

Selection of Controls

The control subjects were chosen from among, asymptomatic outpatients with hyperlipidemia who had no cardiac or infectious diseases. The absence of atherosclerosis in the controls was assessed as follows: normal 12-lead ECG, normal findings on echocardiography, < 25% stenosis of the carotid arteries on Doppler ultrasonography, and normal lower limb arteries on physical examination. A history of cardiac disease meant exclusion from the control group.

Laboratory Tests

Peripheral venous blood specimens and serum samples were obtained from all participants and stored at -80°C until analysis.

C. pneumoniae DNA was isolated from PBMC using the Smitest EX-R&D (Genome Science Laboratories, Fukushima, Japan) in accordance with the manufacturer's recommendations. PCR for the detection of *C. pneumoniae* was done using a *C. pneumoniae*-specific pair of primers (CP1/CP2 and CPC/CPD)¹⁶⁾.

C. pneumoniae IgG and IgA antibodies were measured with enzyme-linked immunosorbent assay (ELISA) kits (Hitazyme *C. pneumoniae*, Hitachi Chemical Co., Ltd., Tokyo, Japan), as described previously^{8)9)17)~19)}. The IgG and IgA were positive when indices were ≥ 1.10 ¹⁷⁾. When the IgG and IgA antibody detection rates by ELISA were compared with those for the microimmunofluorescence method, sensitivity was 90.4% for IgG and 84.6% for IgA, while specificity was 89.9% for IgG and 86.7% for IgA¹⁸⁾. The rate of agreement between ELISA and Western blotting was 80.0% for IgG and 87.5% for IgA¹⁹⁾. All laboratory tests were done in a blinded fashion.

Statistical Analysis

The mean levels of numerical variables were compared by the Mann-Whitney U test, while categorical variables were compared by the chi-square test or Fisher's exact test, as was

appropriate. A P value < 0.05 was considered to indicate statistical significance.

Results

The characteristics of the participants are summarized in Table 1.

Compared with the controls, the patients with atherosclerosis were significantly older and were more likely to be men, to be current smokers, and to have a history of hypertension. *C. pneumoniae* DNA was found in the PBMC of 11 controls (41%), while IgG was positive in 13 controls (48%) and IgA was detected in 7 controls (26%).

The DNA detection rates of the patients with atherosclerosis and controls showed no significant difference, but the IgA positive rate was significantly higher in the patients with atherosclerosis. In contrast, the IgG positive rate was not significantly different between the two groups.

C. pneumoniae DNA was detected in the PBMC of 19 patients (28%) with CA, while IgG for *C. pneumoniae* was found in for 30 patients (44%) and IgA was detected in 32 patients (47%). *C. pneumoniae* DNA was detected in the PBMC of 10 patients (35%) with SA, while IgG for *C. pneumoniae* was found in 14 patients (48%) and IgA was detected in 15 patients (52%). *C. pneumoniae* DNA was detected in the PBMC of 9 patients (23%) with ACS, while IgG for *C. pneumoniae* was found in 16 patients (41%) and IgA was detected in 24 patients (62%). *C. pneumoniae* DNA was detected in the PBMC of 9 patients (28%) with OMI, while IgG for *C. pneumoniae* was found in 14 patients (44%) and IgA was detected in 20 patients (63%). The IgA positive rate of the patients with CAD (SA, ACS, and OMI) was significantly higher than the controls ($p < 0.05$, $p < 0.01$, $p < 0.01$, respectively).

The relationship between the *C. pneumoniae* DNA and IgG or IgA antibodies of the patients with atherosclerosis is shown in Table 2.

IgG for *C. pneumoniae* with atherosclerosis was found in 79 patients (42%) and IgA was

detected in 98 patients (58%), while IgG was positive in 13 controls (48%) and IgA was detected in 7 controls (29%). There was no significant difference between the two groups. IgA positivity was significantly more common in the patients with atherosclerotic diseases (58%) than in the controls (29%) ($P < 0.05$). Among the patients with atherosclerotic diseases, the *C. pneumoniae* IgA antibody positive rate was significantly higher in the patients with DNA than in those without it (71% vs. 18%, $P < 0.05$).

The associations between *C. pneumoniae* DNA and antibody for *C. pneumoniae* IgG or IgA

among the patients with each type of atherosclerotic disease and the controls are shown in Table 3.

The *C. pneumoniae* IgA-positive rate was significantly higher for CA patients who had *C. pneumoniae* DNA in their PBMC than for those without it (74% vs. 18%, $P < 0.05$). In contrast, the *C. pneumoniae* IgG-positive rate was not significantly higher for CA patients who had *C. pneumoniae* DNA than for those without it (47% vs. 64%). Among the patients with CAD (SA + ACS + OMI), the *C. pneumoniae* IgA antibody positive rate was significantly higher for the

Table 1 Characteristics of 168 patients with atherosclerotic diseases and 27 controls

Variables	Atherosclerotic diseases					Controls (n=27)
	Total (n=168)	CA (n=68)	CAD			
			SA (n=29)	ACS (n=39)	OMI (n=32)	
Age, years	67 ± 9**	65 ± 8**	69 ± 8**	68 ± 10**	66 ± 11*	59 ± 7
Male	109 (65)**	25 (37)	20 (69)**	24 (62)**	28 (82)**	6 (22)
Current Smoker	65 (36)*	23 (34)*	11 (38)*	19 (49)**	12 (38)*	3 (11)
Diabetes mellitus	61 (36)	24 (32)	9 (31)	15 (39)	13 (41)	7 (26)
Hypertension	92 (55)**	35 (52)**	16 (55)**	22 (56)**	19 (59)**	5 (19)
<i>C. pneumoniae</i> DNA in PBMC	48 (29)	19 (28)	10 (35)	9 (23)	9 (28)	11 (41)
<i>C. pneumoniae</i> IgG ≥ 1.10	79 (47)	30 (44)	14 (48)	16 (41)	14 (44)	13 (48)
<i>C. pneumoniae</i> IgA ≥ 1.10	98 (58)**	32 (47)	15 (52)*	24 (62)**	20 (63)**	7 (26)

Values are represented as the mean ± SD or number (%).

CA, carotid atherosclerosis; SA, stable angina; ACS, acute coronary syndrome; OMI, old myocardial infarction

Chlamydomydia pneumoniae; *C. pneumoniae*

* $P < 0.05$ vs. controls

** $P < 0.01$ vs. controls

Table 2 Relationship between *C. pneumoniae* DNA and IgG or IgA seropositivity; atherosclerosis patients versus controls

<i>C. pneumoniae</i> antibody	Atherosclerotic disease patients			Controls		
	Total (n=168)	DNA + (n=47)	DNA - (n=121)	Total (n=27)	DNA + (n=11)	DNA - (n=16)
<i>C. pneumoniae</i> IgG ≥ 1.10	79 (42)	27 (59)	41 (34)	13 (48)	7 (64)	6 (36)
<i>C. pneumoniae</i> IgA ≥ 1.10	98 (58)*	33 (71)*	53 (44)	7 (29)	2 (18)	5 (31)

Values are represented as numbers (%).

DNA +, DNA positive; DNA -, DNA negative

* $P < 0.05$ vs. controls

Table 3 Relationship between *C. pneumoniae* DNA and seropositivity by type of atherosclerosis

<i>C. pneumoniae</i> antibody	CA		CAD		Controls	
	DNA + (n=19)	DNA - (n=49)	DNA + (n=28)	DNA - (n=72)	DNA + (n=11)	DNA - (n=16)
<i>C. pneumoniae</i> IgG ≥ 1.10	9 (47)	21 (43)	18 (64)	26 (36)	7 (64)	6 (38)
<i>C. pneumoniae</i> IgA ≥ 1.10	14 (74)*	18 (37)	19 (68)*	40 (56)	2 (18)	5 (31)

Values are represented as numbers (%).

DNA +, DNA positive; DNA -, DNA negative

* $P < 0.05$ vs. controls

patients with DNA than for those without it (68% vs. 18%, $P < 0.05$). However, the *C. pneumoniae* IgG positive rate was not significantly higher for the patients who had *C. pneumoniae* DNA than for those without it (64% vs. 64%).

Discussion

The present case-control study adds new information to the growing pool of data regarding the association between atherosclerosis and *C. pneumoniae* infection. We demonstrated that a high *C. pneumoniae* IgA antibody titer and *C. pneumoniae* DNA positivity are associated with an increased risk of various atherosclerotic diseases. When we investigated whether or not detection of *C. pneumoniae* DNA (in PBMC and atherosclerotic lesions) or antibodies was associated with atherosclerosis, we found a strong association between *C. pneumoniae* DNA in PBMC and advanced atherosclerosis. In fact, both *C. pneumoniae* DNA and the *C. pneumoniae* IgA positive rate were significantly higher for patients with CAD than for controls and the IgA seropositive rate was significantly higher for patients with advanced atherosclerosis than for the controls.

Previous studies²⁰⁻²³ have found *C. pneumoniae* DNA in PBMC at significantly higher rates in patients with atherosclerosis than in controls, but these reports have also shown that the prevalence of circulating *C. pneumoniae* DNA varies widely, being 8.8-59.4% of patients with atherosclerosis and 0.0-46.1% of controls. This wide variation of the *C. pneumoniae* positive rate was found both by using different methods and when independent investigators used similar methods. *C. pneumoniae* generally infects the respiratory tract initially, and may then be disseminated systemically by infected macrophages¹¹⁾¹²⁾. A recent interesting study²⁴⁾ clearly reported that *C. pneumoniae* could not survive in macrophages for long term. Other group also demonstrated that lymphocytes have an important role as host cells for *C. pneumoniae*²⁵⁾. Our results indicate that

detection of chlamydiae in the bloodstream may be a general phenomenon among the adult population rather than being related to the stage of atherosclerosis, so the detection of *C. pneumoniae* DNA may not be a valid marker of current infection.

We found no correlation between the *C. pneumoniae* DNA positivity and seropositivity or antibody titers, as was previously observed in other studies²²⁾²³⁾, even though IgA-positive patients were significantly more likely to have *C. pneumoniae* DNA in their PBMC than were IgA-negative patients. Most of the previous studies²²⁾²³⁾, as well as our study, have shown a higher prevalence of *C. pneumoniae* antibodies than *C. pneumoniae* antigen. Therefore, current infection with *C. pneumoniae* may be indicated by the presence of antibodies in an antigen-positive patient. From our study, measurement of *C. pneumoniae* antibody seemed to be a more sensitive diagnostic method for infection than the detection of *C. pneumoniae* DNA by PCR. The presence of *C. pneumoniae* in atherosclerotic tissues is beyond doubt, but it is difficult to determine whether it is a primary cause of disease or a secondary invader, as well as whether it behaves innocently or aggressively in the latter case. The present results suggested that *C. pneumoniae* may be a secondary invader of atherosclerotic lesions and not the primary cause of atherosclerosis.

Our previous study⁹⁾ showed that *C. pneumoniae* infection reduced the effect of lipid-lowering therapy on carotid atherosclerosis and that this organism may play a role in the progression of the atherosclerosis of hypercholesterolemic patients with advanced CA, although no association was found between *C. pneumoniae* seropositivity and mild atherosclerosis (such as early CA) in the general population⁸⁾. In the present study, we also found a significantly greater prevalence of IgA seropositivity in patients with more advanced atherosclerosis than in the controls, indicating that *C. pneumoniae* IgA positivity may be

associated with advanced atherosclerosis. Future research should take into account the fact that the lesions of atherosclerosis are not sterile, and studies such as clinical antibiotic intervention trials will be necessary.

The main limitation of the present study was its case-control design. Although care was taken to avoid potential biases, it is well known that retrospective studies are often unable to reproduce the associations detected in case-control studies, so further prospective studies are needed to confirm our findings.

In conclusion, a high *C. pneumoniae* IgA antibody titer and *C. pneumoniae* DNA positivity are associated with atherosclerosis but further studies are required to confirm whether or not chronic *C. pneumoniae* infection is actually an independent risk factor for atherosclerosis.

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PBMC 中の *Chlamydomphila pneumoniae* DNA と *Chlamydomphila pneumoniae* 抗体との関連

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Chlamydomphila pneumoniae (*C. pneumoniae*) 感染は動脈硬化に関与しているといわれているが、末梢血中の *C. pneumoniae* DNA と *C. pneumoniae* 抗体との関連を示した報告は少ない。私共は 168 例の動脈硬化性疾患群と 27 例のコントロール群に対して、ELISA 法による *C. pneumoniae* 抗体測定と同時に、PCR 法を用いて末梢血中の *C. pneumoniae* DNA を測定し比較検討した。動脈硬化性疾患群において、*C. pneumoniae* の DNA の検出率は 48/168 (29%)、IgG 抗体陽性率は 79/168 (47%)、IgA 抗体陽性率は 98/168 (58%) であった。一方、コントロール群では、DNA の検出率は 11/27 (41%)、IgG 抗体陽性率は 13/27 (48%)、IgA 抗体陽性率は 7/27 (26%) であった。DNA の検出率は、両群に有意差は認めなかったが、末梢血中の DNA 陽性例における IgA 抗体陽性率は、動脈硬化性疾患群 (74%) では、コントロール (18%) と比較して有意に高く ($p < 0.05$)、その中の冠動脈疾患 (急性冠症候群, 安定狭心症, 不安定狭心症) においても、コントロールと比較して有意に高かった ($p < 0.05$)。これらの結果より *C. pneumoniae* における IgA 抗体高値かつ DNA 陽性の場合、血管内 *C. pneumoniae* 感染による動脈硬化性疾患発症の危険因子に深く関与している可能性が示唆された。



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The longitudinal quantitative assessment by transient elastography of chronic hepatitis C patients treated with pegylated interferon alpha-2b and ribavirin

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ABSTRACT

The aim of this study was to assess the association between liver stiffness measured by transient elastography (FibroScan[®]) and the efficacy of pegylated interferon alpha-2b plus ribavirin combination treatment for patients with chronic hepatitis C virus (HCV) infection. We prospectively studied 145 Japanese patients with chronic HCV infection. FibroScan was done at baseline, at the end of treatment, and at 48 and 96 weeks after the end of treatment. The FibroScan values were significantly decreased for sustained virological response (SVR) patients (the mean rate of change; -16.2% , -32.2% and -43.5%) in comparison with non-SVR patients (-7.2% , -2.1% and $+17.3\%$) at the end of treatment ($P=0.0127$), and 48 weeks ($P<0.0001$) and 96 weeks ($P<0.0001$) after the end of treatment. Among the non-SVR patients, the FibroScan values were significantly decreased for patients with biochemical response (BR) (-17.9% , -30.0% and -27.1%) in comparison with non-BR (-4.1% , $+6.4\%$ and $+30.6\%$) at the end of treatment ($P=0.0270$), and 48 weeks ($P<0.0001$) and 96 weeks ($P<0.0001$) after the end of treatment. The FibroScan values may predict a progressively better clinical outcome for patients with successful virological and biochemical responses.

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1. Introduction

Hepatitis C virus (HCV) infection is a main cause of chronic viral hepatitis worldwide. Chronic hepatitis can lead to cirrhosis and hepatocellular carcinoma (HCC) (Seeff, 2002; Hayashi et al., 2000). Antiviral treatment with interferon (IFN) for chronic HCV infection can induce viral clearance and biochemical and histological improvement (Davis et al., 1989; Hayashi et al., 1994). Pegylated interferon (PEG-IFN) alpha in combination with ribavirin (RBV), which aims at viral eradication (Poynard et al., 2002a; Furusyo et al., 2008), has contributed to a reduction in the relapse rate and a significant increase in the rate of sustained virological response (SVR) compared with standard IFN monotherapy (Hayashi et al., 1994; Zeuzem et al., 2000; Lindsay et al., 2001). IFN treatment has been reported to be responsible for the regression of liver fibrosis in patients with SVR (Shiratori et al., 2000; Furusyo et al., 1997). Even if a virological response with IFN treatment was not obtained, the deterioration of compensated cirrhosis was prevented and the development of HCC was inhibited (Nishiguchi et al., 1995; Veldt

et al., 2004; Kashiwagi et al., 2003), thereby increasing the survival rate (Poynard et al., 2002a).

Liver biopsy had long remained the gold standard for staging fibrosis. However, liver biopsy is no more considered as a perfect methodology because of the invasive nature of the procedure, sampling error and inter-observer variability (Regev et al., 2002; Manning and Afdhal, 2008). Therefore, further testing strategies are needed for assessment of the liver status of patients with liver diseases.

Transient elastography (FibroScan[®]; Echosens, Paris, France) has been proposed as a promising, rapid, noninvasive and reproducible method for measuring liver stiffness (Sandrin et al., 2002). We previously reported a clinical assessment of FibroScan among patients with chronic hepatitis B and C (Ogawa et al., 2007). The values measured by FibroScan (FibroScan values) have been significantly correlated with histopathological staging of percutaneous liver biopsy and have been shown to be more accurate than biochemical scores such as the aspartate aminotransferase (AST)-to-platelet ratio (APRI) and markers of liver fibrosis (e.g. hyaluronic acid and type IV collagen), which are products of the degradation or synthesis of the extracellular matrix.

It has been repeatedly observed that at least two biomarkers FibroTest[®] (Biopredictive, Paris, France) and FibroScan (Manning and Afdhal, 2008; Calès et al., 2008; Friedrich-Rust et al., 2008; Poynard et al., 2008; Shaheen et al., 2007) have the same

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diagnostic value as a 20–25 mm liver biopsy. In case of discordance between FibroTest and liver biopsy, half of failures are attributable to FibroTest and half to the biopsy (Poynard et al., 2004; Ngo et al., 2006). Moreover, FibroTest has already demonstrated a similar prognostic value as biopsy (Ngo et al., 2006). In a few countries, biomarkers are recommended by health authorities as first line test in patients with chronic hepatitis C, and biopsy only if biomarkers are not interpretable (Manning and Afdhal, 2008).

The aim of the present long-term prospective study was to evaluate the association between liver stiffness measured by FibroScan and the efficacy of combined PEG-IFN alpha-2b plus RBV treatment for patients with chronic HCV infection.

2. Materials and methods

2.1. Patients

We prospectively studied a total of 145 patients infected with chronic HCV infection. Of the 145 patients, 19 (13.1%) refused antiviral treatment because of financial problems or anxiety about the possibility of adverse effects and low initial fibrosis degree (low FibroScan values); however, 4 (2.1%) of them underwent liver biopsy. The 19 untreated patients were followed for the same 3-year period as the treated patients. Of the remaining 126 patients, 118 (93.7%) underwent liver biopsy, all of whom were treated with PEG-IFN alpha-2b plus RBV treatment between January 2005 and July 2006 and followed up for 2 years after the end of treatment.

All patients satisfied the following criteria: (1) chronically infected with HCV and (2) a history of an increased alanine aminotransferase (ALT) level for over 6 months. Exclusion criteria for the study were: (1) positivity for antibody to human immunodeficiency virus (HIV) or positivity for both hepatitis B surface antigen and anti-HCV; (2) clinical or biochemical evidence of hepatic decompensation; (3) excessive active alcohol (i.e. ethanol) consumption (>60 g/day) or drug abuse; (4) suspected hepatocellular carcinoma; or (5) treatment with antiviral or immunosuppressive agents prior to enrollment. Patients who fulfilled the above criteria were recruited at Kyushu University Hospital.

Informed consent was obtained from all patients before enrollment. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and the International Conference on Harmonization of guidelines for good clinical practice.

2.2. Clinical and laboratory assessment

Clinical parameters included aspartate aminotransferase, ALT, platelet count, type IV collagen, prothrombin time, HCV genotype and HCV RNA. We also calculated APRI, using 45 IU/L as the upper limit of the normal AST range (ULN), as previously recommended for evaluating liver fibrosis (Wai et al., 2003):

$$\text{APRI} = \frac{\text{AST} (\text{/ULN}) \times 100}{\text{platelet count} (\times 10^9/\text{L})}$$

Body mass index (BMI) was calculated as weight in kilograms/height in square meters. Serum levels of AST, ALT, type IV collagen and HCV RNA, platelet counts, prothrombin time and HCV genotype were measured by standard laboratory techniques at a commercial laboratory (MBC Laboratory, Tokyo, Japan).

2.3. Transient elastography (FibroScan)

FibroScan was done in the right lobe of the liver through the intercostal spaces with the patient lying in the dorsal decubitus position with the right arm in maximal position. The tip of the probe transducer was covered with coupling gel and placed on

the skin, between the ribs at the level of the right lobe of the liver. The operator, assisted by an ultrasonic time-motion image, located a liver portion at least 6 cm thick and free of large vascular structures. Once the measurement area had been located, the operator pressed the probe button to start acquisition. The elasticity was automatically calculated by the apparatus and the data were shown as kiloPascal (kPa). All examinations were performed by four accomplished operators (EO, KT, HT, and SO) of our department who individually experienced over 100 examinations. Only liver stiffness measurements obtained with at least six successful acquisitions and a success rate of at least 60% were considered reliable. The validity of FibroScan values depends on an interquartile range of all successful measurements (IQR/M) of less than 30% of median values (Poynard et al., 2008; Lucidarme et al., 2008). The mean IQR/M of the present study was 21.6% and no case with IQR/M > 30% was found. The first measurement of liver stiffness by FibroScan was performed within 2 weeks before liver biopsy examination.

2.4. Liver histology and quantification of liver biopsy

Liver biopsy was done for 122 (84.1%) of the 145 chronic HCV infected patients and was performed by experienced hepatologists with a 16-G disposable needle (Bard® Monopty®; C.R. Bard, Covington, GA) under ultrasound guidance. The median liver biopsy length was 18 mm (minimal length was 15 mm). Liver biopsy specimens were fixed in formalin and paraffin was embedded. All biopsy specimens were analyzed by two experienced pathologists who were blinded to the clinical data. For each specimen, the stage of fibrosis and the grade of activity were established according to the following criteria (Bedossa and Poynard, 1996). Fibrosis was staged on a 0–4 scale as follows: F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis and few septa, F3 = numerous septa without cirrhosis, and F4 = cirrhosis. The grading of activity, including the intensity of the necroinflammation, was scored as follows: A0 = no histological activity, A1 = mild activity, A2 = moderate activity, and A3 = severe activity.

2.5. Therapeutic protocol

All patients were treated with a weight-based, 1.5 µg/kg weekly dose of subcutaneous PEG-IFN alpha-2b (Pegintron A®; Schering-Plough, Osaka, Japan). In combination with PEG-IFN alpha-2b, RBV (Rebetol®; Schering-Plough) was given orally at a daily dose of 600–1000 mg based on bodyweight (600 mg for patients weighing < 60 kg, 800 mg for those weighing 60–80 kg, and 1000 mg for those weighing > 80 kg). The length of treatment was 48 weeks for genotype 1b and 24 weeks for genotype 2. The above durations and dosages are those approved by the Japanese Ministry of Health, Labor and Welfare. Patients were considered to have RBV-induced anemia if the hemoglobin level decreased to < 100 g/L. In such cases, a reduction in the dose of RBV was required. Some patients also had PEG-IFN alpha-2b induced psychological adverse effects or a decrease of white blood cell and platelet count. In such cases, a reduction in the dosage of PEG-IFN alpha-2b was required. Both PEG-IFN alpha-2b and RBV were discontinued if the hemoglobin level, white blood cell count, or platelet count fell below 85 g/L, $1 \times 10^9/\text{L}$, or $2.5 \times 10^9/\text{L}$, respectively. The treatment was discontinued if severe general fatigue, hyperthyroidism, interstitial pneumonia, or severe hemolytic problems developed, continuation of treatment was judged not to be possible by the attending physician, or the patient desired discontinuation of treatment. All patients received at least 80% or more of the target dosage of PEG-IFN alpha-2b and 60% or over of the RBV, because the condition under the sufficient dosage were needed to estimate between treatment response and liver disease progression accurately.

Table 1
Baseline characteristics of 145 patients with chronic HCV infection.

Characteristics	Non-treated (n = 19)	PEG-IFN alpha-2b combination with RBV treatment		
		SVR (n = 57)	Non-SVR (n = 69)	P-value*
Male/Female (n)	9/10	30/27	25/44	0.0655
Age (years)	63.8 ± 9.2	52.7 ± 13.2	60.3 ± 9.3	0.0003
Body mass index (kg/m ²)	22.2 ± 3.4	23.1 ± 3.1	22.8 ± 3.1	0.5912
Aspartate aminotransferase (AST) (IU/L)	61.2 ± 29.5	65.0 ± 39.2	65.8 ± 42.4	0.9192
Alanine aminotransferase (IU/L)	69.7 ± 50.9	88.3 ± 73.7	72.7 ± 53.2	0.1962
Platelet count (10 ⁹ /L)	154 ± 52	155 ± 45	154 ± 51	0.9294
Type IV collagen (ng/mL)	180 ± 68	172 ± 69	192 ± 88	0.1750
AST/Platelet count	1.39 ± 0.89	1.37 ± 0.99	1.35 ± 1.19	0.9100
Prothrombin time (%)	94.4 ± 15.9	90.2 ± 8.7	88.2 ± 13.1	0.3414
HCV genotypes 1b/2 n	19/0	34/23	65/4	<0.0001
Serum HCV RNA level (kIU/mL)	3207 ± 1542	1565 ± 1645	2014 ± 1455	0.1062
Liver histology				0.7225
Stage of fibrosis				
F0 (%)	1 (25.0)	7 (13.2)	7 (10.8)	
F1 (%)	0 (0.0)	10 (18.9)	17 (26.2)	
F2 (%)	1 (25.0)	19 (35.8)	16 (24.6)	
F3 (%)	2 (50.0)	11 (20.8)	15 (23.1)	
F4 (%)	0 (0.0)	6 (11.3)	10 (15.4)	
Grade of activity				0.7243
A0 (%)	0 (0.0)	0 (0.0)	0 (0.0)	
A1 (%)	1 (25.0)	17 (32.1)	21 (32.3)	
A2 (%)	2 (50.0)	34 (64.2)	39 (60.0)	
A3 (%)	1 (25.0)	2 (3.8)	5 (7.7)	
Not determined	15	4	4	

Data are shown by the mean ± standard deviation. PEG-IFN, pegylated interferon; RBV, ribavirin.

* P-values were analyzed between SVR and non-SVR patients.

2.6. Efficacy of treatment

SVR was defined as serum HCV RNA undetectable at 24 weeks after the end of treatment. This efficacy variable, SVR, was defined as non-detectable HCV RNA as measured by the COBAS[®] Amplicor[®] HCV Monitor Test (version 2.0), and the results were labeled as positive or negative. The lower limit of detection was 50 IU/mL (0.5 kIU/mL) (Lee et al., 2000).

2.7. Assessment of biochemical response (BR) among non-SVR patients

We evaluated BR among non-SVR patients after the end of treatment. Patients who had continuous ALT levels under 30 IU/L every month for 96 weeks after the end of treatment were defined as BR.

2.8. Determination of HCV RNA level and HCV genotype

During the treatment period, HCV RNA was analyzed by the COBAS[®] Amplicor[®] HCV Monitor assay (version 2.0; Roche Diagnostics, Tokyo, Japan), with a lower limit of quantitation of 5000 IU/mL and an outer limit of quantitation of 5,100,000 IU (5100 kIU)/mL. The COBAS[®] Amplicor[®] HCV Monitor assay (version 2.0) is a semi-automated nucleic acid amplification assay, consisting of manual sample preparation and automated reverse transcription (RT), amplification and detection steps on the COBAS[®] Amplicor[®] Analyzer. HCV genotype was determined by RT-PCR using universal and type-specific primers from the putative C gene of the HCV genome, according to Okamoto et al. (1992) and the genotype was classified into the type 1b or type 2a or 2b based on Simmonds et al. (1994).

2.9. Statistical analysis

Statistical analysis was done with BMDP statistical software for the IBM 3090 system computer (BMBD Statistical Software,

Inc., Los Angeles, CA). Continuous data were expressed as mean values, mean ± standard deviation (SD) of the mean. The paired *t*-test, unpaired *t*-test, Mann-Whitney *U*-test or Kruskal-Wallis non-parametric analysis of variance was used for the analysis. The area under the receiver operating characteristic curve (AUROC) analysis was done to evaluate the relationship between histological findings and FibroScan values. The cutoff values were selected from the receiver operating characteristic (ROC) curve to maximize total sensitivity and specificity. A *P*-value less than 0.05 was regarded as statistically significant.

3. Results

3.1. Characteristics of patients

The major clinical and biochemical parameters of the patients at entry (baseline) are summarized in Table 1. The mean FibroScan values were significantly higher in the treated group (10.2 kPa) than in the non-treated group (7.6 kPa) ($P=0.0406$). Of the 126 treated patients, 57 (45.2%) achieved SVR. The median age was significantly younger in the SVR group (52.7 years) than in the non-SVR group (60.3 years) ($P=0.0003$). The rate of SVR was higher for patients with genotype 2 (85.2%) than for those with genotype 1b (34.3%) ($P<0.0001$). No significant differences between the SVR and non-SVR groups were found for gender, BMI, AST, ALT, platelet count, type IV collagen, APRI, prothrombin time, or serum HCV RNA level.

3.2. Relationship between liver fibrosis and FibroScan values

Fig. 1a and b shows the distribution of FibroScan values according to fibrosis stage and activity grade, respectively. The median values of the patients were 4.8 kPa, 7.1 kPa, 8.5 kPa, 12.8 kPa and 19.5 kPa for F0, F1, F2, F3 and F4, respectively. The FibroScan values were significantly correlated with fibrosis stage ($r=0.807$, $P<0.0001$) and were also significantly increased in accordance with

Table 2

Optimal cutoff of FibroScan values for the determination of histological fibrosis stage in 122 biopsy-received patients with chronic HCV infection.

	Histological fibrosis stage by liver biopsy			
	F=1 (F0 vs. F1)	F=2 (F1 vs. F2)	F=3 (F2 vs. F3)	F=4 (F3 vs. F4)
Number	15/27	27/36	36/28	28/16
Cutoff value ^a (kPa)	6.1	7.2	10.3	14.9
AUROC	0.81	0.66	0.88	0.94
Sensitivity (%)	63.0	66.7	89.3	100.0
Specificity (%)	86.7	66.7	77.8	82.1
Positive predictive value (%)	89.5	66.7	75.8	76.2
Negative predictive value (%)	56.5	60.0	90.3	100.0
Positive likelihood ratio	4.72	2.00	4.02	5.60

AUROC, area under the receiver operating characteristic curve.

^a The optimal cutoff value is the one that gives the higher total sensitivity and specificity.

the grade of activity for the patients ($r=0.343$, $P<0.0001$). Table 2 shows the optimal liver stiffness cutoff values obtained for sensitivity and specificity. In analyses of adjacent fibrosis stages (F0 vs. F1, F1 vs. F2, F2 vs. F3 and F3 vs. F4), four threshold FibroScan values were identified: ≥ 6.1 kPa for F1 (sensitivity 63.0%, specificity 86.7%); ≥ 7.2 kPa for F2 (sensitivity 66.7%, specificity 66.7%); ≥ 10.3 kPa for F3 (sensitivity 89.3%, specificity 77.8%) and 14.9 kPa for F4 (sensitivity 100%, specificity 82.1%). The corresponding AUROC were 0.81 for F0 vs. F1, 0.66 for F1 vs. F2, 0.88 for F2 vs. F3 and 0.94 for F3 vs. F4.

3.3. The longitudinal FibroScan values for the PEG-IFN alpha-2b plus RBV combination treatment (Fig. 2a and b)

The baseline mean FibroScan values were 10.3 ± 4.8 kPa, 10.0 ± 5.5 kPa, and 7.6 ± 3.9 kPa for SVR ($n=57$), non-SVR ($n=69$), and non-treated patients ($n=19$), respectively. For SVR patients, the mean FibroScan values were 8.3 kPa, 6.6 kPa and 5.4 kPa at week 0 and at 48 and 96 weeks after the end of treatment, respectively. For non-SVR patients, the mean FibroScan values were 9.0 kPa, 9.5 kPa and 11.4 kPa at week 0 and at 48 and 96 weeks after the end of treatment, respectively.

The changes of the FibroScan values of each patient at the end of treatment, 48 weeks and 96 weeks after the end of treatment were calculated with the entry values as the estimated standard. Significant differences were found between SVR (-16.2% , -32.2% and -43.5%) and non-SVR patients (-7.2% , -2.1% and $+17.3\%$) in the mean rate of change of FibroScan values between each testing point ($P=0.0127$, $P<0.0001$ and $P<0.0001$, respectively). For the untreated patients, the mean FibroScan values increased to 8.3 kPa, 9.8 kPa and 10.6 kPa ($+12.9\%$, $+38.0\%$ and $+49.1\%$, respectively) at each testing point. Significant differences were also found between treated patients (SVR and non-SVR) and untreated patients in the mean rate of change of FibroScan values.

3.4. The longitudinal FibroScan values classified according to the BR and non-BR of non-SVR patients (Fig. 3a and b)

Of the 69 non-SVR group patients, 16 (23.2%) achieved BR. The baseline mean FibroScan values were 9.3 ± 5.2 kPa and 10.2 ± 5.6 kPa for BR ($n=16$) and non-BR ($n=53$) patients, respectively. For BR patients, the mean FibroScan values were 7.4 kPa, 6.2 kPa and 6.7 kPa at week 0 and at 48 and 96 weeks after the end of treatment, respectively. For non-BR patients, the mean FibroScan

Table 3

Differences of the mean rate of changes of FibroScan values after PEG-IFN alpha-2b plus RBV treatment, classified by age group and gender.

	n	PEG-IFN alpha-2b combination with RBV treatment					
		Week 0	P-value*	Week 48	P-value*	Week 96	P-value*
SVR patients							
Age group			0.7995		0.4128		0.3874
<60 years	22	-16.3		-30.7		-42.1	
≥ 60 years	35	-15.9		-34.6		-45.9	
Gender			0.4517		0.6278		0.1740
Men	30	-17.7		-31.1		-46.3	
Women	27	-14.4		-33.4		-40.5	
Fibrosis stage			0.6653		0.2369		0.0927
$\leq F2$	36	-16.1		-31.1		-42.0	
$\geq F3$	17	-18.3		-37.4		-50.0	
Non-SVR patients							
Age group			0.7995		0.3478		0.3998
<60 years	27	-6.4		-6.9		11.5	
≥ 60 years	42	-7.8		1.1		20.9	
Gender			0.3218		0.1625		0.4507
Men	25	-10.8		-9.7		11.8	
Women	44	-5.3		2.3		20.4	
Fibrosis stage			0.6720		0.4893		0.4309
$\leq F2$	40	-7.3		-5.0		13.9	
$\geq F3$	25	-9.7		1.1		23.2	

PEG-IFN, pegylated interferon; RBV, ribavirin; SVR, sustained virological response; week 0, at the end of treatment; week 48, 48 weeks after the end of treatment; week 96, 96 weeks after the end of treatment. The rates of changes of FibroScan values of each patient at the end of treatment, 48 weeks and 96 weeks after the end of treatment were calculated by the entry values as the estimated standard.

* P-values were analyzed at each point between age groups, between genders and between fibrosis stages.

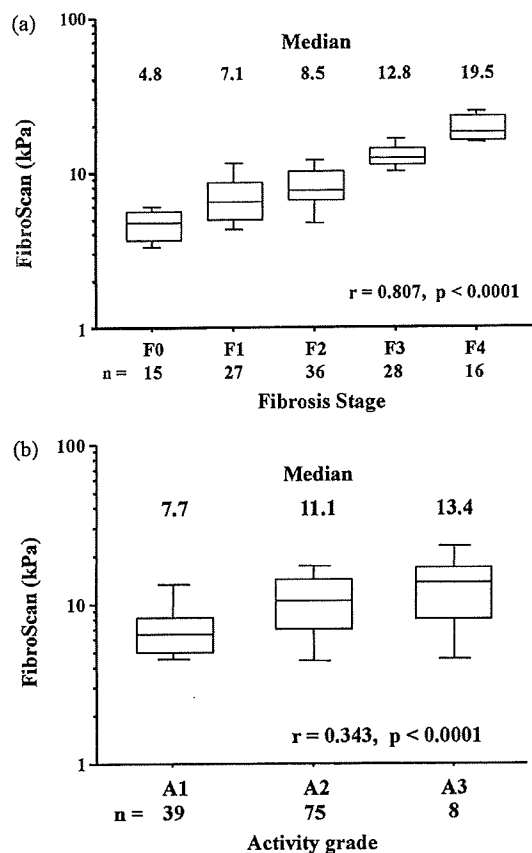


Fig. 1. FibroScan values for each fibrosis stage (a) and activity grade (b) of 122 biopsy-received patients with chronic hepatitis C virus infection. The vertical axis is a logarithmic scale. The bottom and the top boxes are the first and third quartiles, respectively. The length of the box represents the interquartile range within which 50% of the values are located. The lines through the middle of the boxes represent the median. The error bars are the minimum and maximum values.

values were 9.5 kPa, 10.5 kPa and 12.8 kPa at week 0 and at 48 and 96 weeks after the end of treatment, respectively. For BR patients, the mean rate of change of FibroScan values indicated improvement (-17.9% , -30.0% and -27.1% at weeks 0, 48 and 96 after the end of treatment, respectively) with antiviral treatment, in spite of there not being a virological effect. On the other hand, for non-BR patients, the mean rate of change of FibroScan values showed worsening over time (-4.1% , $+6.4\%$ and $+30.6\%$ at weeks 0, 48 and 96 after the end of treatment, respectively). A significant difference was found between BR and non-BR patients in the mean rate of change of FibroScan values in each period ($P = 0.0270$, $P < 0.0001$ and $P < 0.0001$, respectively). Moreover, although a significant difference was found between non-BR ($+6.4\%$) and non-treated patients ($+38.0\%$) at week 48 after the end of treatment ($P < 0.0001$), no significant difference was found between non-BR ($+30.6\%$) and non-treated patients ($+49.1\%$) at week 96 after the end of treatment ($P = 0.0835$) in the mean rate of change of FibroScan values. In other analyses, no significant difference was found between the longitudinal rate of change of FibroScan values and age (< 60 and ≥ 60), gender or fibrosis stage ($\leq F2$ and $\geq F3$) for both SVR and non-SVR patients, as shown in Table 3.

4. Discussion

The present prospective study consists of a demonstration of the association between liver stiffness measured by FibroScan and the efficacy of PEG-IFN alpha-2b plus RBV treatment. Recent reports have shown that treatment for chronic hepatitis C is associated with

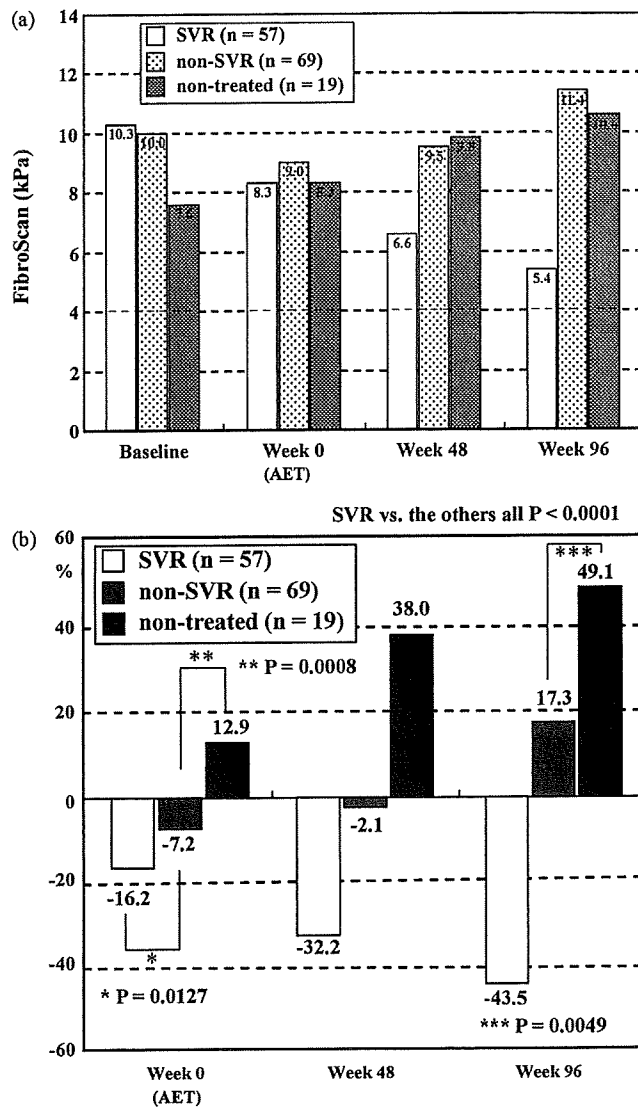


Fig. 2. The longitudinal mean FibroScan values (a) and the mean rate of change of FibroScan values (b) of 126 treated patients after pegylated interferon alpha-2b plus ribavirin combination treatment and 19 non-treated patients. The rates of change of the FibroScan values of each patient at the end of treatment and at 48 and 96 weeks after the end of treatment were calculated using the entry values as the estimated standard. The length of treatment was 48 and 24 weeks for HCV genotypes 1 and 2 patients, respectively. Weeks 0, 48 and 96 for the treated patients indicate the end of treatment and 48 and 96 weeks after the end of treatment, respectively. Weeks 0, 48 and 96 for the non-treated patients indicate 48, 96 and 144 weeks from entry, respectively. SVR, sustained virological response; AET, at the end of treatment.

an improvement of FibroScan values at the end of treatment and 6 months later, whatever the virological response (Vergniol et al., 2008, 2009). However, we investigated the association between an efficacy of the antiviral treatment and FibroScan values related to both virological and biochemical response for a longer period after the treatment than the previous reports. Consequently, we demonstrated that the liver elasticity of SVR patients markedly improved over time and that the liver elasticity of non-SVR patients with biochemical response also improved.

In PEG-IFN alpha plus RBV treatment for chronic hepatitis C, we previously reported that it was necessary to administer $\geq 80\%$ of the target dosage of PEG-IFN alpha-2b plus $\geq 60\%$ of the target dosage of RBV throughout the treatment in order to achieve virological efficacy in Japanese patients (Furusyo et al., 2008). Each patient in the present study was analyzed under this treatment dosage.

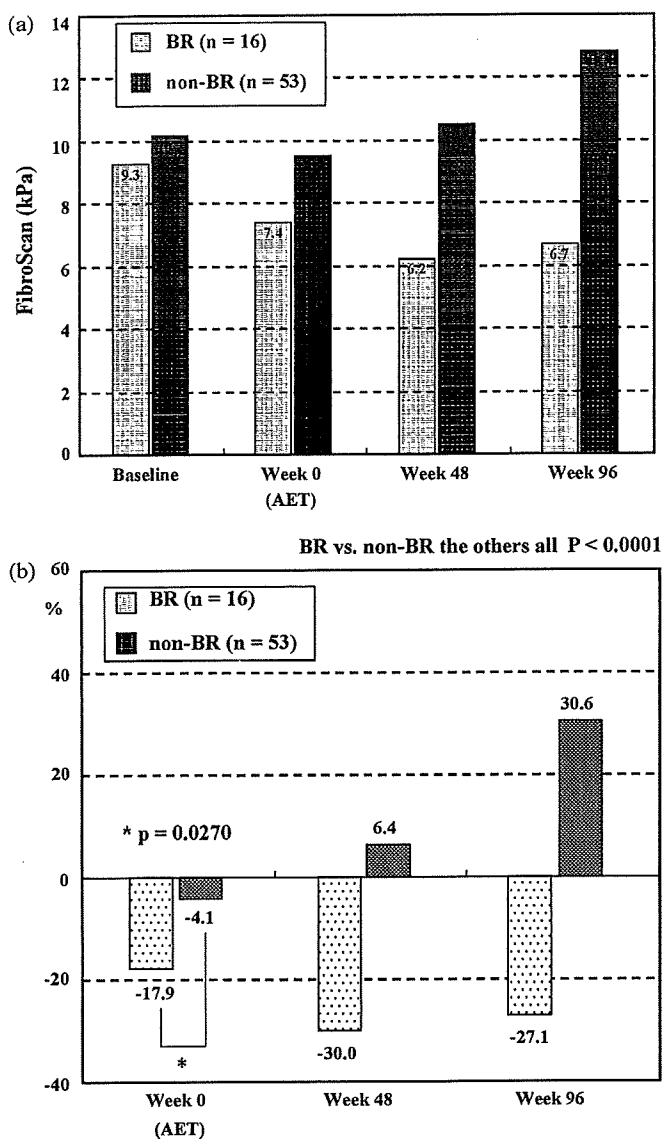


Fig. 3. The longitudinal mean FibroScan values (a) and the mean rate of change of FibroScan values (b) of the non-sustained virological response patients after pegylated interferon alpha-2b plus ribavirin combination treatment, classified by biological response (BR). The rates of change of the FibroScan values of each patient at the end of treatment and at 48 and 96 weeks after the end of treatment were calculated using the entry values as the estimated standard. Weeks 48 and 96 indicate 48 and 96 weeks after the end of treatment, respectively. AET, at the end of treatment.

FibroScan represents a novel clinical methodology based on ultrasound. This can survey a larger sample of the liver parenchyma than liver biopsy and thus more accurately estimates liver fibrosis across a wide range of liver disorders (Ziol et al., 2005; Ogawa et al., 2007). According to meta-analysis, FibroScan could be performed with excellent diagnostic accuracy at distinguishing liver cirrhosis (F4) or no liver cirrhosis (\leq F3), with a mean AUROC of 0.94, and at distinguishing \geq F2 and \leq F1 with a mean AUROC of 0.84 (Friedrich-Rust et al., 2008). The optimal cutoff values for the diagnosis of F4 and \geq F2 suggested from the summary ROC are 13.01 kPa and 7.65 kPa, respectively (Friedrich-Rust et al., 2008). Thus, prior studies have already shown that FibroScan can distinguish absent or mild fibrosis from advanced fibrosis in both HCV-mono-infected and HIV-/HCV-co-infected individuals, but it seems to be less accurate at differentiating between intermediate stages. However, Macías et al. (2008) suggested that the usefulness

of FibroScan could be enhanced using two different cutoff values (6.0 kPa and 9.0 kPa) to identify with \leq F1 and \geq F2, respectively, in HIV- and HCV-co-infected patients.

Biomarkers such as FibroTest demonstrated similar results as FibroScan in patients treated with IFN and RBV (Poynard et al., 2002b, 2003; Ngo et al., 2006; Patel et al., 2009) and the combination of FibroTest and ActiTest[®] (which is a modification of the FibroTest) give not only the fibrosis estimate but also the activity estimate (Poynard et al., 2003). FibroScan has never been demonstrated better than FibroTest for F4 vs. \leq F3 according to evidence-based data (Castéra et al., 2005; Shaheen et al., 2007; Manning and Afdhal, 2008; Poynard et al., 2008; Calès et al., 2008).

While natural history of liver fibrosis progression can vary depending on gender and alcohol consumption, age is the main risk factor for liver fibrosis (Poynard et al., 1997). We recommended that all our patients stop drinking alcohol while under treatment and during follow-up. In our study, the baseline mean value of FibroScan was 7.6 kPa, almost the same as the F2 stage, with a value of 10.5 kPa, nearly equal to the F3 stage, after 3 years in non-treated patients (median age 63.8 years). Poynard et al. (1997) suggested that the rate of liver fibrosis progression of untreated patients was highest in individuals older than 50 years (at a rate of 0.333 stage/year). The findings obtained in the present study were similar to those of the above report.

Many previous reports have shown that IFN treatment achieves biochemical and histological improvement with viral suppression by patients with chronic hepatitis C (Poynard et al., 2002a; Furusyo et al., 1997; Cammá et al., 1998; Bruno et al., 2007; Furusyo et al., 2008). Several potential mechanisms have been hypothesized for the anti-fibrotic effect of IFN, including that IFN alpha can directly reduce fibrogenesis. Shiratori et al. (2000) showed that the fibrosis stage improved from -0.60 at <3 years of follow-up to -0.88 at >3 years follow-up (a rate of -0.28 /years) for SVR patients treated with non-pegylated IFN monotherapy. However, Everson et al. (2008) showed that the fibrosis stage improved -1.00 between before treatment and 6 months after the end of treatment of SVR patients receiving PEG-IFN alpha monotherapy. Poynard et al. (2002a) showed that the mean fibrosis stage 6 months after the end of treatment was 1.9 ± 0.9 (SD) for SVR patients with compensated cirrhosis treated with PEG-IFN alpha monotherapy or in combination with RBV. There was no significant difference between PEG-IFN alpha plus RBV and PEG-IFN alpha alone for the 48-week regimen as to an anti-fibrotic effect (Poynard et al., 2002a). Therefore, PEG-IFN alpha itself may have a stronger anti-fibrotic effect than non-pegylated IFN and RBV, due to a pharmaco-dynamic advantage.

In the present study, we demonstrated a dramatic reduction of FibroScan values in both SVR and BR patients. Firstly, our results confirmed, from the viewpoint of liver fibrosis, that IFN treatment is of long-term benefit for chronic hepatitis C patients. Moreover, such reduction was observed at an early stage of PEG-IFN alpha plus RBV treatment by SVR and BR patients. It is probable that such early reduction of FibroScan values means not only the improvement of fibrosis but also of inflammation in the liver as a result of treatment, because FibroScan values were significantly correlated with the grade of activity in the liver in the present study. Compared with the rate of change of FibroScan values between non-advanced fibrosis ($F \leq 2$) and advanced fibrosis ($F \geq 3$), no significant differences were found at each testing point. Secondly, another important result was that FibroScan values decreased even for BR patients without HCV clearance by treatment. The findings of the present study can explain why the rate of development of HCC is lower in BR patients treated with IFN. Because HCC tends to develop in patients with advanced liver fibrosis (cirrhosis), BR patients who have a reduction of fibrosis probably will not as quickly develop HCC in the future. Thirdly, although the virological treatment itself may produce good

results in terms of a short-term anti-fibrotic effect of the liver, the FibroScan values of non-BR patients became progressively worse in the long-term course after the antiviral treatment. In the case of a high serum ALT level for non-SVR patients, an additional antiviral retreatment has to be considered. In fact, we recommended antiviral treatment for the studied non-treated patients after the completion of this study, and then most of them have started to receive IFN treatment. A long-term IFN treatment regimen was effective in a smaller trial of the anti-fibrotic effect (Arase et al., 2004); therefore, we believe that treatment with careful attention to IFN-related adverse effects could be usefully introduced to help patients avoid progressing to liver fibrosis and HCC. Thus, the FibroScan is a very interesting tool for the follow-up of chronic HCV carriers, as it is easier to use than liver biopsy in clinical settings. These results are of great interest for the understanding of the effects of IFN treatment and of HCV-related liver disease.

In conclusion, our study shows that transient elastography (FibroScan) is a useful tool for the longitudinal assessment of IFN treatment of chronic hepatitis C patients.

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臨 牀 研 究

食 後 高 脂 血 症 に 対 す る エ ゼ チ ミ ブ の 効 果

— 食事負荷試験 (クッキーテスト) を用いて —

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緒 言

わが国での死因の約30%は心血管疾患であり、原因の大部分は動脈硬化によるものである。著者らは超音波装置を用いて頸動脈の内膜中膜厚により動脈硬化を診断し、いくつかの地域で疫学的に調査している¹⁾²⁾。その結果、動脈硬化の危険因子としては空腹時 low density lipoprotein-cholesterol (LDL-C) 高値、空腹時 high density lipoprotein-cholesterol (HDL-C) 低値、糖尿病、高血圧、喫煙などで、空腹時の血清中性脂肪は多変量解析では抽出されなかった。その原因としてはいくつか考えられるが、血清中性脂肪は前回の食事内容によっても異なり、また食後長時間にわたって高値を示す例の存在も指摘され、いわゆる空腹時の血清中性脂肪の正確な値を獲得することが困難である面も考えられる。

近年、動脈硬化進展に寄与する因子として空腹時高血糖より食後高血糖が重要とされ、また食後高脂血症も注目されてきた。このようなことからわが国では、腸管からの脂質吸収を阻害し食後高脂血症を改善させるエゼチミブ³⁾の投与が2007年から認可され、その用い方に議論がなされているところである。また、食後高脂血症の判定基準はなく、種々の食事負荷試験がなされている^{4)~8)}。著者らはアビメディカル株式会社製のクッキー^{9)~11)}を用いて食後高脂血症と診断された例に対して、エゼチミブ単独あるいはプラバスタチンの併用投与を行い、エゼチミブの食後高脂血症に対する有用性を検討したので報告する。

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I. 対 象 お よ び 方 法

LDL-Cが120mg/dl以上の110例について、食事負荷試験 (クッキーテスト;クッキー115g, 総カロリー592Kcal:蛋白質8.0g, 脂肪28.5g, 糖質75.0g)^{9)~11)}を行い、食事負荷後2時間の血清中性脂肪が前値に比較して、66mg/dl以上増加した54例を食後高脂血症例として本研究に対象として登録した。この54例のうち無作為に25例にはエゼチミブ10mgが単独投与され (単独群)、29例にはエゼチミブ10mgとプラバスタチン10mgが併用投与された (併用群)。

観察期間は24週で、治療開始前と治療24週後での空腹時総コレステロール (TC), HDL-C, 中性脂肪 (TG), レムナント・コレステロール (RLP-C), 遊離脂肪酸 (FFA), HbA_{1c}, 高感度CRPを測定した。また食事負荷試験を治療24週後でも行い、食後高脂血症の推移を検討した。

両群の対象の臨床的特徴を表1に示しているが、単独群では平均年齢59歳、男性52%、平均BMI 23%、平均血圧130/79mmHg、高血圧症40%、喫煙32%、糖尿病28%、耐糖能異常28%で、併用群での平均年齢64歳、男性34%、平均BMI 24%、平均血圧132/79mmHg、高血圧症62%、喫煙41%、糖尿病31%、耐糖能異常24%と有意な差はみられなかった。

II. 成 績

治療前と24週後の食事負荷試験の成績を比較した。単独群では、治療前と比較して24週後の血清TG値は負荷前では136mg/dlから121mg/dlおよび負荷後1時間では170mg/dlから141mg/dlと有意に

表 1 エゼチミブ単独投与群およびエゼチミブ+プラバスタチン併用投与群のベースライン

	エゼチミブ単独群 (n=25)	エゼチミブ+ プラバスタチン併用群 (n=29)
年齢(y)	59±11	64±12
性(%)	13(52%)	10(34%)
BMI(kg/m ²)	23±3	24±3
血圧(mmHg)		
収縮期	130±15	132±19
拡張期	79±9	79±10
糖尿病(%)	7(28%)	9(31%)
喫煙(%)	8(32%)	12(41%)
高血圧症(%)	10(40%)	18(62%)
耐糖能異常(%)	7(28%)	7(24%)

表 2 エゼチミブ単独投与群における食事負荷試験の推移

	治療前 (n=25)	24週後 (n=25)	P value
TG(0h)	136±89	121±78	0.05
TG(1h)	170±89	141±78	0.0304
TG(2h)	238±112	194±97	0.18
ΔTG(0-2h)	102±38	73±36	0.0025
RLP(0h)	6.7±6.8	6.2±5.8	0.14
RLP(2h)	9.8±6.6	8.3±5.8	0.0350
ΔRLP-C(0-2h)	3.1±2.1	2.1±1.3	0.21
FFA(0h)	0.60±0.22	0.60±0.19	0.44
FFA(1h)	0.42±0.18	0.39±0.17	0.53
FFA(2h)	0.51±0.23	0.47±0.19	0.15

減少し、また、負荷前から負荷後2時間までの増加値(ΔTG/0~2時間)も102mg/dlから73mg/dlと有意に減少していた。RLP-Cは負荷後2時間で9.8mg/dlから8.3mg/dlと有意な減少がみられたが、FFAについては治療前後の差はみられなかった(表2)。

併用群では治療前と比較して24週後の血清TG値は負荷前では151mg/dlから109mg/dl、負荷後1時間では172mg/dlから121mg/dlおよび負荷後2時間では246mg/dlから167mg/dlと有意に減少し、また、負荷前から負荷後2時間までの増加値も94mg/dlから58mg/dlと有意に減少していた。RLP-Cは負荷前では8.0mg/dlから4.6mg/dlおよび負荷後2時間では11.1mg/dlから6.1mg/dlと有意な減少がみられ、また、負荷前から負荷後2時間までの増加値(ΔRLP-C/0~2時間)も3.1mg/dlから1.5mg/dlと有意に減少していた。FFAについては治療前後の差はみられなかった(表3)。単独群お

表 3 エゼチミブ+プラバスタチン併用投与群における食事負荷試験の推移

	治療前 (n=29)	24週後 (n=29)	P value
TG(0h)	151±55	109±44	0.014
TG(1h)	172±54	121±78	0.0034
TG(2h)	246±69	167±56	0.0001
ΔTG(0-2h)	94±25	58±32	0.0006
RLP(0h)	8.0±4.1	4.6±2.6	0.0053
RLP(2h)	11.1±4.6	6.1±3.1	0.0005
ΔRLP-C(0-2h)	3.1±1.9	1.5±1.4	0.0085
FFA(0h)	0.74±0.30	0.80±0.40	0.62
FFA(1h)	0.45±0.17	0.39±0.09	0.12
FFA(2h)	0.54±0.19	0.46±0.13	0.27

表 4 エゼチミブ単独投与群における生活習慣病関連のバイオマーカーの推移

	治療前 (n=25)	24週後 (n=25)	P value
TC	225±23	206±26	0.0497
LDL-C	139±15	124±12	0.0039
HDL-C	53±14	56±12	0.19
HOMA-R	1.8±1.3	2.4±2.3	0.27
HbA _{1c}	5.5±0.6	5.7±0.9	0.15
hs CRP	0.061±0.055	0.082±0.148	0.05

び併用群のいずれも食後高脂血症を抑制していたが、特に併用群で顕著であった。

その他の生活習慣病マーカーについて、治療前と24週後と比較した。単独群では治療前と比較して24週後の空腹時のTC値は225mg/dlから206mg/dlおよびLDL-Cは139mg/dlから124mg/dlと有意に減少していた。その他、HDL-C、HOMA-R、HbA_{1c}、高感度CRPに有意な変化はなかった(表4)。併用群では治療前と比較して24週後の空腹時のTC値は245mg/dlから206mg/dlおよびLDL-Cは151mg/dlから105mg/dlと有意に減少していた。また、HDL-Cは55mg/dlから58mg/dlと有意に増加していた。その他、HOMA-R、HbA_{1c}および高感度CRPに有意な変化はなかった(表5)。

Ⅲ. 考 察

食後高脂血症は、食後に血清中性脂肪濃度が一定以上に増加することを指しており、1979年 Zilvermit¹²⁾が食後高脂血症と動脈硬化との関連を指摘して以来、注目されてきた。食後の血清中性脂肪の高値は動脈硬化を促進するRLP-Cの存

在を示唆しており、Nordestgaardら¹³⁾はデンマークのコペンハーゲンの一般住民のコホート研究から食後高脂血症は心筋梗塞、虚血性心疾患および死亡を予測因子であること報告し、Bansalら¹⁴⁾は米国の女性のコホート研究から食後中性脂肪レベルが空腹時レベルより心血管イベントと関連していることを報告している。また、Isoら¹⁵⁾はTC低値の日本人においても食後高脂血症が心血管疾患の予測危険因子であることを示している。

食後の血清中性脂肪の推移は個人差が大きく、食後高脂血症の診断基準は未だ決まっていない。以上のことから、本研究では食事負荷試験に脂質が28.5g含まれているクッキーを用いて、負荷後2時間の血清中性脂肪が負荷前より66mg/dl増加した例を食後高脂血症例と判定した。わが国では食後高脂血症の評価を行うため、新鮮な生クリームを体表面積(m²)あたり30g負荷する方法が最も一般的であったが⁶⁾⁷⁾、新鮮な生クリームが必用であり、また負荷量が多すぎて被検者によっては、吐気、嘔吐、下痢などの症状が出現するなどの問題があった。その後、種々の脂肪負荷試験食が試みられているが、本試験に用いたクッキーは75gトレランGと同等の糖負荷試験結果になることも証明されており¹⁰⁾、負荷後の不快感も全くなく優良な負荷試験食と考えられる。

本研究の対象者はいずれも食事負荷試験により食後高脂血症と診断された患者で、腸管からの脂質吸収阻害薬であるエゼチミブ¹⁶⁾の投与に適していると思われる。特に糖尿病患者では腸管のmicrosomal triglyceride transfer protein (MTP)、Niemann-Pick C 1 like 1 protein (NPC1L1)のmRNAの発現が増加し、ATP-binding cassette transporters G5およびG8 (ABCG5およびABCG8)のmRNAの発現が低下していることも明らかとなっている。エゼチミブの作用するNPC1L1はコレステロールの腸管から体内への流入に、ABCG5およびABCG8は体内からの流出に関与する。また、MTPはカイロミクロンの生成、assemblyに関与する¹⁷⁾。著者らもメタボリック・マーカーを多く有する患者で、エゼチミブが有効であることを報告したが¹⁸⁾、糖尿病患者で高コレステロール血症を有する患者へのエゼチミブ投与は理にかなっていると思われる。すなわち亢進したNPC1L1やMTPによるカイロミクロンの産生増大を是正することにより、コレステロールの体内への流入を抑制し、その結果脂質代謝が改善される。

表5 エゼチミブ+プラバスタチン併用投与群における生活習慣病関連のバイオマーカーの推移

	治療前 (n=29)	24週後 (n=29)	P value
TC	245±34	206±26	0.0003
LDL-C	151±32	105±25	0.0006
HDL-C	55±11	58±11	0.0168
HOMA-R	1.8±1.4	2.2±2.3	0.72
HbA _{1c}	5.7±0.5	5.8±0.5	0.023
hs CRP	0.122±0.148	0.068±0.068	0.23

また、本研究でもHMG-Co還元酵素阻害薬であるスタチン系のプラバスタチン併用群でより良い成績が得られた。プラバスタチンはHMG-Co還元酵素を阻害することにより、肝でのコレステロールの合成を抑制し、肝臓内でのコレステロール貯蔵量を減少させ、肝細胞でのLDL-C受容体を増加させることにより、血中LDL-Cを低下させる。すなわち、エゼチミブが脂質の腸管からの吸収抑制作用に、スタチンのコレステロール合成作用とが相乗的に働いたものと考えられる。さらに、本研究では併用群の方が食後高脂血症の改善も著明であった。これはプラバスタチンのインスリン抵抗性改善作用も加わっているためと考えられる⁹⁾。インスリンの作用が不足することで、acyl-cholesterol acyltransferaseの活性化が増加し、コレステロールからコレステロールエステルへの反応が亢進し、腸管からのカイロミクロンの吸収が増加する可能性も指摘されている。すなわち、インスリン抵抗性が改善することにより、脂質の腸管からの吸収も減少しているため、エゼチミブ単独群より食後高脂血症の改善の程度が良いのではないかと考えられた¹⁹⁾。

スタチンの単独投与との比較は本研究では試みていないが、スタチン系であるアトルバスタチンおよびシンバスタチンでは腸管からの脂質の吸収が亢進することが報告されており²⁰⁾、食後高脂血症患者を対象とした場合には、スタチン系の増加投与は不的確と考えられた。

スタチンは高脂血症改善だけでなく動脈硬化の改善がみられることは知られており²¹⁾、また、糖尿病患者は心血管病のリスクが高く、スタチン療法はこれを有意に減少させることも証明され、糖尿病患者にはスタチンの投与が推奨されている²²⁾。一方、糖尿病患者では食後高脂血症の患者が多く、本研究の対象でも50%以上は糖尿病あるいは耐糖能異常であったが、これらに対する脂質改善療法

にスタチンを投与する場合, エゼチミブの併用を考慮すべきではないかと考えられた。

結 語

スタチンが脂質の吸収を亢進することを考えると, 食後高脂血症の患者にはエゼチミブの投与, 抗動脈硬化も考慮した場合エゼチミブと通常用量のスタチンとの併用が適していると考えられた。

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たんじゅうさん

胆汁酸今昔物語

「胎児—新生児期の胆汁酸に魅せられて」藤間貞彦……2

鼎談

「代謝ホルモンとして機能する胆汁酸—代謝性疾患から癌まで—」

榎島 誠／渡辺光博／松崎靖司（司会）……3

新しい視点：特集「Genericの現状」

「C型慢性肝炎に対するグリチルリチン注射剤の有効性とその後発品の功罪」林 純ほか……12

「薬学的観点から後発医薬品を考える」眞野成康……13

「Generic医薬品の品質について

—ウルソデオキシコール酸剤の例—」堀切勇児ほか……14

「Generic（後発）医薬品の現状と今後」中木敏夫……15

「Generic医薬品使用促進に向けての薬剤師の役割」二神幸次郎……16

診療最前線Q&A

「Q：ウルソ600～900mg/日投与に際し、

下痢の発現が心配なのですが…」回答／滝川 一……17

胆汁酸EBM

「PSCに対するUDCA用量設定に関する多施設臨床試験」菅野啓司ほか……18

ニュース・話題

「医療崩壊」本田 宏……20

胆汁酸研究のあゆみ

「私とウルソ」吉川正英……21

おじゃまします—北から南から—

「ウルソ賞を励みに—臨床だけでなく研究にも興味を」黒木祥司……22

最新のガイドラインを学ぶ

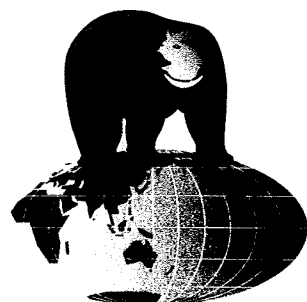
「高血圧」檜垣實男……25

研究会予告

「第25回日本静脈経腸栄養学会」……19 「第31回胆汁酸研究会」……19

「第26回日本胆膵生理機能研究会」……26

お知らせ／スナップ写真……27



表紙の説明

筑波大学附属病院院長時代の院長室での大菅俊明先生。業務激務の中、胆汁酸研究などにつき目を輝かせながら在りし日の先生が思い出されます。（松崎靖司編集委員、写真提供：大菅奈々様）

C型慢性肝炎に対するグリチルリチン注射剤の有効性とその後発品の功罪



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古庄憲浩

本邦ではC型慢性肝炎 (chronic hepatitis C; CHC)患者が多く存在し、その多くが肝癌へと進展するため、CHCに対しては肝癌発症防止効果も期待したインターフェロン療法が主として行われている。しかし、インターフェロン療法で効果が得られない症例や、その副作用のため治療困難な症例もある。このような症例に対する静注グリチルリチンは、肝機能検査の改善だけでなく、肝癌発症を抑制していることも報告されており、CHCにとっては重要な薬剤の一つである。

静注グリチルリチンである強力ネオミノファーゲンC (SNMC: ミノファーゲン社)は、1977年に二重盲検法により、慢性肝炎に有効であることが確認され、CHCに対しても汎用されている。その一方で、政府が勧める医療費削減のためこの薬剤の後発品が多く使用されているが、その効果が疑問視されていた。以上のことから、筆者らはSNMCとその後発品である静注グリチルリチン剤のCHCに対する効果を検討した。対象としたCHC患者はSNMC (60~100ml)を6カ月以上投与され、ALT値が平均151.6 IU/Lから63.2 IU/Lに低下した84例であった。このうち48例は後発品(投与量は変更していない)に変更したが、3カ月後にALT値は約2倍(p<0.0001)に上昇した。一方、SNMC継続投与36例ではALT値は低値に留まっていたことから(図1)、今回使用した後発品はCHCに対する効果はないと考えられた¹⁾。伯井ら²⁾はSNMCと4社の後発品の成分を調査し、主成分であるβグリチルリチンの成分が後発品では20%以上低濃度であると報告している。

一方、著者らは主成分であるβグリチルリチンがSNMCと同等に多く含まれているミノフィット(テルモ社)60mlをCHC患者29例に6カ月間投与し、SNMC投与16例と比較検討した。ALT値はミノフィット群でもSNMC群と同程度に低下していた(図2)。また、SNMCからミノフィットに変更した例でもALT値は上昇しなかった(図3)³⁾。

以上のことから、後発品の製造販売会社は、薬剤の成分および先発品との差を正確に明示し、また、治験も行った後に販売するべきではないと思われる。

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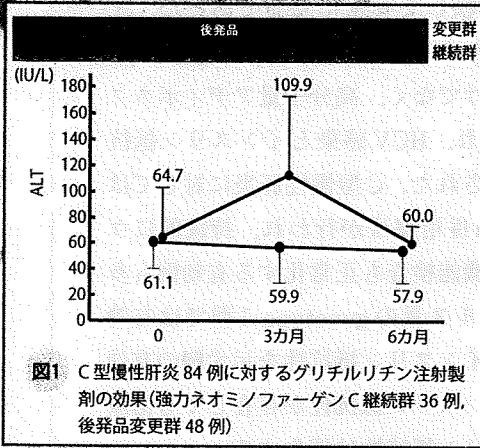


図1 C型慢性肝炎 84例に対するグリチルリチン注射剤の効果(強力ネオミノファーゲンC継続群 36例, 後発品変更群 48例)

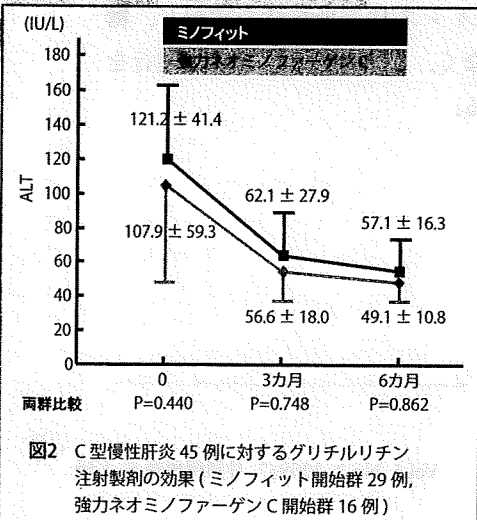


図2 C型慢性肝炎 45例に対するグリチルリチン注射剤の効果(ミノフィット開始群 29例, 強力ネオミノファーゲンC開始群 16例)

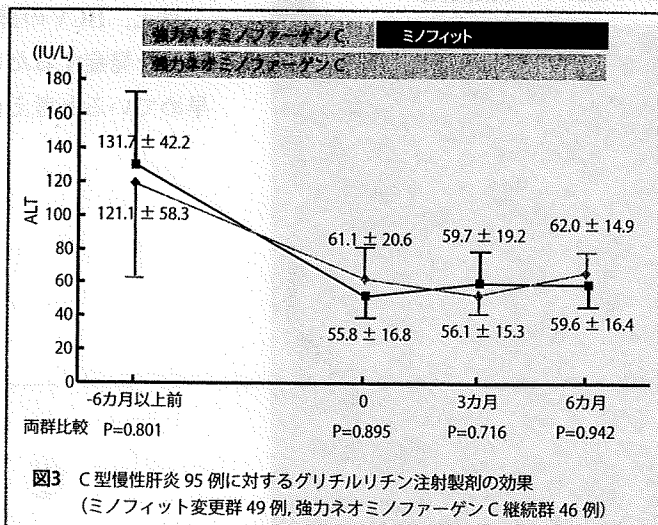


図3 C型慢性肝炎 95例に対するグリチルリチン注射剤の効果(ミノフィット変更群 49例, 強力ネオミノファーゲンC継続群 46例)