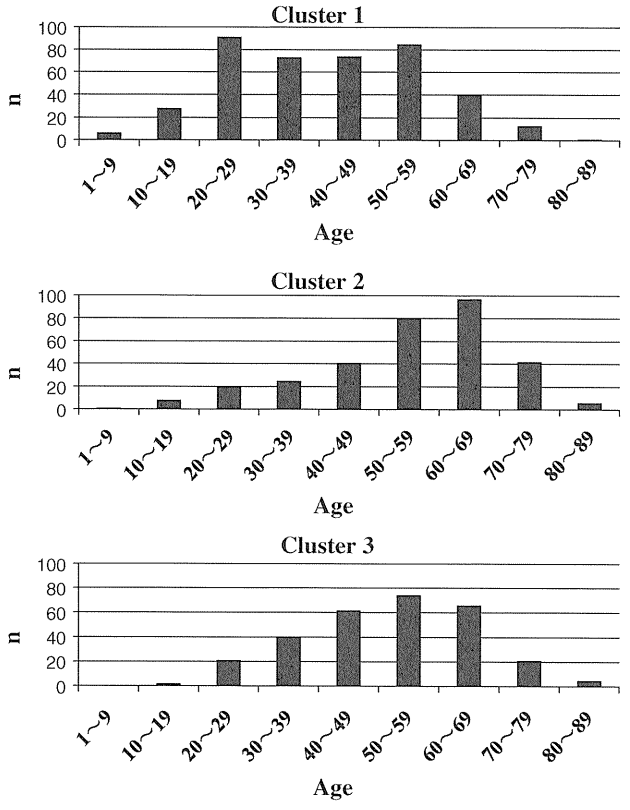

Fig. 1 Distribution of the ages (years) of acute liver failure patients classified according to the clustering analysis based on the self-organizing map

differed more obviously among the clusters as compared with the differences between the disease types determined based only on the interval from the onset of the hepatitis symptoms and the development of hepatic encephalopathy (Table 1). A total of 141 (66.8%) of the 211 patients treated by LT were classified into cluster-1, and the survival rate of these patients was 90.1%. Also, the survival rate of those treated conservatively without LT was extremely high (94.4%) in this cluster. In contrast, the percentage of the patients treated by LT was extremely low in cluster-2 and cluster-3, and 78.8% (252/320) and 81.1% (236/291) of the patients, respectively, who were treated conservatively without LT died. The factors that seemed to be responsible for the differences among these clusters were the sex and age of the patients, the presence of the underlying diseases and history of previous medications, the etiology of the liver diseases, and the number of complications developing during acute liver failure, as well as the interval between the onset of the hepatitis symptoms and the development of hepatic encephalopathy. The patients with such factors, among which complex multi-dimensional relationships may exist, could be classified into the clusters reflecting their outcome only through SOM analysis, a form of artificial neural network.

Our classification is also useful for speculating on the possible cause of acute liver failure in the patients with indeterminate etiology. Of the 319 patients with indeterminate etiology, 131 (41%) were classified into cluster-2, in which 61 and 46% of the patients in whom the acute liver failure was due to autoimmune hepatitis and drug-induced liver injury, respectively, were also classified. The total percentage of patients with indeterminate etiology and those whose acute liver failure was due to autoimmune hepatitis and drug-induced liver injury was 72% in this cluster. In contrast, 124 (39%) of the patients with indeterminate etiology were classified into cluster-1 and 64 (20%) into cluster-3. Viral infection was the most prevalent etiology in both clusters (53 and 64%, respectively, in cluster-1 and cluster-3), but the causative virus differed between these clusters; transient infections of HAV and HBV were dominant in cluster-1, while HBV carriers showing acute hepatitis exacerbation prevailed in cluster-3. Considering the characteristics of the patients with indeterminate etiology in each cluster, possible etiologies in these patients were transient unknown virus infection in cluster-1, autoimmune hepatitis or drug-induced liver injury in cluster-2, and HBV occult infection in cluster-3. These possibilities should be further investigated.

Although our classification may be useful to predict the outcome of patients with acute liver failure and the possible causes of the condition in those with indeterminate etiology, there still exist problems that need to be resolved preceding the application of the system for the benefit of hepatologists in Japan. First, the patients can be classified into the three clusters only through the SOM technique using the IBM Intelligent Miner software. Physicians and/or transplant surgeons are required to input the data sets on a total of 104 items collected from the patients using personal computers, and the clusters that the patients are categorized into are shown on the websites through a blind decision process. Such complicated procedures might prevent our system from becoming prevalent for clinical use by hepatologists nationwide. Simple algorithms, using which any physicians and/or surgeons can predict the outcome of the patients based on the clusters that they belong to, with no reference to the websites, should be established using data-mining analysis methods, such as the decision tree [17]. Secondly, the purpose of our system is to categorize patients with acute liver failure depending on the demographic and clinical features of the patients as well as their prognosis, suggesting that such a system may not always be suitable for determination of the indications for LT. For example, regarding cluster-1, in which the survival rate of the patients was extremely high, one-third of the patients were rescued after being treated by LT, while two-thirds survived following conservative treatment without transplantation. Thus, a system to directly predict

the prognosis of the patients should be established based on the data sets exclusively derived from the patients treated conservatively without LT. The radial basis function model [17, 18] and/or the back propagation model [19], one form of artificial neural network, would seem to be suitable for such analysis with the data-mining procedures. Also, precise information on past medical history, especially regarding underlying diseases and previous medication (which are important factors that determine the character of each cluster), should be analyzed. These projects should be undertaken in the future.

In the present study, patients with acute liver failure diagnosed according to the definition established in Japan were analyzed through the SOM technique. Analyses using the SOM technique in patients with acute liver failure in the United States and Europe also merit consideration, since the outcome prognosis of the patients may be determined through complex multidimensional relationships including the etiology, as well as the intervals between the onset of hepatitis symptoms and the development of hepatic encephalopathy [4, 20, 21]. There seems to be a higher percentage of patients that can be rescued without LT in these countries than in Japan, possibly because of the difference in the definition criteria of acute liver failure, especially in relation to the differences in criteria for prothrombin time among the countries [1–4]. Such patients may be identified through data-mining analysis using the SOM technique. Thus, such an approach may contribute to the overcoming of problems of organ shortage in the field of LT, which also exist in Europe and the United States.

In conclusion, patients with acute liver failure in Japan were classified into three clusters independent of the disease types depending on the interval between the disease onset and the development of encephalopathy, using the SOM technique. This technique may be useful for establishing prognosis prediction models, since the outcome of the patients differed markedly among the clusters.

Acknowledgments This study was supported by Health Labor Sciences Research Grant, Research on Measures for Intractable Diseases, Ministry of Health, Labor and Welfare of Japan.

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＜原　著＞

北海道東部の釧路市および根室市におけるE型肝炎ウイルス感染の疫学調査：感染の地域差と食文化の相違について

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要旨：我が国においてE型肝炎患者数が最も多い北海道におけるE型肝炎ウイルス（HEV）感染の地域差の有無を検討するため，釧路市721例および根室市687例の血清検体についてIgG型HEV抗体を測定し，既報の札幌市および北見市の住民での測定結果と比較した．抗体陽性率は釧路市で5.4%，根室市で2.0%であり，両市とも男性で有意に高率であった（釧路市，男性8.5% vs. 女性3.0%，P＝0.0010；根室市，4.0% vs. 0.5%，P＝0.0012）．40歳以上の年代で各市の抗体陽性率を比較すると，釧路市と北見市，札幌市との間で有意差は認められなかったが（それぞれ7.9%，12.1%，6.4%），根室市では2.1%に過ぎず，北見市，札幌市および釧路市よりも有意に低率であった．道内4都市での感染率の地域差は地域産業および食文化の相違を背景にしたブタ肉・内臓消費量の違いに由来すると推測された．

索引用語：　E型急性肝炎　　北海道　　感染の地域差　　地域産業　　食文化

はじめに

E型肝炎はE型肝炎ウイルス（HEV）の経口感染に起因するウイルス肝炎であり，従来，我が国では公衆衛生インフラの整備が遅れた発展途上国からの輸入感染症の一つと考えられていた．しかし，2001年に国内で海外渡航歴のないE型肝炎患者から3型HEV株（JRA1株）が分離され[1]，飼育ブタでHEV感染が蔓延状態にあることが報告されて以来[2]，我が国に土着のHEV株によるE型肝炎症例が全国から報告され[3)~15)]，土着HEV株によるE型劇症肝炎の存在も明らかになって[16]，俄かに国内感染E型肝炎が注目されることになった．加えて，生あるいは加熱不十分な動物の肉・内臓の喫食後のE型肝炎の発症が相次いで報告され，我が国において動物由来感染症（Zoonosis）としてのE型肝炎が世界に先駆けて認知された[17)~21)]．

全国の健常成人22,027例を対象にしたHEV感染調査の結果では，IgG型HEV抗体陽性率は全体で5.3%であったが，北海道，東北地方で高く，西日本で低い東高西低の傾向が認められた[22]．原因として東日本ではHEV感染のリザーバーであるブタの肉の消費割合が牛肉よりも高く，反対に西日本の多くの地域では豚肉よりも牛肉を好むという食文化の差異が想定された[22]．北海道ではIgG型HEV抗体の陽性率が7.4%と全国で最も高く，HEVの高度浸潤地域であることを示すデータであったが，この調査の検体は主として札幌市と北見市で採取されたものであった．

2006年1月末の時点で集積されたE型肝炎の全国集計[23]では，北は北海道から南は沖縄まで全国で患者が発生しているが，HEV遺伝子型が判明した228例のうち123例は北海道からの報告であり，北海道において患者数が突出していることが明らかになった．また，重症化しやすい4型HEVが感染したE型肝炎症例が多いことも北海道の特徴である[24]．

北海道内の合計43の医療機関が参加する「北海道E型肝炎研究会」の4年間（2007年～2010年）のprospective studyでは，非ABC型肝炎340例中69例（20%）がE型肝炎であり，近年の発生数の増加が指摘されている．症例発表は札幌圏，北見・網走，函館等に多くみられる[25]．

北海道におけるHEVの感染経路は，E型肝炎患者に

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＜受付日2011年7月1日＞＜採択日2011年7月14日＞

Table 1 Age and gender-specific prevalence of anti-HEV IgG among 1,408 individuals in Kushiro and Nemuro cities in Hokkaido

Age (years)	No. (%) of individuals with anti-HEV IgG					
	Kushiro			Nemuro		
	Total	Male	Female	Total	Male	Female
10-19	0/6	0/2	0/4	0	0	0
20-29	1/138 (0.7)	1/51 (2.0)	0/87	0/7	0/3	0/4
30-39	2/123 (1.6)	2/49 (4.1)	0/74	0/28	0/20	0/8
40-49	6/116 (5.2)	5/53 (9.4)	1/63 (1.6)	2/70 (2.9)	2/37 (5.4)	0/33
50-59	9/110 (8.2)	5/52 (9.6)	4/58 (6.9)	2/125 (1.6)	2/58 (3.4)	0/67
60-69	9/123 (7.3)	7/57 (12.3)	2/66 (3.0)	3/192 (1.6)	3/75 (4.0)	0/117
70-79	12/104 (11.5)	7/52 (13.5)	5/52 (9.6)	5/181 (2.8)	3/68 (4.4)	2/113 (1.8)
≥80	0/1	0	0/1	2/84 (2.4)	2/37 (5.4)	0/47
Total	39/721 (5.4)	27/316 (8.5)	12/405 (3.0)	14/687 (2.0)	12/298 (4.0)	2/389 (0.5)

おけるデータ［90%（9/10），ないし93%（25/27）が発症1～2カ月前のブタ肉・内臓の摂取歴あり］[18)24)]，およびHEV NAT陽性献血者におけるデータ［71%（102/143）が献血前の2カ月間に動物内臓肉を食していたこと］[26)]から主としてHEV陽性のブタの肉や内臓の摂食であると考えられている．しかし，北見市や網走市でのブタ内臓肉を摂取した会食者におけるmini-outbreakが報告されている一方で[11)27)]，2009年9月から10月にかけて札幌圏内で発生した同一クラスターに属する4型HEVによる小流行事例では共通する感染源が特定されていない[28)]．したがって，地域の産業や食文化が異なると，HEVの感染状況にも影響が及ぶことが想定される．今回我々はこれまで調査が行なわれていなかった北海道東部の釧路市と根室市の住民を対象にしてHEV感染の疫学調査を行い，地域産業が異なる北海道内の2都市（札幌市と北見市）での調査結果と比較し，食文化とHEV感染リスクとの関連について検討したので報告する．

対象および方法

HEV感染調査に同意が得られた住民を対象として本調査を行なった．釧路市での調査では，釧路協立病院における2003年5月から8月までの外来通院患者355人と同時期の職員366人の計721人（男性316人，女性405人，年齢48.1±17.2歳），根室市での調査では，2010年11月から2011年4月までに採血された，10年以上同市に居住している住民（道東勤医協ねむろ病院の外来患者）687人（男性298人，女性389人，年齢64.3±13.7歳）を対象とした．根室市では2011年3月時点の人口が約3万人であり，20歳以上の住民の3%弱に相当する．両地域の調査対象者のなかには，急性肝炎の臨床症状並びに生化学所見を示した症例はなかった．

IgG型HEV抗体は上述の全国調査[22)]と同じく既報のELISA法[29)]を用いて測定した．またIgG型HEV抗体陽性検体についてはIgM型およびIgA型HEV抗体も追加測定した[29)]．

成　績

IgG型HEV抗体の陽性率は釧路市で5.4%（39/721）であったのに対して，根室市では2.0%（14/687）に過ぎなかった（Table 1）．性差については，釧路市では男性8.5%（27/316），女性3.0%（12/405），および根室市では男4.0%（12/298），女性0.5%（2/389）であり（Table 1），両市とも男性で有意に高率であった（各P=0.0010およびP=0.0012）．

釧路市においては加齢とともに抗体陽性率の上昇が認められたが，根室市では20–39歳で全員が抗体陰性，40歳以上でも1.6–2.9%の陽性率に留まり，年齢増加による明らかな抗体陽性率上昇の傾向は認められなかった（Table 1）．10歳以降の年代別，性別のIgG型HEV抗体陽性率を札幌市，北見市，および全国調査のデータ[22)]と対比しFig. 1に示す．男性では，北見市の住民が最も高率で，50歳代，60歳代，70歳以降の年齢層でそれぞれ15.1%，17.2%，25.8%の陽性率であった．それに対して，根室市は全国平均よりも低く，特に50歳代

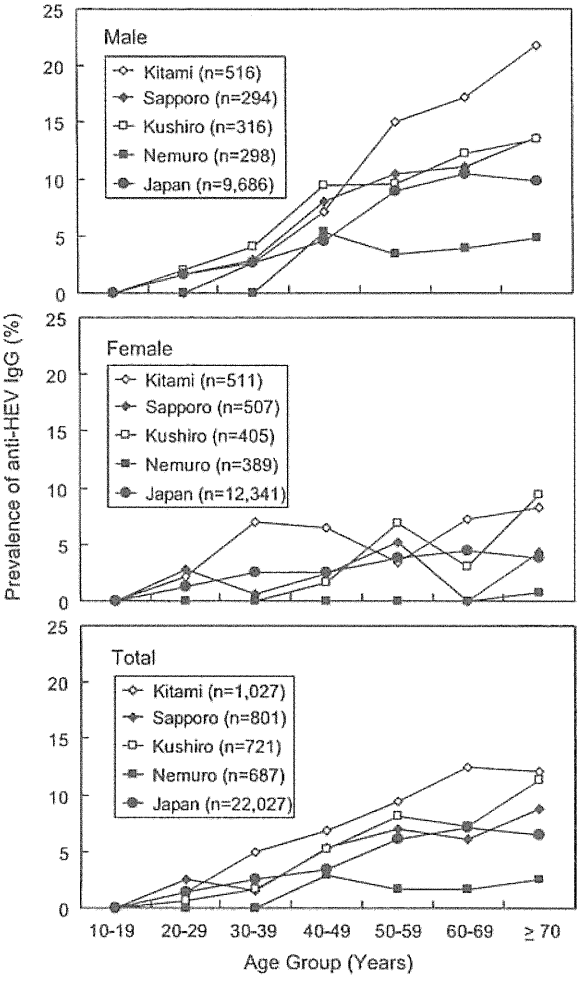

Fig. 1 Comparison of the gender- and age-dependent prevalence of anti-HEV IgG among individuals living in Kitami, Sapporo, Kushiro, and Nemuro in Hokkaido as well as in the whole Japan. Data in Sapporo, Kitami, and the whole Japan were retrieved from our previous report[22].

以上の年齢層で 3.4–5.4% に過ぎず，顕著に低率であった．一方，釧路市は各年代において全国平均よりも高く，札幌市とほぼ同等の陽性率であった．女性では全国平均を含め，どの 4 地域でも 10% を超えることはなかったが，北見市は全体として高率であった．釧路市では 50 歳代と 70 歳以降の年代でそれぞれ 6.9%，9.6% と北見市よりも高率であり，他の年代でも全国平均に近似した陽性率を示した．それに対して，根室市では，10 歳代から 60 歳代まで全ての年代で陽性者は認められず，70 歳以上の 160 名中わずか 2 名（1.3%）が抗体を保有しているに過ぎなかった．男女のデータを合わせた全体での抗体陽性率の集計結果を Fig. 1 の最下段に示すが，男性での傾向に近似しており，北見市が最も高く，札幌市と釧路市がほぼ同等でそれに次ぎ，全国平均よりも高い陽性率を示した．根室市は抗体陽性者が認められた 40 歳以降の年代においても全ての年代で最も低い抗体陽性率であった．

40 歳以上の住民での各市の IgG 型 HEV 抗体陽性率を男女別に比較した結果を Table 2 に示す．釧路市と北見市，札幌市との間で抗体陽性率に男女ともに有意な差は認められなかったが，根室市では北見市，札幌市および釧路市と比べて統計学的に有意に抗体陽性率が低いことが確認された（Table 2）．

IgG 型 HEV 抗体陽性の釧路検体（39 検体）および根室検体（14 検体）について IgM 型および IgA 型 HEV 抗体を測定したがいずれも陰性であり，過去の感染既往によって IgG 型 HEV 抗体が検出されたものと推測された．

考　案

HEV が主として水系を介して糞口感染する発展途上国とは違い，先進諸国における E 型肝炎の多くは，ブタ，イノシシおよびシカ等をリザーバーとして感染動物の肉や内臓を食することにより HEV に感染する人獣共通感染症である[30]．北海道は全国的に見て E 型肝炎患者数が最も多く，IgG 型 HEV 抗体陽性率も高率であり，HEV の高度浸潤地域と看做されている[3)22)23)26)]．ブタでの HEV 感染は世界各地で広く蔓延しており，我が国でも全国的に飼育ブタでの HEV 感染が拡がっており[31]，北海道も例外ではない．また，ヒトへの主要な感染源の一つと考えられている豚レバーでの HEV RNA 陽性頻度は北海道の市販ブタレバーについての調査で 1.9%（7/363）であり[18]，北見市における劇症肝炎による死亡症例を含む遺伝子型 4 型 HEV の集団感染事例もその原因は加熱不十分な豚レバーの摂食が原因であると考えられている[11]．

2009 年度の総務省統計局調査（http://www.stat.go.jp/data/kakei/index.html）によると，生鮮肉への支出金額における肉消費地方別特化係数（全国平均に対する比率）は北海道において牛肉は 0.49 で全国最低であり，豚肉は 1.27 で東北の 1.37 に次いで多かった．牛肉と豚肉の消費支出比較では北海道民は牛肉の 3.2 倍を豚肉にかけている．これが北海道を E 型肝炎高度浸潤地域にした一つの要因と思われる．イノシシおよびシカもブタと同様にヒトへの HEV 感染の感染源となりうることが明らかになっている[17)19)~21)]．しかしながら，北海

Table 2 Comparison of the prevalence of anti-HEV IgG among individuals aged ≥40 years among four cities (Kitami, Sapporo, Kushiro, and Nemuro) in Hokkaido[a]

Region	Prevalence of anti-HEV IgG	P value
Male		
Kitami vs. Sapporo	77/452 (17.0%) vs. 16/165 (9.7%)	0.0241
Kushiro	77/452 (17.0%) vs. 24/214 (11.2%)	NS
Nemuro	77/452 (17.0%) vs. 12/275 (4.4%)	<0.0001
Sapporo vs. Kushiro	16/165 (9.7%) vs. 24/214 (11.2%)	NS
Nemuro	16/165 (9.7%) vs. 12/275 (4.4%)	0.0265
Kushiro vs. Nemuro	24/214 (11.2%) vs. 12/275 (4.4%)	0.0040
Female		
Kitami vs. Sapporo	29/422 (6.9%) vs. 7/197 (3.6%)	NS
Kushiro	29/422 (6.9%) vs. 12/240 (5.0%)	NS
Nemuro	29/422 (6.9%) vs. 2/377 (0.5%)	<0.0001
Sapporo vs. Kushiro	7/197 (3.6%) vs. 12/240 (5.0%)	NS
Nemuro	7/197 (3.6%) vs. 2/377 (0.5%)	0.0056
Kushiro vs. Nemuro	12/240 (5.0%) vs. 2/377 (0.5%)	0.0003
Total		
Kitami vs. Sapporo	106/874 (12.1%) vs. 23/362 (6.4%)	0.0025
Kushiro	106/874 (12.1%) vs. 36/454 (7.9%)	0.0188
Nemuro	106/874 (12.1%) vs. 14/652 (2.1%)	<0.0001
Sapporo vs. Kushiro	23/362 (6.4%) vs. 36/454 (7.9%)	NS
Nemuro	23/362 (6.4%) vs. 14/652 (2.1%)	0.0006
Kushiro vs. Nemuro	36/454 (7.9%) vs. 14/652 (2.1%)	<0.0001

[a] Data in Kitami and Sapporo were retrieved from our previous study[22].

道はイノシシの自然生域の北限を超えており野生種は存在せず，道内では限局して飼育されているのみであり，エゾシカの肉は，食用として広くは流通していない．

北海道は広大な土地面積を有し，地域によって産業も異なる．北見地域は，畜産業（養豚）が基幹産業の一つであり，また，「焼肉の街」と言われるように，焼肉料理店の数が北海道でも突出して多く，漁業は存在するが，その主体（87%）はホタテ貝の生産である．一方，釧路市および根室市では地域産業としては水産業が主体であり，養豚場は存在しない．両市とも漁業水揚げ量が全国でも有数の漁港があり，根室市は漁業従事者数が圧倒的に多くみられ，「魚の街」として知られている．釧路市は圏内30万人の人口集中地であり，漁業の占める割合からみると北見市と根室市の中間の街として位置づけられ，焼肉店の数も多い．札幌は191万人の大都市であり「消費の街」である．2009年度政府統計（市町村の姿）(http://www.machimura.maff.go.jp/machi/）から釧路市，根室市，北見市および札幌市の4都市の人口，漁業従事者数，農家人口，家畜の飼育頭数（ブタおよびウシ）を引用し，また電話帳タウンページから焼肉店の数を調査し，比較検討した(Table 3)．人口1万人当たりのブタの飼育頭数は北見市711頭，札幌市13.6頭で，釧路市と根室市ではブタは飼育されていない．漁業従事者数比率は北見市で0.4%，札幌市で0%，釧路市で0.4%と低値であったが，根室市では7.0%と突出して高かった(Table 3)．根室市は地理的に市内主要地域が半島に存在し，隣市の釧路まで120 kmの距離があり，孤立した地域環境にある．北海道内の都市別肉消費量に関するデータを入手することが困難であったことから，焼肉専門店数がブタ肉消費量をある程度反映すると仮定し，電話帳タウンページ（2011年度）で「焼肉店」を検索したところ，人口1万人あたりの焼肉店の数は，抗体陽性率が高い北見市で4.9店と特に多く，釧路市がそれに次ぐ3.1店であった．しかし，予想に反して札幌市で1.8店，根室市で1.7店と，両市は人口比では近似した店数であった．人口約191万人を抱える札幌市と人口僅か3万人弱の根室

Table 3 Comparison of four cities (Kitami, Sapporo, Kushiro, and Nemuro) in Hokkaido with regard to various features

Feature	Kitami	Sapporo	Kushiro	Nemuro
Population[a]	125,628	1,914,434	181,206	29,192
Rotisserie[b]	61	347	57	5
(No. of stores/10,000 population)	(4.9)	(1.8)	(3.1)	(1.7)
Livestock[a]				
Pigs (/10,000 population)	8,930 (711)	2,610 (13.6)	0	0
Cows (/10,000 population)[c]	11,580 (922)	1,530 (8.0)	17,280 (954)	13,010 (4,457)
Occupation[a]				
Agriculture	5,439 (4.3%)	3,657 (0.2%)	1,083 (0.6%)	576 (2.0%)
Fishery	445 (0.4%)[d]	0	813 (0.4%)	2,037 (7.0%)
Hepatitis E[e]				
No. of reported cases (/10,000 population)	25 (1.99)	50 (0.26)	2 (0.11)	0

[a] Available from http://www.machimura.maff.go.jp/machi/.

[b] Available from http://biglobe-tel.goo.ne.jp/.

[c] Milk cows account for 72% in Kitami, 91% in Sapporo, 78% in Kushiro, and 96% in Nemuro.

[d] The majority (87%) of fishermen are engaged in the production of Japanese scallop, compared to those in Kushiro and Nemuro, where most fishermen are engaged in the capture of sea fish.

[e] No. of reported cases with hepatitis E (from 2004 to the 24th week of 2011), which is available from http://www.iph.pref.hokkaido.jp/kansen/434/data.html.

市では店の規模，1 店舗当たりの集客数や肉消費量などが異なり，単純に人口比店舗数を比較しただけでは実際の肉消費量の多寡の試算が難しいことが上記のような数値として現れているものと考えられた．一方，根室市は漁業が基幹産業の大部分を占め，200 海里問題が発生する 1977 年以前は現在の約 2 倍の漁獲量が記録され（http://www.city.nemuro.hokkaido.jp/），住民の間では「魚は買う物でなく，もらう物」と言われるほどであった．現在でも食材として魚が容易に入手し易い状況下にある．今回の調査に参加し，無作為に選ばれた 100 人に対する食事アンケート調査の結果，11 人が肉を食べたことが無いと回答した事実からも類推できるように，根室市は魚食に大きく依存しており，養豚場が存在しないこと，地理的にほぼ隔離された地域であることなどが抗体陽性率の低さに関係していると考えられた．釧路市も漁業が主要産業の一つであるが，漁業従事者数比率は低く，人口集中地域で消費地としての性格を有し，焼肉店も多いことから，根室市とは異なり，北見市や札幌市と同じ傾向を示したものと思われる．

E 型肝炎は 2003 年 11 月 5 日より施行されている改正感染症法（感染症の予防及び感染症の患者に対する法律）において 4 類感染症に分類され，診断した医師は届出義務がある．ウイルス性肝炎が疑われる患者にはA 型，B 型および C 型と同様にルーチンに E 型の検査も行うべきであるが，保険適用となっている HEV マーカーはいまだ存在しない．そのような状況下での届出件数であり，相当数見逃されていると推測されるが，2004 年から 2011 年 24 週目までに道内保健所に報告された E 型肝炎症例は 130 例であり，そのうち 50 例が札幌市，25 例が北見市の患者である（http://www.iph.pref.hokkaido.jp/kansen/434/data.html）．北海道にありながら，IgG 型 HEV 抗体の陽性率が全国平均よりも大きく下回っている根室市では E 型肝炎の届出件数はゼロであり，本研究で得られた調査結果を支持している．また，釧路市も未だ僅か 2 例に過ぎないが，人口 1 万人当たりの患者数として比較すると，釧路市は 0.11 人で，1.99 人の北見市には及ばないが，札幌市（0.26 人）の約半数である（Table 3）．本研究で明らかになった IgG 型 HEV 抗体の陽性率から推測される 3 都市間の患者発生件数に矛盾しない．しかしながら，全数把握からは程遠いのが現状であり，1 日も早く保険適用になった E 型肝炎の検査試薬が臨床現場に登場し，真の意味での E 型肝炎発生状況の全容の把握が可能となること

を望むものである．

結　語

北海道東部の釧路市および根室市におけるHEV感染の疫学調査を行い，根室市では北海道内の北見市，札幌市および釧路市よりもHEV感染既往率が低いことを明らかにした．その原因として地域産業の違いによる食文化の違い，即ち魚食が多く，ブタ肉の摂食量が少ないことが背景にあるものと推測された．全国的に見て，感染源や感染経路を特定できていないE型肝炎症例が過半数を占めている状況[23]を鑑み，食習慣や食文化とHEV感染の関係は今後もさらに深く検証されるべき重要な課題であると思われる．

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