belonging to type 1b and type 2 (type 2a and 2b were considered as the same group with 2), 70 samples were belonging to type 1b, 41 samples were belonging to type 2, and only one was of type 3a. Therefore, for the sake of simplicity, we decided to use one dimensional feature to represent the genotype. Type 1b was assigned by -1, type 2 was assigned by $+1$, and the only one sample of type 3a was assigned by 0. Of course this is not always the optimal choice for each situation, but this is considered to be the best and simplest representation for the data used in current experiment. In the case of "hepatobiopsy," although they were nominal data, they showed transition phase. Thus the values of this attribute could be considered to have order among them. This characteristic motivated us to use numerical representation, by assigning the best condition F0 by 0, F1 by 1, F2 by 2, F3 by 3, and the worst condition F4 by value of 4, respectively.

Classification of interferon treatment efficacy

One hundred and twelve patients were divided into two groups according to the results of HCV-RNA test six months after the end of interferon therapy. The patients with undetectable HCV-RNA were defined as the effective group, and those with detectable HCV-RNA were defined as the ineffective group. Among 112 patients, 66 belonged to the effective group; the remaining 46 belonged to the ineffective group.

Methods

The prediction model is constructed of two parts: Feature Subset Selection (FSS) and Support Vector Machine (SVM) as classifier, as depicted in Fig. 1.

Feature Subset Selection (FSS) is the preprocessing part of the model that works to select features useful for the classification. In this study, the selection was conducted based on the individual advantages of each feature. Fisher criterion was used to measure the significance of each feature [9].

Let us denote the D-dimensional input vector as $\vec{x} = (x_1, x_2, \ldots, x_j, \ldots, x_D)^T$. Where the number of examples belonging to the effective group is n_{+1}, and the examples belonging to the ineffective group is n_{-1}, the mean of j^{th} feature of the effective group is $\mu_{j,+1}$, the mean of j^{th} feature of ineffective group is $\mu_{j,-1}$, and their standard deviations are $\sigma_{j,+1}$ and $\sigma_{j,-1}$, respectively. The significance of each feature x_j is measured by the following equation:

$$F(x_j) = \frac{n_{+1}n_{-1}}{n_{+1}+n_{-1}} \frac{(\mu_{j,+1}-\mu_{j,-1})^2}{n_{j,+1}\sigma_{j,+1}^2 + n_{j,-1}\sigma_{j,-1}^2} \tag{1}$$

This criterion can be interpreted as finding one single feature that best discriminates both of the groups in the feature space. The greater this score is, the better is the discrimination power of the feature. Based on this score, each feature was assigned by rank of significance. The feature selection was conducted by selecting a certain number of features from the top.

The second part of the model is the Support Vector Machine (SVM) [10]. SVM has currently received increasing attention due to its promising performance in many situations. In principal, SVM is a linear classifier that is trained to obtain an optimal classification hyperplane on the feature space. The optimal hyperplane is obtained by maximization of the "margin"; a criterion defined by the distance between the training samples and the hyperplane.

Let us denote each example as $\vec{x}_i \in \Re^d$, $i = 1, 2, \ldots, l$. Where l is the number of examples, and each example is labeled by $y_i \in \{-1, +1\}$; -1 represents the ineffective group and $+1$ the effective group. It is assumed that both the effective and ineffective groups are perfectly separated by a hyperplane in D-dimensional feature space. This hyperplane is represented by $\vec{w}.\vec{x}_i + b = 0$. Examples $\vec{x}_i$ that belong to the ineffective group should satisfy $\vec{w}.\vec{x}_i + b \leq -1$, and those that belong to the effective group should satisfy $\vec{w}.\vec{x}_i + b \geq +1$. The optimal margin is obtained by maximizing the distance between the hyperplane and the closest pattern, which is formulated by $1/\|\vec{w}\|$ ($\|\vec{w}\|$ is the norm of vector $\vec{w}$). This can be formulated as a Quadratic Programming (QP) problem, by minimizing Eq. (2) under constraint (3). Minimize:

$$\|\vec{w}\|^2 \tag{2}$$

Subject to:

$$y_i(\vec{x}_i.\vec{w} + b) - 1 \geq 0, \quad \forall i \tag{3}$$

The solution to this problem can be obtained through the Lagrange multiplier.

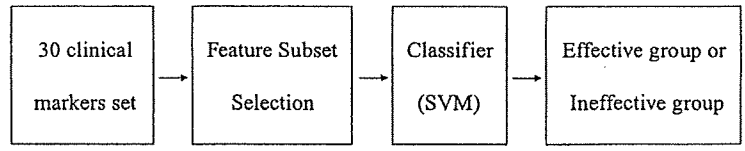

Fig. 1 Predictor model

Table 2 Results of SVM

Dim	SV	Overall ratio of discrimination [%]	Ineffective group ($n = 46$) Errors	Ineffective group ($n = 46$) Ratio of discrimination [%]	Effective group ($n = 66$) Errors	Effective group ($n = 66$) Ratio of discrimination [%]
5	70	84	8	83	10	85
10	76	76	18	61	9	86
15	88	81	12	74	9	86
20	85	82	11	76	9	86
25	89	81	13	72	8	88
30	106	78	14	70	11	83

Note. SV (Support Vectors): it shows how many is the support vectors after the training phase completed.

In this study, due to the complexity of the data, we used a non-linear SVM. To work with non-linear problem, the example $\vec{x}$ is mapped onto a higher dimensional feature space by a mapping function $\Phi(\vec{x})$. By this transformation, both groups will become linearly separable in the new feature space. The training phase in SVM is conducted based on the optimization problem as mentioned in the linear SVM. The computation in this optimization must calculate the dot product of two examples in the new feature space, which is denoted by $\Phi(\vec{x}_i).\Phi(\vec{x}_j)$. This computation could be obtained indirectly, without knowing the tranformation function Φ. This strategy is called the *Kernel Trick*. Instead of computing the dot product in the new feature space, it is possible to use the following kernel function, as given by Eq. (4):

$$K(\vec{x}_i, \vec{x}_j) = \Phi(\vec{x}_i).\Phi(\vec{x}_j) \tag{4}$$

The experiments in this study used the Gaussian kernel. The decision function of test sample $\vec{x}$ by non-linear SVM is obtained as follows:

$$f(\Phi(\vec{x})) = \sum_{i=1,\vec{x}_i \in SV}^{l} \alpha_i y_i K(\vec{x}, \vec{x}_i) + b \tag{5}$$

α_i is the Lagrange multiplier corresponding to example $\vec{x}_i$ that takes zero or positive values. SV (Support Vectors) is the subset of training set $\vec{x}_i$ with corresponding $\alpha_i \neq 0$.

In this paper, the classification accuracy of SVM was tested by the leave-one-out cross validation (LOO-CV) method, which can be applied when the samples are small. The procedure consists of picking up one example for testing while the rest of the data are used to train the classifiers, and then testing the removed example. After testing, the classification result is recorded. The process is repeated until all examples have been tested. This method estimates the generalization error of the classifier with a set of tuning parameter β, The tuning parameter set β consisted of : the number of features, and two SVM parameters (soft margin parameter C and Gaussian Kernel function σ). We attempted to evaluate several combination of these three parameters to obtain the best combination for the model. When it is applied to an independent test sample. The final score is obtained by taking the average of the classification rate of each part:

$$\text{LOO} - \text{CV}(\beta) = \frac{1}{l}\sum_{i=1}^{l} \text{correct\_classif\_rate(i, } \beta) \tag{6}$$

In each part of LOO-CV, Fisher Criterion—based Feature Subset Selection (FSS) was applied to the training set, and the significance of the features is ranked based on the score defined by Eq. (1). A number of top-ranked features were selected and used for training by SVM.

We have also attempted the same experiments using *k*-Nearest Neighbour (k-NN) [9] Classifier for comparison with SVM. To classify one example using k-Nearest Neighbour Classifier, first we measure the distance (e.g. Euclidean Distance) between the example from the test set and the whole data of the training set. A number of k examples with the smallest distance are chosen. These data is the nearest neighbours to the test example. The classification of test sample is made by examining the class on the selected k nearest neighbours and taking a vote.

Results

In SVM experiment, When 5, 10, 15, 20, 25 and 30 features were selected, the best identification rate of each dimensionality was presented in Table 2. When five features are selected, the result was optimal: overall identification rate of 84%, identification rate of 85% for the effective group, and identification rate of 83% for the ineffective group.

In *k*-Nearest Neighbor Classifier (k-NN) experiment. When 5, 10, 15, 20, 25 and 30 features are selected, the optimal identification rate by k-NN of each dimensionality is presented in Table 3. The optimal overall identification rate was 81%.

In the study, the rank of the features was further analyzed in each part of the leave-one-out test. The total score of significance is simply defined by the sum of the rank of each feature in each part. The lower score shows the greater the contribution of the feature to the classification task.

Table 3 Results of K-NN

Dim	Overall ratio of Discrimination [%]	Ineffective group ($n = 46$) Errors	Ineffective group ($n = 46$) Ratio of Discrimination [%]	Effective group ($n = 66$) Errors	Effective group ($n = 66$) Ratio of Discrimination [%]
5	81	12	74	9	86
10	78	12	74	13	80
15	69	20	56	15	77
20	71	18	61	15	77
25	74	18	61	11	83
30	71	18	61	14	79

HCV-RNA level, hepatobiopsy, HCV genotype, ALP and CHE are chosen in the top rank of the features. The list of significance of the features is presented in Table 4.

Discussion

The hepatitis C virus (HCV) is one of the main reasons for the human hepatitis virus [21]. Hepatitis C readily tends to become chronic and develop into liver cirrhosis and liver cancer. At present, there are about 170,000,000 HCV-infected people, and this number increases by 1/100,000 to 3/100,000 each year [11]. Once they are ill, about 80% will become chronic [12, 13]. Interferon is the most effective drug to treat CHC, but it is very expensive and has various side effects. Therefore, the early prediction of interferon treatment efficacy is very important.

During interferon treatment, the factors that affect its treatment efficacy include HCV-RNA level, HCV genotype, liver damage level, IFN dosage, and treatment interval, among which the HCV-RNA level and HCV genotype are the most important. Many studies reported that a low HCV-RNA level before treatment can be an index for predicting effective of interferon treatment, Interferon treatment efficacy is better for low HCV-RNA patients [14, 24]. Referring to the influence of IFN treatment efficacy by HCV genotype, some research has demonstrated that 1b type efficacy is not as good as those of 1a, 2a, 2b and 3 types [15–18]. The response rate of HCV-1b patients to α—IFN is only 20%–40% whereas that of HCV-2a reaches 70%–80% [19]. As to the liver damage level, the IFN treatment efficacy for patients with liver cirrhosis or obvious fibrosis clearly decreases. Interferon treatment efficacy is better for mild chronic hepatitis, i.e. no fibrosis or liver cirrhosis [20]. In treatment, some studies show that increasing the dosage and treatment course can help eradicate the virus and promote the IFN treatment efficacy [22, 23], The joint use of IFN and ribavirin has cooperative action and better treatment efficacy.

Table 4 shows the list of significance of the features, of which the HCV-RNA level, hepatobiopsy and HCV genotype are the top-ranked features, in agreement with clinical opinion. Treatment interval and ribavirin were expected to rank higher, but it was not reflected in the available dataset. In this study, several types of interferon were used. The interferon dosage cannot be determined each time in comparison with the treatment efficacy, because the patients were not treated with the same type of interferon. Therefore, the total interferon dosage each time is not included in the analysis.

Table 3 and Table 2 show that the difference of overall identification rate between five features and 30 features in k-Nearest Neighbor Classifier is 10%, while that of SVM is 6%. The difference of overall identification rate in SVM is less than k-Nearest Neighbour. This result shows that SVM is more robust to the existance of irrelevant features, rather than k-Nearest Neighbour Classifier. This is because SVM works by mapping the data into new higher dimensional feature space, that makes it easier in finding the discrimination hyperplane on the new space. The results of SVM and k-NN

Table 4 List of significance of the features

Rank	Features	Rank	Features	Rank	Features
1	HCV-RNA level	11	A/G	21	Total Cholesterol
2	Hepatobiopsy	12	Treatment interval (weeks)	22	APTT (hours)
3	HCV genotype	13	GPT	23	PT (hours)
4	ALP	14	HPT (hours)	24	Hb
5	CHE	15	PT (%)	25	LAP
6	GOT	16	Sex	26	Total Protein
7	Platelet	17	Ribavirin	27	Lymphocyte
8	GOT/GPT	18	APTT (%)	28	γ-GTP
9	LDH	19	Total Bilirubin	29	HPT (%)
10	Age	20	WBC	30	RBC

Note. 1 ~ 30 denotes significance rank of the features. 1 represents the most significance.

clearly illustrated that the SVM performance was better than that of k-NN.

We observed identification rate of SVM if one of the features is removed. Accordingly, we did the same experiments by using the best 4 features. The result obtained by leave one out cross validation scheme showed that the performance of SVM was overall identification rate of 82 % (identification rate of 85% for the effective group, and identification rate of 78% for the ineffective group, SV: 87 samples). This result showed that taking off one marker worsened the performance of SVM. From this result we concluded that five markers are significant to discriminate the interferon efficacy.

At present, only a few studies have predicted the efficacy of the interferon treatment for CHC patients. In the present study, a different method, which is a combination of the Feature Subset Selection (FSS) and the Support Vector Machine (SVM) was used. System performance was estimated by leave-one-out cross validation. And a higher identification rate of 85% for the effective group, and 83% of for the ineffective group by using the top 5 ranked features was obtained.

In this study, because of the small sample, the reliability of the features selected was limited, Future issues to be addressed include establishing larger clinical databases, reducing the number of support vectors, and finding a better way to assign significance rank to the features for evaluation, in order to obtain the best outcome.

Conclusion

This study clearly showed that a simple model consisting of 5 clinical data with FSS-SVM could identify CHC patients with interferon treatment efficacy (effective group and ineffective group) with a higher degree of accuracy. Thus, the application of this model can be a useful reference for doctors when making decisions regarding interferon treatment.

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Original Article

Assessment of nutritional status of patients with hepatitis C virus-related liver cirrhosis

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Aim: Nutrition support for patients with liver cirrhosis, such as late evening snacks and branched-chain amino acids, has been demonstrated to be effective. However, the assessment of the malnutrition of liver cirrhosis is still a problem. The aim of this study was to assess the nutritional status of patients with liver cirrhosis due to hepatitis C virus by six methods and to test the sensitivity and specificity of these methods.

Methods: In total, 86 patients with liver cirrhosis due to hepatitis C virus were assessed for nutritional status by triceps skinfold thickness (TSF), arm muscle circumference (AMC), subjective global assessment (SGA), nutritional risk index (NRI), Maastricht index (MI), and instant nutritional assessment (INA).

Results: Malnutrition was found in 11 (12.8%) patients by TSF, 15 (17.4%) by AMC, 22 (25.6%) by SGA, 52 (60.5%) by the NRI, 66 (76.7%) by the MI, and in 54 (62.8%) by INA. The MI detected malnutrition at a significantly higher rate compared with the other five methods. Sixty-two patients were diagnosed as malnourished by the combined index, which defines the patients as malnourished when any two of the NRI, MI, and INA also define them as malnourished. The misclassification rate compared with the combined indexes was significantly lower in the MI (4.7%) than in any of the TSF (59.3%), AMC (59.3%), SGA (46.5%), NRI (16.3%), and INA (14.0%).

Conclusion: The MI was the best single score to identify the patients who had malnutrition, including early stage, and may benefit from nutrition support.

Key words: cirrhosis, malnutrition, hepatitis C virus, Maastricht index, nutritional risk index, instant nutritional assessment

INTRODUCTION

PATIENTS WITH LIVER diseases often suffer from malnutrition because of reduced nutrient intake or impaired metabolism in liver.[1,2] Malnourished patients with liver diseases have been recognized as being at greater risk for increased mortality and postoperative complications.[3-5] In cirrhotic patients, both nutrient intake and metabolism are likely to be impaired. Some parameters used for the nutrition assessment are influenced by liver disease and its complications, and the others are influenced by nutrient intake, although it is difficult to separate these two groups of parameters completely. Thus accurate assessments of nutritional status are not easily obtained in patients with cirrhosis,[6,7] making it difficult to identify those at risk for malnutrition and to evaluate the need and efficacy of nutritional intervention.

Nutritional approaches, such as late evening snacks (LES)[8] and enteral branched chain amino acid (BCAA) supplementation,[9] have been demonstrated to be effective for malnutrition of liver cirrhosis. It should be elucidated what percentages of the patients with liver cirrhosis suffer from malnutrition, and benefit from these approaches. The methods to assess the nutritional status of patients with liver cirrhosis are needed to apply these approaches.

There have been many suggested methods to assess the nutritional status. In the present study, six methods, triceps skinfold thickness (TSF),[7] arm muscle circumference (AMC),[6] subjective global assessment (SGA),[10] nutritional risk index (NRI),[11] Maastricht index (MI),[12]

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Table 1 Characteristics of 86 patients with liver cirrhosis by hepatitis C virus

Variables	All patients
Sex (male/female)	46/40
Age (years)	65 ± 8
Serum albumin (g/dL; 4.0–5.0)	3.7 ± 0.6
Serum total cholesterol (mg/dL; 128–219)	149 ± 32
Serum cholinesterase (IU/l; 3500–6900)	2854 ± 1155
Serum total bilirubin (mg/dL; 0.3–1.2)	1.3 ± 0.7
Serum creatinine (mg/dL; 0.4–0.7)	0.7 ± 0.2
Serum pre-albumin (mg/dL; 22–40)	13 ± 6
Branched chain amino acids/tyrosine ratio (5.0–9.5)	4.07 ± 1.51
Prothrombin time (%; 80–135)	78 ± 12
Ammonia (μg/dL; 35–100)	54 ± 31
White blood cell count (cells/μL; 4000–9400)	4109 ± 1547
Blood platelet count (×10⁴/μL; 15.3–35.0)	9.2 ± 3.7
Blood lymphocyte count (cells/μL; 1500–4000)	1423 ± 636
Body mass index	23.2 ± 3.7
Triceps skinfold thickness (mm)	14.9 ± 8.4
Arm muscle circumference (cm)	22.4 ± 2.8
Child–Pugh classification (A/B/C)	61/19/6
Presence of hepatocellular carcinoma (+/−)	31/55
Presence o f diabetes mellitus (+/−)	31/55

and instant nutritional assessment (INA),[13] were applied to assess the nutritional status of patients with liver cirrhosis due to hepatitis C virus (HCV), and their sensitivity and specificity were tested.

METHODS

Patients

EIGHTY-SIX PATIENTS with liver cirrhosis due to HCV were analyzed (Table 1). Cirrhosis was diagnosed by ultrasound sonography or computed tomography. All patients were positive for HCV RNA in serum. Thirty-one patients had hepatocellular carcinoma. Thirty-one patients had diabetes mellitus. This study was approved by the local committee on medical ethics and adhered to the guidelines of the 1975 Declaration of Helsinki.

Nutritional assessment

The nutritional assessment was performed by TSF, AMC, SGA, the NRI, MI, and INA.

In the assessment of TSF and AMC, malnutrition was diagnosed when the measurement was below the fifth percentile.

SGA was performed by a trained nutritionist using a standard questionnaire concerning food intake and complements, such as recent body-weight changes, gastrointestinal symptoms, edema, ascites, dehydration, and functional capacity. On the basis of these data, the nutritionist classified the patients as not, moderately, or severely malnourished.

The NRI is derived from the serum albumin concentration and the ratio of actual to usual weight using the equation:

$$\text{NRI} = (1.519 \times \text{serum albumin [g/dL]}) + 41.7 \times (\text{present weight/usual weight}).$$

A NRI >100 indicates that the patient is not malnourished, 97.5–100 is mildly malnourished, 83.5–<97.5 is moderately malnourished, and <83.5 is severely malnourished. The usual weight was defined as the stable weight 6 or more months before.

The MI uses serum albumin, serum pre-albumin, blood lymphocyte count, and percentage of ideal weight; a positive value indicates malnutrition with the following equation:

$$\text{MI} = 20.68 - (2.4 \times \text{serum albumin [g/dL]}) - (0.1921 \times \text{pre-albumin [mg/dL]}) - (0.00186 \times \text{blood lymphocyte count [cells/mm}^3\text{]}) - (4 \times [\text{present weight/ideal weight}]).$$

Ideal weight was determined by the equation:

$$\text{Ideal weight} = 22 \times (\text{height [m]}^2).$$

We defined a MI of >0–3 as mildly malnourished, a MI of >3–6 as moderately malnourished, and a MI of >6 as severely malnourished, although the degree of malnutrition was not defined in the original report.[12]

INA uses serum albumin and blood lymphocyte count for nutritional assessment. On the basis of these data, patients were classified in four degrees of nutritional state: first degree (serum albumin ≥3.5 g/dL; blood lymphocyte count ≥1500 cells/mm³), second degree (serum albumin ≥3.5 g/dL; blood lymphocyte count <1500 cells/mm³), third degree (serum albumin <3.5 g/dL; blood lymphocyte count ≥1500 cells/mm³), and fourth degree (serum albumin <3.5 g/dL; blood lymphocyte count <1500 cells/mm³). Second, third, and fourth degrees were considered as malnourished.

The results of the NRI, MI, and INA were merged into a single combined index. The patients were considered to be malnourished in the combined index if they were

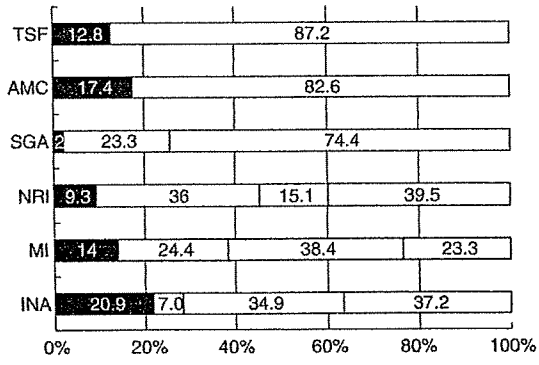

Figure 1 Assessment of malnutrition by six different methods. Number in each bar is a percentage of patients diagnosed as respective nutritional grade. By triceps skinfold thickness (TSF) and arm muscle circumference (AMC), the patients were diagnosed into only two grades: no malnutrition and malnourished. By subjective global assessment (SGA), the patients were diagnosed into three grades: no malnutrition, moderate malnutrition, and severe malnutrition. By nutritional risk index (NRI), Maastricht index (MI), and instant nutritional assessment (INA), the patients were diagnosed into four grades: (□) no malnutrition, (□) mild malnutrition, (□) moderate malnutrition, (■) and severe malnutrition.

malnourished to any degree according to at least any two of the NRI, MI, and INA.

Statistical analyses

The statistical analyses were done by χ^2-test and Fisher's exact test. A *P*-value of less than 0.05 was considered significant. When a multiple comparison was performed, statistical analyses were done by Kruskal–Wallis rank sum test and Bonferroni's methods, in which a *P*-value of less than 0.0083 was considered significant.

RESULTS

Assessment of malnutrition

MALNUTRITION WAS FOUND in 11 patients (12.8%) by TSF, in 15 (17.4%) by AMC, in 22 (25.6%) by SGA, in 52 (60.5%) by the NRI, in 66 (76.7%) by the MI, and in 54 (62.8%) by INA (Fig. 1). The MI detected malnutrition at a significantly higher rate compared with the other five methods; TSF ($P < 0.0001$), AMC ($P < 0.0001$), SGA ($P < 0.0001$), the NRI ($P = 0.0214$), and INA ($P = 0.0463$).

Statistical evaluation of nutritional scores according to the combined index

Sixty-two patients (72.1%) who were defined as malnourished by two of the NRI, MI, and INA were considered malnourished by the combined index. The misclassification rate was 59.3% in TSF, 59.3% in AMC, 46.5% in SGA, 16.3% in the NRI, 4.7% in MI, and 14.0% in INA, when the results were compared with that of the combined index (Table 2). The misclassification rate was significantly lower in the MI than the other five methods: TSF ($P < 0.0001$), AMC ($P < 0.0001$), SGA ($P < 0.0001$), the NRI ($P = 0.0127$), and INA ($P = 0.0357$).

Comparison of nutritional assessment by MI with the other five methods

The patients diagnosed as malnourished by the MI tended to be more frequently diagnosed as malnourished by TSF (17%) than those diagnosed as no malnutrition by the MI (0%; $P = 0.0606$; Table 3).

The patients diagnosed as suffering from moderate or severe malnutrition by the MI tended to be more frequently diagnosed as malnourished by AMC (27%)

Table 2 Statistical evaluation of nutritional scores according to combined index

	TSF	AMC	SGA	NRI	MI	INA
Sensitivity	17.7%	21.0%	35.5%	80.6%	100%	83.9%
Specificity	100%	91.7%	100%	91.7%	83.3%	91.7%
Positive predictive value	100%	86.7%	100%	96.2%	93.9%	96.3%
Negative predictive value	32.0%	31.0%	37.5%	64.7%	100%	68.8%
False positive	0	2	0	2	4	2
False negative	51	49	40	12	0	10
Misclassification	59.3%	59.3%	46.5%	16.3%	4.7%	14.0%

AMC, arm muscle circumference; INA, instant nutritional assessment; MI, Maastricht index; NRI, nutritional risk index; SGA, subjective global assessment; TSF, triceps skinfold thickness.

Table 3 Comparison of nutritional assessment by Maastricht index (MI) with other five methods

	No malnutrition	Malnutrition		
		Mild	Moderate	Severe
No. patients	20	33	21	12
TSF below the fifth percentile†	0 (0%)	6 (18%)	4 (19%)	1 (8%)
AMC below the fifth percentile‡	2 (10%)	4 (12%)	5 (24%)	4 (33%)
SGA (not/moderately/severely malnourished)§	20/0/0	21/12/0	17/3/1	6/5/1
NRI (not/mildly/moderately/severely malnourished)¶	18/2/0/0	15/8/10/0	1/3/16/1	0/0/5/7
INA (not/mildly/moderately/severely malnourished)††	18/2/0/0	14/15/4/0	0/12/2/7	0/1/0/11

†Malnutrition rate assessed by triceps skinfold thickness (TSF) tended to be higher in the patients diagnosed as malnutrition by the MI (17%) than in those diagnosed as no malnutrition (0%; $P = 0.0606$). ‡Patients diagnosed as moderate or severe malnutrition by the MI tended to be more frequently diagnosed as malnourished by arm muscle circumference (AMC; 27%) than those diagnosed as no or mild malnutrition by the MI (11%; $P = 0.0580$). §Patients diagnosed as malnourished by the MI were significantly more frequently diagnosed as malnourished by subjective global assessment (SGA; 33%) than those diagnosed as no malnutrition by the MI (0%; $P = 0.0028$). ¶Severer malnutrition assessed by the nutritional risk index (NRI) was significantly less prevalent in the patients with no malnutrition than in those with mild malnutrition ($P = 0.0034$), in those with mild malnutrition than in those with moderate malnutrition ($P = 0.0018$), and in those with moderate malnutrition than in those with severe malnutrition ($P = 0.0052$) assessed by the MI. ††Severer malnutrition assessed by , instant nutritional assessment (INA) was significantly less prevalent in the patients with no malnutrition than in those with mild malnutrition ($P = 0.0025$), in those with mild malnutrition than in those with moderate malnutrition ($P = 0.0001$), and in those with moderate malnutrition than in those with severe malnutrition ($P = 0.0052$) assessed by the MI.

than those diagnosed as having no or mild malnutrition by the MI (11%) ($P = 0.0580$).

The patients diagnosed as malnourished by the MI were significantly more frequently diagnosed as malnourished by SGA (33%) than those diagnosed as no malnutrition by the MI (0%; $P = 0.0028$).

The severer malnutrition assessed by the NRI was significantly less prevalent in the patients with milder malnutrition than in those with severer malnutrition assessed by the MI (no *vs* mild, $P = 0.0034$; mild *vs* moderate, $P = 0.0018$; moderate *vs* severe, $P = 0.0052$).

The severer malnutrition assessed by INA was significantly less prevalent in the patients with milder malnutrition than in those with severer malnutrition assessed by the MI (no *vs* mild, $P = 0.0025$; mild *vs* moderate, $P = 0.0001$; moderate *vs* severe, $P = 0.0052$).

Comparison of various parameters among different nutritional status assessed by MI

Serum albumin was significantly higher in the patients with milder malnutrition than in those with severer malnutrition assessed by the MI (no *vs* mild, $P = 0.0001$; mild *vs* moderate, $P < 0.0001$; moderate *vs* severe, $P < 0.0001$; Table 4).

Serum pre-albumin was significantly higher in the patients with milder malnutrition than in those with severer malnutrition assessed by the MI (no *vs* mild, $P < 0.0001$; moderate *vs* severe, $P = 0.0014$).

The BCAA/tyrosine ratio was significantly higher in the patients with milder malnutrition than in those with severer malnutrition assessed by the MI (no *vs* mild, $P = 0.0080$; mild *vs* moderate, $P = 0.0039$).

The blood lymphocyte count was significantly higher in the patients with milder malnutrition than in those with severer malnutrition assessed by the MI (no *vs* mild, $P = 0.0009$; mild *vs* moderate, $P < 0.0001$).

Serum cholinesterase, white blood cell count, blood platelet count, and body mass index were significantly higher in the patients with no malnutrition than in those with mild malnutrition ($P < 0.0001$, $P < 0.0001$, $P = 0.0029$, and $P = 0.0059$, respectively).

Prothrombin time was significantly higher in the patients with mild malnutrition than in those with moderate malnutrition ($P = 0.0019$).

The higher grade of the Child–Pugh classification was significantly less prevalent in the patients with mild malnutrition than in those with moderate malnutrition ($P = 0.0002$).

Receiver operator characteristics curve analysis

The receiver operator characteristics (ROC) curve analysis was applied to compare the performance of detecting the malnutrition of the MI, serum albumin, serum total cholesterol, serum cholinesterase, serum total bilirubin, serum creatinine, serum pre-albumin, BCAA/tyrosine

Table 4 Characteristics of four groups of patients with different grades of nutrition assessed by Maastricht index (MI)

	No malnutrition	Malnutrition		
		Mild	Moderate	Severe
No. patients	20	33	21	12
Sex (male/female)	12/8	22/11	8/13	4/8
Age (years)	65 ± 9	67 ± 7	65 ± 9	65 ± 11
Serum albumin (g/dL; 4.0–5.0)	4.3 ± 0.3[a]	3.9 ± 0.3[a]	3.4 ± 0.3[a]	2.8 ± 0.3[a]
Serum total cholesterol (mg/d; 128–219 L)	162 ± 27	154 ± 29	141 ± 30	131 ± 41
Serum cholinesterase (IU/l; 3500–6900)	4166 ± 731[b]	2833 ± 914[b]	2314 ± 806	1884 ± 887
Serum total bilirubin (mg/dL; 0.3–1.2)	1.1 ± 0.7	1.1 ± 0.4	1.4 ± 0.9	1.9 ± 0.9
Serum creatinine (mg/d; 0.4–0.7)	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2
Serum pre-albumin (mg/dL; 22–40)	19 ± 4[c]	13 ± 4[c]	10 ± 3[c]	6 ± 2[c]
Branched chain amino acids/tyrosine ratio (5.0–9.5)	5.3 ± 1.7[d]	4.4 ± 1.2[d]	3.4 ± 0.9[d]	2.6 ± 0.8
Prothrombin time (%; 80–135)	85 ± 6	83 ± 9[e]	74 ± 10[e]	66 ± 1
Ammonia (μg/dL; 35–100)	43 ± 14	51 ± 27	63 ± 43	65 ± 29
White blood cell count (cells/μL; 4000–9400)	5695 ± 1613[f]	3991 ± 1043[f]	3362 ± 1211	3208 ± 1382
Blood platelet count (× 10^4/μL; 15.3–35.0)	12.1 ± 3.7[g]	9.2 ± 3.0[g]	7.8 ± 3.3	6.8 ± 3.1
Blood lymphocyte count (cells/μL; 1500–4000)	2035 ± 556[h]	1581 ± 520[h]	1035 ± 335[h]	760 ± 270
Body mass index	25 ± 3[i]	22 ± 3[i]	23 ± 5	23 ± 3
Child–Pugh classification (A/B/C)	19/1/0	31/2/0[j]	9/11/1[j]	2/5/5
Presence of hepatocellular carcinoma (+/−)	4/16	13/20	9/12	5/7
Presence o f diabetes mellitus (+/−)	8/12	11/22	7/14	5/7

[a]Serum albumin was significantly higher in the patients with milder malnutrition than in those with severer malnutrition assessed by the MI (no *vs* mild, $P = 0.0001$; mild *vs* moderate, $P < 0.0001$; moderate *vs* severe, $P < 0.0001$). [b]Serum cholinesterase was significantly higher in the patients with no malnutrition than in those with mild malnutrition ($P < 0.0001$). [c]Serum pre-albumin was significantly higher in the patients with milder malnutrition than in those with severer malnutrition assessed by the MI (no *vs* mild, $P < 0.0001$; moderate *vs* severe, $P = 0.0014$). [d]BCAA/tyrosine ratio was significantly higher in the patients with milder malnutrition than in those with severer malnutrition assessed by the MI (no *vs* mild, $P = 0.0080$; mild *vs* moderate, $P = 0.0039$). [e]Prothrombin time was significantly higher in the patients with mild malnutrition than in those with moderate malnutrition ($P = 0.0019$). [f]White blood cell count was significantly higher in the patients with no malnutrition than in those with mild malnutrition ($P < 0.0001$). [g]Blood platelet count was significantly higher in the patients with no malnutrition than in those with mild malnutrition ($P = 0.0029$). [h]Blood lymphocyte count was significantly higher in the patients with milder malnutrition than in those with severer malnutrition assessed by the MI (no *vs* mild, $P = 0.0009$; mild *vs* moderate, $P < 0.0001$). [i]Body mass index was significantly higher in the patients with no malnutrition than in those with mild malnutrition ($P = 0.0059$). [j]Higher grade of Child–Pugh classification was significantly less prevalent in the patients with mild malnutrition than in those with moderate malnutrition ($P = 0.0002$).

ratio, prothrombin time, ammonia, white blood cell count, blood platelet count, blood lymphocyte count, and body mass index using the combined index as the standard. The area under the ROC curve of the MI (0.96) was the largest. Serum albumin (0.90), serum cholinesterase (0.88), blood lymphocyte count (0.85), white blood cell count (0.82), blood platelet count (0.80), serum pre-albumin (0.80), BCAA/tyrosine ratio (0.77), and prothrombin time (0.74) followed.

DISCUSSION

SINGLE OBJECTIVE ASSESSMENT variables, such as percentage ideal body weight or serum albumin are not appropriate for the assessing nutritional status in patients with cirrhosis because of the innate confounding effects of fluid retention and alterations in protein metabolism.[14–16] Composite methods of assessment that include multiple appropriate variables and obviate the problems in the use of single techniques, thereby increasing the accuracy of the assessment, are needed to optimize nutritional management in patients with cirrhosis.

Some variables used for nutritional assessment are influenced by liver disease and its complications, and the others are influenced by nutrient intake. It is difficult to separate these two groups of variables completely. Thus, accurate assessments of nutritional status are not easily obtained in patients with cirrhosis.[6,7] Alberino *et al.* found that malnutrition, defined using percentile thresholds for AMC and/or TSF, was an independent predictor of survival.[4] SGA was used for assessing

patients with liver disease in categorizing risk groups with some success.[17,18] In the present study, malnutrition was found in 13% by TSF, in 17% by AMC, and in 26% by SGA. The MI, NRI, or INA, which had not been used for assessing nutritional status in cirrhotic patients, detected malnutrition at higher rates: 77%, 61%, and 63%, respectively.

The MI detected malnutrition at the highest rate among the six methods. The misclassification rate of the MI in comparison with the combined index was lowest among the six methods. Thus, the MI was the best single score to identify patients who had malnutrition and who may benefit from nutrition support. The MI was developed by comparing objective nutritional variables in 50 patients selected for parenteral nutrition, with the same variables in 38 patients selected for elective minor surgical procedures in Maastricht, the Netherlands.[12] The severity of malnutrition of the non-surgical patients assessed by the MI predicted the occurrence of complications during their hospital stay.[19] The present study did not deal with the outcome of the patients, which is needed to assess whether the cirrhotic patients diagnosed as malnourished by the MI will have the worst outcome.

Figueiredo *et al.* reported a high rate of malnutrition in cirrhotic patients by body composition analysis; 34% in Child A, 69% in B, and 94% in C.[20] In the present study, the MI detected an even higher rate of malnutrition: 69% in Child–Pugh A, 95% in B, and 100% in C. The high rate of malnutrition assessed by the MI is probably the result of detecting milder malnutrition in early stage of cirrhosis. The malnutrition in the patients with cirrhosis caused by HCV usually progressed, unless HCV was eradicated by interferon treatment. Thus, it is important to detect the patients with early stage of malnutrition and to start the treatment, such as LES and enteral BCAA administration, to prevent the progression of malnutrition.[21] It is necessary to study whether the nutritional intervention for the patients with early-stage malnutrition prevents the further progression of malnutrition.

The MI uses blood lymphocyte count, which can be affected by the stage of liver disease and portal hypertension.[22] Thus, there is possible overestimation of the prevalence and degree of malnutrition when the MI is used for the patients with cirrhosis. The blood lymphocyte count also has been known to be decreased by deficient nutrient intake.[23] Further studies are necessary to clarify whether the decreased blood lymphocyte count in cirrhosis is caused by malnutrition and can be restored by nutrition support.

The NRI and INA also detected malnutrition at reasonably high rates, and the results assessed by both methods correlated significantly with that assessed by the MI. The NRI was developed by calculating the association of various nutritional indexes with postoperative complications.[11] INA was developed by assessing parameters associated with markedly increased morbidity and mortality in a series of 500 patients.[13] Thus, it is probable that the patients diagnosed as malnourished by the NRI or INA will have the worst outcome. TSF, AMC, and SGA detected malnutrition at a low rate, and the results of these three methods also correlated with that of the MI.

The MI uses serum albumin, serum pre-albumin, blood lymphocyte count, and percentage of ideal weight. Serum albumin, serum pre-albumin, and blood lymphocyte count significantly correlated with the results of the MI. Serum cholinesterase, BCAA/tyrosine ratio, prothrombin time, white blood cell count, and blood platelet count also partially correlated with the results of the MI. Thus, single variables may be easily used for assessing nutritional status. However, the ROC curve analysis demonstrated that a composite method, such as the MI, is superior for detecting malnutrition to single variables, such as serum albumin or serum cholinesterase. Therefore, the MI is appropriate for the assessment of the nutritional status of cirrhotic patients.

The MI detected malnutrition in 77% of cirrhotic patients due to HCV. The MI was demonstrated to be the best single score to identify the patients who had malnutrition, including early stage, and who would benefit from nutrition support.

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HEPATOLOGY

Efficacy of ribavirin plus interferon-α in patients aged ≥60 years with chronic hepatitis C

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Key words
age group, hepatitis C virus, interferon, ribavirin, sustained virologic response.

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Abstract

Background: In Japan, patients with hepatitis C virus (HCV)-associated liver disease are getting older, and thus the number of deaths due to such disease is increasing. The efficacy of combination therapy with ribavirin and interferon for chronic HCV infection in elderly patients has not been fully clarified. The aim of the present study was to evaluate the efficacy and tolerability of combination therapy in such patients.

Methods: Two hundred and twenty consecutive patients with chronic hepatitis C were treated with combination therapy. These patients were divided into two groups according to age: patients ≥ 60 years ($n = 66$) and patients < 60 years ($n = 154$). Clinical characteristics, the sustained virologic response (SVR) rate obtained by intention-to-treat analysis, and the rate of reduction or discontinuation of ribavirin were compared between the two groups.

Results: The ribavirin discontinuation rate was significantly higher in the patients aged ≥60 years than in the patients aged <60 years. However, the SVR rates did not differ significantly between patients aged ≥60 years and those aged <60 years (31.8% *vs* 38.3% by intention-to-treat analysis). According to multivariate analysis, genotype and HCV viral load were significantly associated with SVR while patient age did not affect SVR.

Conclusions: Treatment of chronic hepatitis C with combination therapy was comparably effective between patients aged ≥60 years and those aged <60 years, although the ribavirin discontinuation rate was higher among the older patients than the younger patients.

Introduction

Hepatitis C virus (HCV) infection is a widespread viral infection that often leads to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. The need for treatment of chronic HCV infection in the elderly is increasing in Japan and is expected to increase in the USA and other Western countries.[1]

Sustained virologic responders who are negative for serum HCV-RNA 6 months after treatment with interferon (IFN) are reported to be likely to remain in virologic and biochemical remission with histologic improvement.[2,3] Moreover, IFN therapy reduces the risk of hepatocellular carcinoma among virologic or biochemical responders.[4–6] Ribavirin is now generally used in combination with IFN for the treatment of chronic hepatitis C, and this therapy has been reported to be more effective than IFN monotherapy, with a higher rate of HCV eradication.[7–10]

Efficacy of IFN monotherapy in elderly patients with chronic hepatitis C has been reported,[11,12] but efficacy of combination ribavirin and IFN therapy in elderly patients has not been established. We retrospectively evaluated the efficacy and tolerability of ribavirin plus interferon in patients aged ≥60 years with chronic hepatitis C.

Methods

Patients

Two hundred and twenty consecutive patients with chronic hepatitis C with a high viral load (we defined high viral load as serum HCV-RNA level >100 KIU) were treated with IFN and ribavirin in combination between January 2002 and April 2003 at 14 institutions: Nagoya University Hospital and affiliated hospitals. One hundred and twenty-two of 220 patients were naïve patients. All met the following inclusion criteria: <75 years old; positivity for anti-HCV antibody; and serum HCV-RNA level > 100 KIU/mL on

Table 1 Patients treated by combination therapy

	Total patients ($n = 220$)	Age <60 years ($n = 154$)	Age ≥60 years ($n = 66$)	*P*
Sex ratio (male/female)	147/73	109/45	38/28	0.0567
Baseline serum ALT (IU/L)	94.0 ± 68.6	92.4 ± 71.4	97.6 ± 62.0	0.6081
Hemoglobin (g/dL)	14.3 ± 1.3	14.5 ± 1.3	13.9 ± 1.4	0.0056
Creatinine clearance (mL/min)	101.6 ± 24.5	106.5 ± 24.6	85.3 ± 15.2	<0.0001
Genotype (1/2/other)	169/50/1	115/38/1	54/12/0	0.4510
HCV-RNA (KIU/mL)	648.7 ± 339.4	638.8 ± 342.3	671.9 ± 333.9	0.5090
Activity (A0/A1/A2/A3)	6/77/63/18	5/55/44/14	1/22/19/4	0.8405
Fibrosis (F0/F1/F2/F3/F4)	8/74/45/26/10	6/54/34/17/7	2/20/11/9/3	0.9199

ALT, alanine aminotransferase; HCV-RNA, hepatitis C virus RNA.

quantitative polymerase chain reaction (PCR) assay (Amplicor Monitor Assay; Roche Molecular Systems, Pleasanton, CA, USA) within 12 weeks preceding the therapeutic period. Exclusion criteria included pretreatment hemoglobin level < 10 g/dL, positivity for serum hepatitis B surface antigen, drug addiction, alcohol abuse, autoimmune hepatitis, primary biliary cirrhosis, coexisting serious psychiatric or medical illness, and pregnancy. To exclude any patient bias, only complete cohorts from each hospital were enrolled. HCV genotypes were determined by PCR with genotype-specific primers.[13,14]

All patients were treated with 6–10 MU IFN-α-2b (Intron A, Schering Plough, Osaka, Japan) daily for 2 weeks, followed by the same dose of IFN three times a week for 22–46 weeks. We conducted 24 weeks of treatment at first. In the last 44 patients treatment duration was elongated to 48 weeks because this produced higher efficacy than 24 weeks of treatment. Oral ribavirin (Rebetol, Schering-Plough, Kenilworth, NJ, USA) was administered for 24 weeks at 600 mg/day for patients who weighed ≤60 kg and at 800 mg/day for those who weighed >60 kg during the treatment period. The dose of ribavirin was reduced by 200 mg/day when the patient's hemoglobin concentration fell below 10 g/dL because of hemolytic anemia induced by the drug. Ribavirin was discontinued when IFN therapy was discontinued. In Japan, combination with interferon and ribavirin therapy was approved for medical insurance coverage in 2001 with a limit in ribavirin administration of up to 24 weeks. Combination therapy with peg-interferon and ribavirin was not approved for medical insurance coverage in Japan until November 2004.

Liver histology

Pretreatment liver biopsy specimens were classified in terms of fibrosis on a scale of F0–F4 (F0, no fibrosis; F1, portal fibrosis without septa; F2, few septa; F3, numerous septa without cirrhosis; F4, cirrhosis) and in terms of necroinflammatory activity on a scale of A0–A3 (A0, no histologic activity; A1, mild activity; A2, moderate activity; A3, severe activity).[15]

Assessment of efficacy

Virologic response was assessed by qualitative HCV-RNA assay with a lower sensitivity limit of 100 copies/mL (Amplicor HCV version 2.0; Roche Molecular Systems). According to the qualitative HCV-RNA results, responses were defined as follows: sustained virologic response (SVR), no HCV-RNA detected at the end of the 24-week follow-up period after completion of treatment; relapse, no HCV-RNA at end of treatment and reappearance of serum HCV-RNA during the 24 week follow-up period; or non-response (NR), persistent positive serum HCV-RNA throughout treatment.

Comparison of characteristics and efficacy of treatment according to age

Patients were divided by age into two groups: those aged ≥60 years ($n = 66$) and those aged <60 years ($n = 154$). Sex ratio, baseline serum alanine aminotransferase level, pretreatment hemoglobin level, creatinine clearance, HCV genotype and viral load, histologic activity and fibrosis were compared between the two groups (Table 1). End-of-treatment virologic response (ETR) rate and SVR rate obtained by intention-to-treat analysis and per-protocol analysis, and the rate of reduction or discontinuation of ribavirin were compared between the two groups (Table 2).

Comparison of treatment efficacy between combination therapy and monotherapy in older patients

We examined efficacy of combination therapy in comparison to that of monotherapy in patients aged ≥60 years. For this purpose, we included as historical controls 257 patients with chronic hepatitis C with a high viral load treated with IFN-α alone. These were 168 men and 89 women aged 18–69 years (mean ± SD, 50.1 ± 9.9 years) treated at Nagoya University Hospital or Ogaki Municipal Hospital from 1989 to 2001. Forty-seven patients out of 257 were >60 years. All patients were treated with 6–10 MU IFN-α daily for 2 weeks, followed by the same dose of IFN-α three times a week for 22–46 weeks.

The study protocol was approved by the ethics committee of each hospital, and written informed consent was obtained from each patient before therapy.

Statistical analysis

Values are expressed as mean ± SD. Between-group differences in mean quantitative values were analyzed using Student's *t*-test, and differences in non-parametric data were analyzed by Mann–Whitney *U*-test. Differences in proportions were tested using χ^2 test. Multiple logistic regression analysis was used to identify factors related to SVR. All statistical analyses were performed

Table 2 Efficacy of combination therapy

	Total patients ($n = 220$) % (n)	Age <60 years ($n = 154$) % (n)	Age ≥60 years ($n = 66$) % (n)	P
SVR rate (intention-to-treat)	36.4 (80/220)	38.3 (59/154)	31.8 (21/66)	0.3589
SVR rate (per-protocol)	43.7 (80/183)	45.0 (59/131)	40.4 (21/52)	0.5671
ETR rate (intention-to-treat)	71.8 (158/220)	71.4 (110/154)	72.7 (48/66)	0.8444
ETR rate (per-protocol)	81.4 (149/183)	79.4 (104/131)	86.5 (45/52)	0.2621
SVR/relapse/NR/discontinuation	80/69/34/37	59/45/27/23	21/24/7/14	0.2834
Ribavirin discontinuation rate	24.5 (54/220)	20.8 (32/154)	33.3 (22/66)	0.0474
Ribavirin dose reduction rate	33.6 (74/220)	29.9 (46/154)	42.4 (28/66)	0.0709
IFN discontinuation rate	16.8 (37/220)	14.9 (23/154)	21.2 (14/66)	0.2540
IFN dose reduction rate	15.9 (35/220)	15.6 (24/154)	16.7 (11/66)	0.8406
Combination therapy discontinuation rate	16.8 (37/220)	14.9 (23/154)	21.2 (14/66)	0.2540

ETR, end of treatment virologic response; IFN, interferon; NR, non-response; SVR, sustained virologic response.

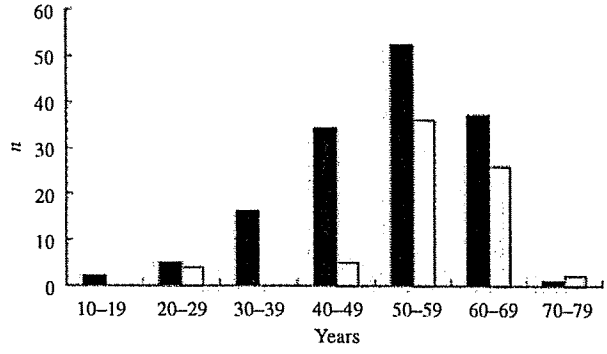

Figure 1 Patient age distribution by decade. (■) Male; (□) female.

using SAS software (SAS Institute, Cary, NC, USA). All P were two-tailed, and $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

Patients were 147 men and 73 women aged 17–71 years (mean ± SD, 53.0 ± 11.1 years). The age distribution of patients treated with combination therapy is shown in Fig. 1. Patients ≥ 60 years comprised 30.0% of the patient population (66/220). The majority of female patients were over age 50 years (87.7%, 64/73). Clinical characteristics of the two study groups are shown in Table 1. The hemoglobin level was significantly lower in patients aged ≥60 years than in patients aged <60 years ($P = 0.0056$). Creatinine clearance in patients aged ≥60 years was worse than that in patients aged <60 years ($P < 0.0001$).

Response to therapy

The ribavirin discontinuation rate was significantly higher in patients aged ≥60 years than in patients aged <60 years ($P = 0.0474$). The dose ribavirin reduction was higher in the patients aged ≥60 years, but the difference did not reach statistical significance (42.4% *vs* 29.9%; $P = 0.0709$). However, the IFN discontinuation and dose reduction rate did not differ significantly between the two groups. The treatment discontinuation rate did not differ significantly between the two groups. As a result, the SVR rate by both intention-to-treat analysis and per-protocol analysis did not differ significantly between the two groups. And ETR rate by both intention-to-treat analysis and per-protocol analysis also did not differ significantly between the two groups (Table 2).

Histologic factor associated with SVR were determined by univariate analysis. The SVR rate of the F0–1 patients was not different from that of the F2–4 patients (49.3% *vs* 47.7%, $P = 0.8490$ by per-protocol analysis; 43.9% *vs* 38.3%, $P = 0.4651$ by intention-to-treat analysis). Factors associated with SVR in combination therapy were determined by multivariate analysis (Table 3). Genotype ($P < 0.0001$, odds ratio 0.074, 95% confidence interval [CI]: 0.030–0.182), and viral load ($P = 0.0002$, odds ratio 1.002, 95%CI: 1.001–1.004]) were significantly associated with SVR, but age was not significantly associated with SVR.

Clinical characteristics of the 66 patients aged ≥60 years who underwent combination therapy and 47 historical control patients aged ≥60 years who underwent monotherapy are shown in Table 4. The SVR rate with combination therapy was significantly higher than that with monotherapy (31.8%, 21/66 *vs* 10.6%, 5/47, $P = 0.0084$ by intention-to-treat analysis; 40.4%, 21/52 *vs* 10.6%, 5/47, $P = 0.0008$ by per-protocol analysis). Treatment discontinuation rate of combination therapy tends to be higher than that of monotherapy, but there was no significant difference between the two groups. This is because the number of patients undergoing monotherapy was small.

Virologic response to combination therapy and to IFN monotherapy in patients with HCV genotype 1 and a high viral load are shown by age group in Fig. 2.

With monotherapy, the SVR rate decreased with age, but with combination therapy, the SVR rates of patients in their 40s, 50s, and 60s and higher were similar. In patients ≥60 years with genotype 1 and a high viral load, the SVR rate with combination therapy was significantly higher than that with monotherapy (27.5% *vs* 6.7%, $P = 0.0322$ by per-protocol analysis).

Virologic responses to combination therapy and to IFN monotherapy in patients with HCV genotype 2 and a high viral load are shown by age group in Fig. 3.

Table 3 Factors associated with SVR to combination therapy ($n = 220$; multivariate analysis)

Variable		Odds ratio (95%CI)	*P*
Sex	Male *vs* female	0.808 (0.365–1.789)	0.5985
Age (years)		1.015 (0.983–1.048)	0.3677
Baseline serum ALT (IU/L)		0.997 (0.992–1.002)	0.1973
Genotype	1 *vs* 2	0.074 (0.030–0.182)	<0.0001
Viral load (KIU/mL)		1.002 (1.001–1.004)	0.0002

ALT, alanine aminotransferase; CI, confidence interval; SVR, sustained virologic response.

Table 4 Treatment efficacy in patients aged ≥60 years

	Combination therapy ($n = 66$)	Monotherapy ($n = 47$)	*P*
Sex ratio (male/female)	38/28	30/17	0.5033
Baseline serum ALT(IU/L)	97.6 ± 62.0	100.0 ± 71.8	0.8536
Genotype (1/2)	54/12	34/13	0.2316
Activity (A0/A1/A2/A3)	1/22/19/4	1/18/25/0	0.1593
Fibrosis (F0/F1/F2/F3/F4)	2/20/11/9/3	2/16/20/7/0	0.1773
SVR rate (intention-to-treat)	31.8 (21/66)	10.6 (5/47)	0.0084
SVR rate (per-protocol)	40.4 (21/52)	10.6 (5/47)	0.0008
SVR/relapse/NR/discontinuation	21/24/7/14	5/23/15/4	<0.0001
Treatment discontinuation rate	21.2 (14/66)	8.5 (4/47)	0.0690

ALT, alanine aminotransferase; NR, non-response; SVR, sustained virologic response.

With combination therapy, the SVR rate was similar for all age groups. In patients ≥60 years with genotype 2 and a high viral load, the SVR rate was significantly higher with combination therapy than with monotherapy (83.3% *vs* 23.1%, $P = 0.0048$ by per-protocol analysis and by intention-to-treat analysis).

Adverse events

For 14 of 74 patients with dose reduction of ribavirin, ribavirin was reduced due to fatigue and anemic symptoms though the hemoglobin levels were above 10 g/dL, which is the level of dose reduction of this study. The combination therapy discontinuation rate was not statistically different between patients aged ≥60 years and those aged <60 years (Table 2). The combination therapy discontinuation rate was higher in combination therapy (21.2%) than in monotherapy (8.5%) among patients aged ≥60 years (Table 4). The reasons for discontinuation of the combination therapy and the times at which the therapy was discontinued are shown in Table 5. If discontinuation of treatment occurred we did not restart therapy after disappearance of the initial symptom or illness. Ribavirin discontinuation was higher in older patients ($P < 0.05$). A serious adverse effect occurred in one patient in each group: infarction of vessel in the retina in the older group and cerebral hemorrhage in the younger group.

Effect of dose reduction and discontinuation of ribavirin or IFN on the SVR rate

Ribavirin dose reduction and discontinuation rates are shown according to age group in Fig. 4. The total of dose reduction and discontinuation rates increased with age. The SVR of patients who completed treatment was 44.7% (51/114). Among patients who had dose reduction, the SVR was 36.5% (19/52). Among patients who discontinued treatment, the SVR was 18.5% (10/54). The SVR was not significantly different between those in whom the dose of ribavirin was reduced and those in whom it was not. Creatinine clearance in patients who needed dose reduction or discontinuation of ribavirin was worse than that in patients who did not (90.2 ± 20.9 mL/min *vs* 107.5 ± 24.2 mL/min, $P < 0.0001$). The SVR in those who completed full treatment was significantly higher than that in those who had reduced-dose IFN (39.5% *vs* 20%, $P = 0.0282$). The SVR in those who completed full treatment was significantly higher than that in those who had discontinued IFN (43.3% *vs* 5%, $P < 0.0001$).

Comparison between 24-week and 48-week treatment

Among the patients with HCV genotype 1, the SVR of 48-week treatment was significantly higher than that of 24-week treatment (48.1% *vs* 24.3%, $P = 0.0148$ by per-protocol analysis; 37.1% *vs* 19.4%, $P = 0.0265$ by intention-to-treat analysis). However, among the patients with HCV genotype 2, the SVR of the 48-week treatment was similar to that of the 24-week treatment (75.0% *vs* 85.0%, $P = 0.4884$ by per-protocol analysis; 75.0% *vs* 81.0%, $P = 0.6997$ by intention-to-treat analysis).

The IFN dose reduction rate for 48-week treatment was significantly higher than that of 24-week treatment (27.3% *vs* 13.1%, $P = 0.0212$). The treatment discontinuation rate for the 48-week course was not statistically different from the 24-week course (20.5% *vs* 17.6%, $P = 0.6621$).

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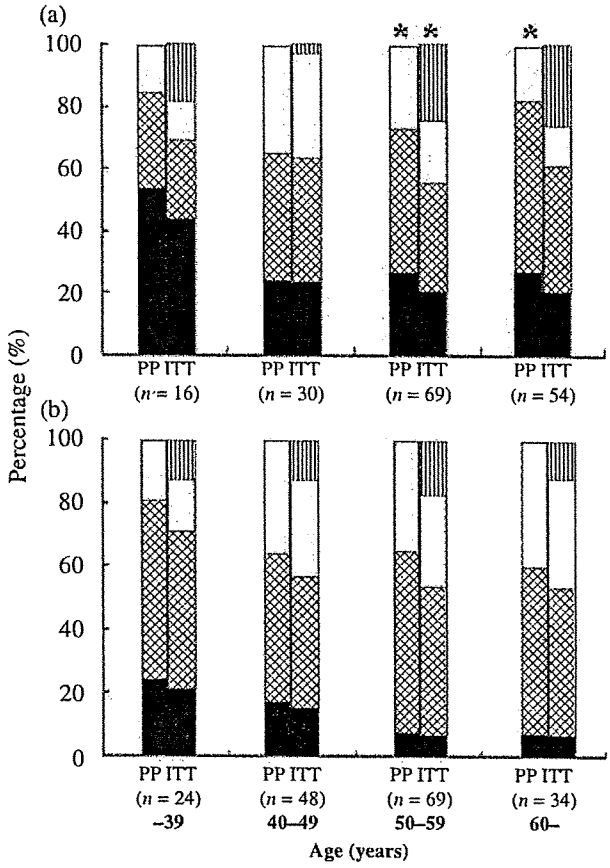

Figure 2 Virologic response to (a) combination therapy and (b) interferon (IFN) monotherapy according to age of patients with genotype 1 and a high viral load. Asterisks indicate significant differences *vs* the respective IFN monotherapy (*$P < 0.05$). (▥) Treatment discontinuation; (□) non-responder; (▨) relapse; (■) sustained virologic response. ITT, intention-to-treat analysis; PP, per-protocol analysis.

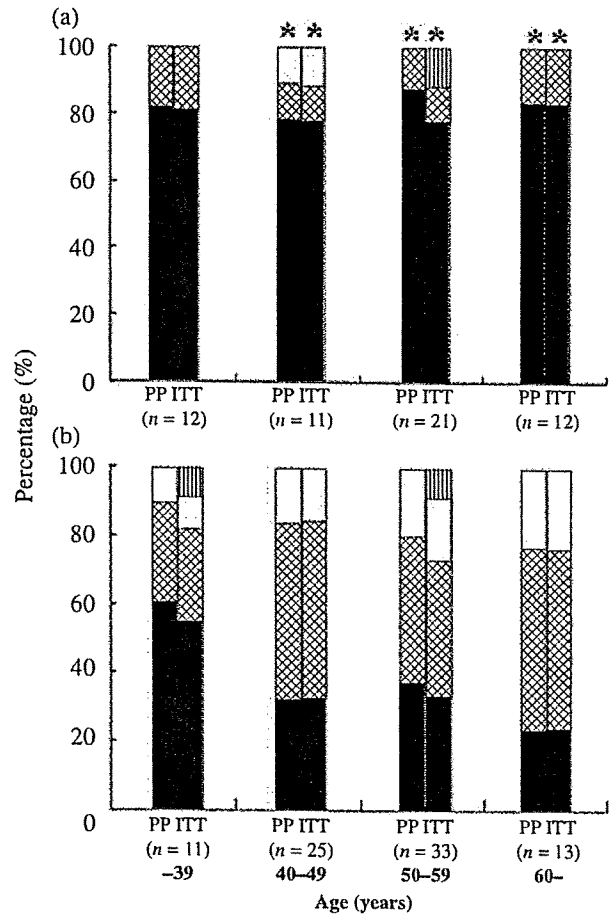

Figure 3 Virologic response to (a) combination therapy and (b) interferon (IFN) monotherapy according to age of patients with genotype 2 and high viral load. Asterisks indicate significant differences *vs* the respective IFN monotherapy (*$P < 0.05$). (▥) Treatment discontinuation; (□) non-responder; (▨) relapse; (■) sustained virologic response. ITT, intention-to-treat analysis; PP, per-protocol analysis.

Discussion

It is important to eradicate HCV by IFN to reduce the risk of hepatocellular carcinoma.[4,5] In addition, IFN reportedly reduces liver-related mortality in chronic hepatitis C patients aged >60 years.[16,17] However, these findings are based on studies of IFN monotherapy. The present study showed the effect of ribavirin and IFN in combination. Ribavirin has been used in combination with IFN to treat chronic hepatitis C, and this combination therapy has been reported to be more effective than IFN monotherapy for eradicating HCV.[7–10] However, ribavirin and IFN or pegylated IFN in combination produce a common adverse effect, that is, hemoglobin levels decrease in 20–36% of treated patients with chronic hepatitis C, necessitating dose reduction or discontinuation.[7,8,18,19]

It has been reported that there is no significant difference in the efficacy of IFN monotherapy between older and younger patients after standardization of their background clinical characteristics, suggesting that age itself does not influence the outcome of IFN monotherapy.[11,12] However, the efficacy and tolerability of combination therapy in the elderly patient has not been clarified. We therefore conducted a multi-institution study to evaluate the efficacy and tolerability of ribavirin plus IFN-α in older patients with chronic hepatitis C.

Multivariate analysis showed baseline viral load and genotype to be the only significant factors associated with SVR. Age was not associated with SVR. Many studies have shown baseline viral load and genotype to be significant factors associated with SVR.[8,19] Our results suggest that the SVR of patients aged ≥60 years is comparable to that of younger patients. Because the SVR differs according to genotype and viral load, we classified patients by genotype and compared the SVR rate for both combination therapy and IFN monotherapy. In patients aged ≥60 years, the SVR rate of combination therapy was significantly increased over that of IFN monotherapy (in patients with genotype 1 and a high

Table 5 Reasons for discontinuation of combination therapy

Patients aged < 60 years			Patients aged ≥ 60 years		
Reason	*n*	Weeks after starting treatment	Reason	*n*	Weeks after starting treatment
Cerebral hemorrhage	**1**	**4**	**Infarction in the retina**	**1**	**14**
Rash	5	1,1,5,22,25	Fatigue	4	4,12,12.14
Fatigue	5	6,12,20,20,21	Anemia	3	10,16, 22
Depression	2	4,10	Anorexia	2	1,19
Anorexia	2	21,23	Nervousness	1	2
Vomiting	1	2	Dizziness	1	6
Anemia	1	4	Vomiting	1	16
Worsening diabetes	1	16	Depression	1	18
Spontaneous pneumothorax	1	17			
Hypothyroidism	1	18			
Uterine cancer	1	20			
Thyroiditis	1	24			
Pancytopenia	1	37			

Bold, serious adverse effect.

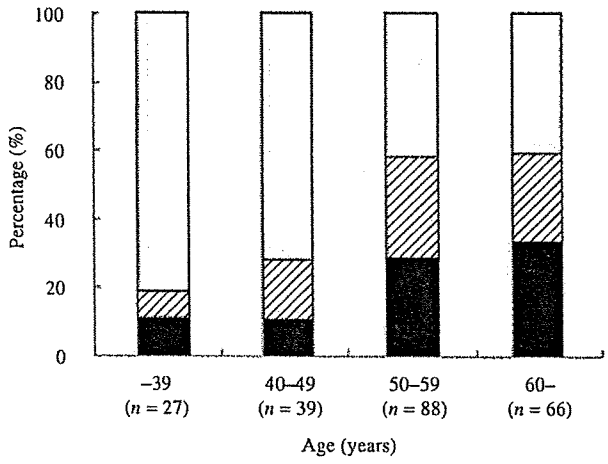

Figure 4 Ribavirin dose reduction and discontinuation rates according to age of patients ($n = 220$). (□) Completion: SVR 44.7% (51/114); (▨) dose reduction: SVR 36.5% (19/52); (■) treatment discontinuation: SVR 18.5% (10/54). SVR, sustained virologic response.

viral load by per-protocol analysis, 27.5% *vs* 6.7%, $P = 0.032$; in patients with genotype 2 and a high viral load by per-protocol analysis, 83.3% *vs* 23.1%, $P = 0.0048$; Figs 2,3). Moreover, the SVR rate among patients aged ≥60 years with HCV genotype 1 did not decrease with age. Neither did the SVR rate change with age for patients ≥60 years with genotype 2. Patients with genotype 2 achieved a high SVR rate of approximately 80% in all age categories. Adverse effects are thought to increase in elderly patients, but adverse effects necessitating discontinuation of IFN and ribavirin did not differ significantly between the older and younger patients (21.2% *vs* 14.9%). In addition, the severe adverse effects were not associated with age. These findings were similar to previously reported findings that there was no difference between young and elderly patients with respect to adverse effects.[11,12] The treatment discontinuation rate tended to be higher in combination therapy (14/66) than in monotherapy (4/47) among patients aged ≥60 years, but there was no significantly difference between the two groups. (Table 4). This is because there was a small number of patients in the monotherapy group. The reason for discontinuation of combination therapy in seven of 14 patients was ribavirin-related adverse effects such as general fatigue or anemia.

The ribavirin dose reduction and discontinuation rates increased with age, but the SVR rate did not differ significantly between patients with and without dose reduction who completed the treatment schedule (36.5% *vs* 44.7%). These findings are consistent with previously reported findings.[19] In patients aged ≥60 years with HCV genotype 1 and a high viral load, the SVR rate did not differ significantly between combination therapy and IFN monotherapy by intention-to-treat analysis, but it did differ significantly by per-protocol analysis. These findings indicate that rather than discontinuing treatment, we should continue as permitted by dose reduction of ribavirin. In groups 50–59 years and >60 years of age the rate of dose reduction and treatment discontinuation was similarly high. In contrast, in groups <50 years of age the rate was low. In the present study we focused on patients aged ≥60 years because 60 years is often used as a cut-off for older patients; if we had focused on patients ≥65 years the number of study patients would have decreased and the comparison would have been difficult. There were high dose-reduction and discontinuation rates in the patients aged ≥50 years, so we should consider dose modification for these patients in advance.

Careful monitoring and appropriate reduction of the ribavirin dose is required to circumvent the need for discontinuation in elderly patients.[20,21] Also, it will be necessary to be careful when treating elderly patients with other diseases commonly observed in this age group, such as diabetes or hypertension. The present study, however, was limited due to being a retrospective analysis and using of historical controls, therefore further prospective studies are needed.

In conclusion, combination therapy was shown to be of comparable efficacy for chronic hepatitis C between patients aged <60 years and those aged ≥60 years, although the rate of ribavirin

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discontinuation was shown to be higher among the older patients than among the younger patients. The efficacy of combination therapy was shown to be greater than that of IFN monotherapy in older patients.

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TNF-α Induces Hepatic Steatosis in Mice by Enhancing Gene Expression of Sterol Regulatory Element Binding Protein-1c (SREBP-1c)

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We investigated the effect of tumor necrosis factor-α (TNF-α), a member of the proinflammatory cytokine family, on steatosis of the mouse liver by analyzing morphological changes and hepatic triglyceride content in response to TNF-α. We also examined expression of the sterol regulatory element binding protein-1c gene. Intraperitoneal injection of TNF-α acutely and dramatically accelerated the accumulation of fat in the liver, as evidenced by histological analysis and hepatic triglyceride content. This treatment increased liver weight, increased serum levels of free fatty acids, and increased fatty acid synthase and sterol regulatory element binding protein-1c mRNA expression. Furthermore, intraperitoneal injection of lipopolysaccaride (LPS) to induce TNF-α expression also accelerated hepatic fat accumulation. Pretreatment with anti-TNF-α antibody attenuated the development of LPS-induced fatty change in the liver. Antibody pretreatment not only decreased sterol regulatory element binding protein-1c expression in LPS-treated mice but also attenuated the expression of suppressors of cytokine signaling-3 mRNA. This study suggests that TNF-α, acting downstream of LPS, increases intrahepatic fat deposition by affecting hepatic lipogenetic metabolism involving sterol regulatory element binding protein-1c. Exp Biol Med 232:614–621, 2007

Key words: TNF-α; LPS; liver; SREBP-1c

Introduction

Proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), have been suggested to cause obesity-related metabolic disorders, including insulin resistance (1). In addition to direct impairment of insulin signaling (2–4), lipotoxicity induced by fat accumulation in the liver also leads to hepatic insulin resistance (5–7). TNF-α may thus affect hepatic lipid metabolism. TNF-α treatment has been shown to accelerate hepatic triglyceride production and hyperlipidemia (8–11). However, the molecular mechanisms underlying TNF-α–induced fatty liver disease are not clear. A previous study suggested that TNF increases triglyceride (TG) production in the liver by providing an increased amount of fatty acids as substrate, since TNF did not increase the activity of triglyceride synthesis enzymes (10).

Fat accumulation in the liver is regulated by a number of lipogenetic and lipolytic factors. In particular, fatty acid synthase (FAS) and sterol regulatory element binding protein-1c (SREBP-1c) play important roles in lipogenetic processes in the liver (12–14). FAS is an enzyme necessary for *de novo* synthesis of fatty acids, which is regulated both transcriptionally and post-transcriptionally in response to nutrients and hormones (14). SREBP-1c, a transcription factor integral to maintaining lipid homeostasis, regulates gene expression related to fatty acid metabolism, including expression of FAS (12, 13, 15). Several studies have demonstrated that these lipogenetic processes are influenced by obesity and insulin-resistant conditions (16–18). Recently, suppressors of cytokine signalling proteins (SOCS) have been suggested to play pathogenetic roles in obesity-related metabolic disorders, including insulin resistance and fatty liver disease, by affecting cytokine signaling (19, 20). Overexpression of SOCS-1 and SOCS-3 in the liver has been shown to induce insulin resistance accompanied by elevation of SREBP-1c (19). This study suggests that proinflammatory cytokines, including TNF-α, may affect steatosis of the liver by modulating SOCS and SREBP-1c.

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