The CD8/CD4 ratio is said to be higher in the liver than in peripheral blood, and French and co-workers [66] demonstrated that CD4+ cells are predominant in zone 3 and CD8+ cells are predominant in zone 1, although both cells are seen in the portal area. MHC class I expression correlates with levels of portal inflammation and interface hepatitis, and MHC class II expression correlates with hepatocyte necrosis and appearance of Mallory bodies. CD29, CD45 RA, and CD45 RO, which are important for recognition of allo antigens or for adhesion, are highly expressed in the necrotic area and/or in Mallory-positive liver. These findings indicate that cytotoxic T cells (CTLs) are important to the progression of alcoholic hepatitis as well as to the progression of viral hepatitis.

Fas-Fas-ligand (FasL)-mediated CTL and TNF-α play important roles in chronic hepatitis C virus (HCV) infection [67], and alcohol abuse may exaggerate the cytotoxic process [68] because alcohol increases Fas/Fas-L expression [69,70]. Expression of another important cytotoxic mediator, perforin-granzyme, requires MHC class I expression, and alcohol intake increases its expression, suggesting that alcohol drinking increases CD8+ T cell-mediated cytotoxicity in the liver [71].

## 5. Role of antibody-dependent cell-mediated cytotoxicity

Antibody-dependent cell-mediated cytotoxicity (ADCC) is thought to be involved in the liver damage in alcoholic hepatitis. Autoantibodies against CYP3A4 and CYP2E1 are found in 20–30% and 10–20%, respectively, of healthy individuals [72], and the titers of these autoantibodies in the Tsukamoto–French model rise to two- to three-fold above the control level 1 month after the start of feeding and they correlate with levels of liver damage. Administration of chlormethiazole, an inhibitor of CYP2E1, reduced CYP2E1 activity as well as the autoantibody titers [73]. A similar phenomenon has also been observed in regard to other autoantibodies, such as anti-hydroxyethyl adduct and anti-malondialdehyde adduct, in both animal models and humans [74]. These adducts are produced during oxidation of ethanol by CYP2E1. Accumulation of the autoantibodies to these adducts is observed on hepatocyte membranes by confocal microscopy [75], suggesting that the ADCC mechanism may operate in alcoholic hepatitis [66,76]. Vidali et al. [77] recently reported that polymorphism in the exon 1 of CTL antigen (CTLA)-4 gene induces disregulation of T-cell proliferation, leading to production of autoantibody against CYP2E1.

## 6. Role of monocytes

The peripheral blood monocytes of alcoholic hepatitis patients produce more TNF-α, both in the presence and absence of stimulation by LPS, than those of healthy controls [31], and TNF receptor expression is increased [30]. The expression levels are always correlated with progression to liver cirrhosis, and the same phenomenon is observed in regard IL-6 production. The production levels and serum levels of MCP-1 and MIP-1 are significantly higher in alcoholic hepatitis patients than in healthy controls [42,78]. A recent study demonstrated that acute alcoholic stimulation inhibits cytokine production from monocytes, but chronic stimulation up-regulates production of cytokines and reactive oxygen from monocytes [79], suggesting that pathophysiological mechanisms are different between acute injury and chronic injury. Thus, inhibition of monocyte activation is now going to be the target of the treatment of alcoholic hepatitis in the future.

Chronic ethanol administration to rats for 6–8 weeks makes hepatocytes more susceptible to injury by TNF-α [80], and the mechanism of the change in susceptibility is attributable to a change in the membrane permeability of mitochondria [81]. Recent studies have shown that liver injury always correlates with genomic polymorphisms of manganese superoxide dismutase [82], while a negative report appeared after then. Further studies are needed in regard to this matter [83].

## 7. Conclusion

We have reviewed the immunological aspects of alcoholic hepatitis. Although many factors such as hypoxia have been implicated to have a significant role in the pathogenesis of alcoholic liver disease [84], immunologic factors seem to be still important for making its individual difference. The major reason why the precise immunological mechanism has not been understood in the pathogenesis of alcoholic hepatitis is a lack of adequate animal models, the same as in viral hepatitis. Integration of human investigations and accumulated information from various animal models will gradually clarify the immunological mechanisms of alcoholic hepatitis in the future.

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# Lifestyle guidance for patients with chronic liver diseases; information provision via educational classes on liver diseases

Shinzo Kato*, Hiromasa Ishii

*Division of Gastroenterology, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo, Japan*

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**Abstract**

Patients with chronic liver disease need information on the disease to keep their good quality of life. Educational class on liver disease is one of solutions for that. We have started the class in 1992, and continued to run it once per month. Exchange of information among patients at group work is also helpful to relief their anxiety related to their disease. Many hospitals are now preparing to establish such classes in Japan. In this article, we present tips on providing information to patients with chronic liver diseases and advice on class management.


## 1. Introduction

Patients with chronic liver diseases require detailed information on the various aspects of their disease [1]. For example, the recent media focus on hepatitis C virus associated with contaminated blood products has led to patients with hepatitis C virus infection becoming anxious about their future. Because they are also worried that the viruses will infect their family and other people, they should be provided with education about prophylaxis [2]. Various new pharmaceuticals, such as interferon and other antiviral agents, have been developed and placed on the market, but their severe side effects are also reported in the media, leading to confusion about the new pharmaceuticals [3]. Because hepatic cancer may develop during their clinical course, patients also suffer from cancer-related anxiety. Once cancer develops, the importance of information provision increases when patients are informed the name of their diseases and when the therapeutic regimen is selected. Various techniques have been developed for treating esophageal varices and liver cancer, which are common complications of liver cirrhosis, and patients need to be involved in the selection of therapeutic regimens. When the cancer advances, palliative treatment is required, and care for patients' families also becomes important [1].

The importance of rest and a high protein diet for patients with chronic liver diseases was overemphasized for many years in Japan, which led some patients to pay too much attention to resting, and others to suffer from hepatic encephalopathy due to an excessive intake of high protein foods. Furthermore, obesity is recently reported to be a risk factor for the development of liver diseases, such as fibrosis and cancer [4–14]. In order to educate about a truly balanced lifestyle, we should work to overturn common misperceptions held by the patients.

Although information provision to patients is difficult, patients require appropriate and comprehensive information on chronic liver diseases. Falvo [15] stated that 'patient education is a right of patients and a duty of medical staff.' However, in Japan, many patients are at a loss what to do due to insufficient information from medical staff.

In order to respond to these patient demands, we started an educational class on liver diseases in 1992, and continue to run this class once per month. This class has been improved so that, information is not only unilaterally provided from doctors and nutritionists, but is also exchanged and shared among the patients. In recent years in Japan, hepatologists

* Corresponding author. Tel.: +81 33353 1211x62298; fax: +81 33353 6247.
*E-mail address:* katos@mvc.biglobe.ne.jp (S. Kato).

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Table 1
Facilities with laboratories for liver disease

| | In operation | Preparation underway | Interested | Total | Facilities certified by the Association of Gastroenterology | Educational facilities certified by the Association of Internal Medicine |
|---|---|---|---|---|---|---|
| Hokkaido | 3 | 1 | 26 | 30 | 39 | 42 |
| Tohoku | 3 | 2 | 25 | 30 | 43 | 61 |
| Kanto/Shin-etsu | 7 | 1 | 11 | 19 | 83 | 126 |
| Tokyo | 5 | 2 | 19 | 26 | 67 | 67 |
| Yokohama | 3 | 2 | 10 | 15 | 34 | 38 |
| Tokai | 3 | 1 | 2 | 6 | 84 | 110 |
| Kyoto/Hokuriku | 5 | 1 | 8 | 14 | 42 | 62 |
| Osaka | 9 | 5 | 37 | 51 | 91 | 115 |
| Chugoku | 6 | 2 | 32 | 40 | 52 | 56 |
| Shikoku | 6 | 4 | 6 | 16 | 24 | 39 |
| Kyushu | 14 | 4 | 11 | 29 | 54 | 75 |
| Total | 64 | 25 | 188 | 277 | 613 | 791 |

Based on research by the Ajinomoto Pharmaceutical Co. Ltd. June 2003.

have belatedly realized the necessity of providing education and information to patients with chronic liver diseases, and about 10% of postgraduate education hospitals in Japan now run educational classes on liver diseases (Table 1). Many hospitals are now preparing to establish such classes. Therefore, we expect that educational classes on liver diseases will quickly become common. In this article, we present tips on providing information to patients with chronic liver diseases and advice on class management.

## 2. Goals of educational classes on liver diseases

The primary goal of educational classes on liver diseases is to improve the quality of life (QOL) of patients with liver diseases. For patients with HIV infection, the necessity of mental health support from counselors has been discussed extensively, but medical staffs rarely recognize that this is also a necessity for patients with HCV or HBV infection [16–19]. It has been reported that patients with HCV-related chronic hepatitis who were informed of their viral infections had a lower QOL than patients who were not informed [20]. However, considering that information disclosure and transparency are presently required, it is unacceptable not to inform patients of their HCV infections. Therefore, the issue is how to provide information without decreasing QOL. Providing information in an appropriate way may help the patients to lead happier, more active lives.

## 3. Preparations for opening classes

Firstly, a room is booked for class, and posters are displayed on the wall of the out-patient clinic in order to inform patients of the class opening. We display posters on the wall of our out-patient clinic to inform patients about our monthly class, which is open-access for patients. We have 30–50 patients a month in our class. The number of patients who are starved of information is larger than we think. We also request that doctors in other departments, such as general surgery and radiology, participate in lectures on liver diseases, which are held once or twice per year. When we broadly announce these lectures using large posters, more than 200 people want to attend, which is over the capacity of the hall. Therefore, we use a pre-registration system for these lectures. When an educational class is opened for the first time, one option is to adopt a pre-registration system, taking into consideration the preparation of documents and the size of the classroom.

The speech content is summarized using presentation software (PowerPoint) [1], and slide content is printed out and given to patients as handouts. Recently, many patients are disappointed or complain if handouts are not prepared. Therefore, it is advisable to prepare handouts, even though the actual information content may only be an overview.

## 4. Frequency and duration of classes and detailed content

We run classes once per month and repeat four themes. Classes are held on Saturday mornings, because it is easy for patients to attend. Each class lasts approximately 2 h, and we give a lecture of 45 min–1 h, including the question period. After the lecture, there is a question-and-answer session (30 min) and a group work session (30 min).

The four themes picked up in classes are (1) lifestyle precautions for patients with liver diseases, (2) what liver examinations are checking for, (3) chronic hepatitis; interferon therapy and antivirus therapy, and (4) complications of liver cirrhosis and their treatments. If there are too many themes, patients will hesitate in attending, and medical staff will also acquire the added burden of extra preparation time. Therefore, we limit the themes to four and repeat the same themes, and gradually add new content in each class. When the same theme is repeated every four classes, even if patients have not been able to attend for 1 month, they are able to attend a class which deals with the same theme after just three more classes.

Some consider that classifying patients by clinical stage or by cause of liver diseases is efficient for class management. However, we aim to operate a class management system that allows patients at all clinical stages of liver diseases to participate and encourage each other. Therefore, we do not specifically target the patients for classes. In fact, at our classes, we sometimes observe patients with liver cirrhosis encouraging patients with chronic hepatitis. It is better to request nutritionists and nurses to join classes, because many of them are interested in patient education. In the Japanese medical system, we can claim health care costs as group nutritional guidance when nutritionists join. We consider that this kind of patient education should eventually be provided by nutritionists and nurses. However, to continue these patient classes in Japan, physicians should first understand their significance.

In Japan, many doctors still emphasize the importance for patients to rest. However, patients with chronic diseases require information about how much exercise they can do without problems, instead of how much rest they should take [21–23]. We attempted to give exercise guidance to patients with chronic hepatitis or liver cirrhosis, and found that they were able to do aerobic exercise without causing deterioration of their liver and nervous functions. When exercise intensity is within 60% of its maximum, exercise does not increase blood ammonia levels. In terms of ammonia metabolism and sugar metabolism, muscles work similarly in the liver and may compensate for hepatic dysfunction. Therefore, in order to maintain muscle mass, it is important to actively instruct patients to do some exercise, rather than to rest for long-periods of time or throughout their life.

## 5. Provision of information among patients: group work

When there are not too many participants (around 20 patients), active participation may be encouraged by the question-and-answer session alone. However, if the number of participants is larger than this, many patients are discouraged from active participation, such as asking questions and presenting their opinions. Therefore, we make groups of approximately five patients and let them actively exchange their opinions in these smaller groups. Group work allows patients to talk about their clinical conditions, examinations, therapies, problems that they have and their strategies for dealing with such problems with other patients (Table 2). The perspective on information that is exchanged among patients is different from that provided by doctors, the former information being more familiar and authentic for patients. In fact, during group work, patients become more active than we had anticipated and confer about a wide range of issues in a positive manner. In these groups, patients discuss issues that they do not usually talk about with doctors. Many people are concerned that group work does not work well in small communities or in rural areas, because there may be many acquaintances in a group. However, the medical staff from an institution in a rural area, who had visited our institution, attempted group work and also reported that it worked well.

At the start of group work, we always explain the rules and present examples of topics (Fig. 1). For this, we present

Table 2
What is group work?

| |
|---|
| Its objective is to utilize information exchange between patients, and not to provide information solely from doctors to the patients |
| In most cases, the information provided to patients from other patients is more beneficial than the information provided by doctors |
| Discussion of experiences, including those regarding liver biopsies, TAE, PEIT and RF |
| How to deal with such problems as leg cramps, lethargy, fatigue, or depression |
| Patients can see the prospects for the future course of their illness by observing other patients |
| Patients with chronic hepatitis will understand the circumstances of the daily life of patients with cirrhosis or HCC |
| Doctors will give advice to the patients when needed |

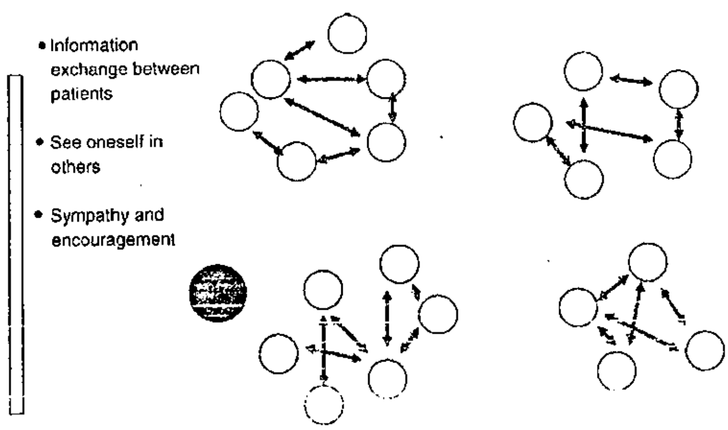


Fig. 1. Group work format. Information exchange between patients. See oneself in others. Sympathy and encouragement.

Table 3
Group work rules

| |
|---|
| 1. Discuss your problem with, and how you are bothered by, having liver disease |
| 2. Give priority to patients with the greatest need for discussion, or to new patients |
| 3. The discussion with each patient will last from 3–5 min |
| 4. Start with a short introduction. You do not have to give your real name; an assumed name or nickname will be fine |
| 5. Your opinion is for reference purposes only. Do not try to push your opinion on others |
| 6. Raise your hand and ask to be recognized when you want to ask for the opinion of a doctor or medical personnel |

the slide shown in Table 3. Attention should be paid not to let discussion place disproportionate weight on one topic, such as folk remedies or the soliciting of patients to join particular religious groups. It is better to mix patients, who have experienced group work several times with patients who have never experienced it. When group discussion is not so active, medical staff needs to help (e.g. by providing a topic that works as a trigger for discussion).

## 6. Collecting fees and countermeasures against costs, including the cost of lectures

At present, our classes are free. We expect that the day will soon come when the necessity of providing education to patients with chronic liver diseases is understood, and when we can claim the medical expense for this education as a health care cost. At present, as previously mentioned, when nutritionists join, costs can be claimed as group nutritional guidance. Additionally, another option is that participation in classes is defined as self-motivating instead of compulsory, and that patients pay for the cost of the room and documents.

## 7. Problems that are frequently faced in class management, and tips for continuing classes long-term

The ultimate goal of these educational classes is to improve the QOL of patients. When medical staff speak to patients in an authoritative manner or preach to them, fewer patients want to continue attending classes, so improvement of their QOL cannot be achieved. In order for classes to continue for a long-time, the term 'empowerment' should be deeply understood by med staffs [14], and in class management, we should always consider how to make the lives of patients with chronic diseases meaningful and satisfying. When too many medical staffs are involved in classes, it is difficult to achieve improved efficiency and simplified health care (which is also a goal of opening classes), and it also becomes difficult to continue classes from a financial perspective.

## 8. Conclusion

Starting educational classes on liver diseases is not as difficult as it seems. When the opportunity arises, it would be a good idea to open classes because of the multiple benefits they bring. Once you open a class, you will encounter problems and will be able to use your experiences to improve future classes. These educational classes will be beneficial and able to continue successfully if patients can become less anxious about their diseases and feel even a slight sense of relief by the end of each class.

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HEPATOLOGY

# Kupffer cell depletion attenuates superoxide anion release into the hepatic sinusoids after lipopolysaccharide treatment

MASAHIKO FUKUDA, HIROKAZU YOKOYAMA, TAKESHI MIZUKAMI, HIDEKI OHGO, YUKISHIGE OKAMURA, YOSHITAKA KAMEGAYA, YOSHINORI HORIE, SHINZO KATO AND HIROMASA ISHII

*Department of Internal Medicine, School of Medicine, Keio University, Japan*

**Abstract**

***Background and Aim***: The mechanisms involved in the beneficial effect of gadolinium chloride against endotoxin-induced liver damage were studied.

***Methods***: Superoxide anions released into the hepatic sinusoids were examined in a liver perfusion model using the cytochrome C method.

***Results***: Gadolinium chloride treatment fully depleted ED2-positive cells from the liver and significantly attenuated superoxide anion release after a lipopolysaccharide or tumor necrosis factor-α (TNF-α) challenge. Moreover, gadolinium chloride treatment resulted in a significant decline in endothelial cell damage in the hepatic sinusoids as assessed by the purine nucleoside phosphorylase/glutamic–pyruvic transaminase ratio in the liver perfusate. Although gadolinium chloride treatment did not affect the level of serum TNF-α, it significantly reduced that of interleukin (IL)-8 and neutrophil migration in the hepatic sinusoids after the lipopolysaccharide challenge.

***Conclusion***: These data suggest that a reduction of the superoxide anion level in the hepatic sinusoids in acute endotoxemia and subsequent reduction of neutrophil migration into the liver may indicate that gadolinium chloride treatment suppresses the progression of liver damage in acute endotoxemia.


**Key words**: free radical, $GdCl_3$, Kupffer cell, macrophages, neutrophils.

## INTRODUCTION

Endotoxin from intestinal bacteria reaches the liver via the portal vein.[1] The endotoxin is mainly eliminated by Kupffer cells, the resident macrophages of the liver. Excessive endotoxin, however, can cause liver injury,[2,3] and there are numerous lines of evidence indicating that Kupffer cells play a central role in the progression of endotoxin-dependent liver injury.[4] The recently prevailing view is that treatment with gadolinium chloride attenuates the progression of endotoxin-related liver damage via inactivation of Kupffer cells.[5,6] However, the exact mechanisms behind this phenomenon are, so far, unclear.

Bautista and Spitzer demonstrated that treatment with lipopolysaccharide (LPS), the main component of endotoxin, results in superoxide anion release into the hepatic sinusoids.[7] They also showed that tumor necrosis factor-α (TNF-α) plays a crucial role in the superoxide anion formation.[8] We were able to confirm these results, and our preliminary data indicated that superoxide anion release into the hepatic sinusoids might cause damage to hepatic sinusoidal endothelial cells.[9] Moreover, Bautista and Spitzer used cultured cells to show that superoxide anions are mainly released from Kupffer cells, whereas the participation of endothelial cells and hepatocytes was minimal, at least after phorbolmyristate ether treatment.[10] Thus, it is conceivable that Kupffer cell depletion diminishes superoxide anion level in the hepatic sinusoids in acute endotoxemia and subsequent neutrophil migration into the liver. This supports the reported finding that gadolinium chloride treatment suppresses the progression of liver damage caused by acute endotoxemia.

Correspondence: Dr H Ishii, Department of Internal Medicine, School of Medicine, Keio University, Japan, 35 Shinanomachi Shinjukuku, Tokyo 160-8582, Japan. Email: hishii@sc.itc.keio.ac.jp
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# METHODS

## Experimental animals and treatments

Male Wistar rats, weighing 250–300 g, were used in the experiments. All animals received humane care including anesthetic, in compliance with the Guidelines for the Care and Use of Laboratory Animals of Keio University School of Medicine (Tokyo, Japan). To deplete Kupffer cells, gadolinium chloride (Sigma Chemical, St.Louis, MO, USA), at 10 mg/kg bodyweight, was injected intravenously from the tail vein twice, 24 h apart.[11] Another group of rats received an equal volume of saline (the non-depleted rats). Twelve hours after the second injection, LPS (from *Escherichia coli* serotype 0111-B4: Sigma Chemical) at 2 mg/kg bodyweight dissolved in saline was injected intravenously into the Kupffer cell-depleted and non-depleted rats. An equal volume of saline without LPS was given to the control group of both the Kupffer cell-depleted and non-depleted rats. In a separate experiment, TNF-α at 5000 U/kg bodyweight was infused continuously for 2 h into the portal vein of both the Kupffer cell-depleted and non-depleted rats.

## Detection of superoxide anions released into the hepatic sinusoids

Superoxide anion release into the hepatic sinusoids was measured according to the method by Bautista *et al.* with some modification.[8] After the LPS or saline administration, the portal vein was cannulated with an 18 G Teflon catheter, and the liver was perfused with Hanks' balanced salt solution (HBSS, Gibco BRL, Grand Island, NY, USA) at a rate of 2–3 mL/min per g wet liver. After the blood had been fully removed from the liver, the infusion solution was changed to HBSS containing 50 μmol/L oxidized cytochrome C (Wako Pure Chemical Industries, Tokyo, Japan, solution A). The perfusate was collected from the inferior vena cava every 2 min for 16–20 min, and the absorbance of each sample was measured at 550 nm[12] with a spectrophotometer (MPS-2000, Shimadzu, Kyoto, Japan). In each series, the difference between the absorbance of solution A and the highest absorbance among the samples was expressed as ΔABS. This reflects the formation of the reduced form of cytochrome C converted from the oxidized form in the presence of superoxide anions, and represents the amount of superoxide anions released into the hepatic sinusoids.[9] The superoxide anion level was examined in rats prepared 5 min, 1 h, 3 h, 12 h, and 24 h after the LPS treatment.

## Population of Kupffer cells and hepatic macrophages in the liver specimens

Three hours after the LPS challenge, liver samples were obtained from each group and embedded in OCT compound (Tissue-Trek; Sakura Fine Technical Tokyo, Japan). Frozen sections (6 μm) were prepared with a cryostat (Tissue-Trek). Immunohistochemical analysis was performed with a monoclonal antibody to rat macrophages (Mar1; Seikagaku, Tokyo, Japan)[13] or an ED 2 antibody, which specifically recognizes Kupffer cells (Seikagaku).[14] The immune reaction was visualized with a peroxidase-labeled affinity purified antibody to mouse IgG (H + L) adsorbed with rat serum (Kirkegaard & Perry Laboratory, Gaithersburg, MD, USA) and 3,3′-diaminobenzidine tetrahydrochloride (Sigma). The number of positive cells per high-power field (×400) was determined by light microscopy. We prepared five specimens in each group and cells were counted in five high-power fields chosen at random in each specimen.

## Neutrophil population in the liver specimens

Liver samples were obtained from each group, fixed with 10% formaldehyde, and embedded in paraffin. Thin sections (3 μm) were prepared and stained with hematoxylin and eosin (H&E). The number of neutrophils per high-power field (×1000) was determined by light microscopy. The cell population was examined as aforementioned in rats prepared 5 min, 1 h, 3 h, 12 h, and 24 h after the LPS treatment.

## Levels of TNF-α-and interleukin-8 in the portal vein

A blood sample was collected from the inferior vena cava of each animal 5 min, 1 h, 3 h, 12 h, and 24 h after the LPS or saline treatment. Time-courses of the TNF-α and interleukin (IL)-8 levels were measured in the sera with ELISA kits (Rat TN-Fα immunoassay; Techne Corporation, Minneapolis, MN, USA; and Rat IL-8 measuring kit, Immuno-Biological Laboratories, Gunma, Japan).

## Purine nucleoside phosphorylase activity/glutamic–pyruvic transaminase activity ratio in liver perfusate

To evaluate the endothelial cell damage in the hepatic sinusoids, the purine nucleoside phosphorylase (PNP)/glutamic–pyruvic transaminase (GPT) ratio[15] was determined in the liver perfusate 3 h after the LPS challenge. The PNP activity was measured by the method described by Hoffee *et al.*[16] Briefly, 100 μL of the liver perfusate was incubated with 100 μL of potassium phosphate buffer (0.05 mol/L, pH 7.4) containing 0.5 nmol/L of inosine (Wako Pure Chemical Industries) and 10 pg/mL of xanthine oxidase (from milk, Sigma) at 37°C. The change in absorbance was measured spectrophotometrically at 293 nm. This represents the PNP activity. The GPT activity in the perfusate was measured with a kit (Unikitrate GPT, Kanto Kagaku, Tokyo, Japan).

## Statistical analysis

The data were expressed as means ± SD. Differences between two groups were assessed by the Student's *t*-test. Comparisons among more than three groups were performed by one-way ANOVA with the Newman–Keuls test. The difference was considered significant at $P < 0.05$.

## RESULTS

To verify that Kupffer cells were successfully depleted from the liver with gadolinium chloride, ED2-positive cells were immunohistochemically examined in liver specimens. As shown in Fig. 1, ED2-positive cells were observed at very low numbers in the livers of rats given gadolinium chloride, even after the LPS challenge. Gadolinium chloride also significantly reduced the population of cells positive for antimacrophage antibody (Fig. 2) and neutrophils (Fig. 3) in the liver, although they were not totally depleted. The results of the morphometric analysis are summarized in Table 1.

The superoxide anion level in the hepatic sinusoids was examined. Absorbance at 550 nm was measured in the liver perfusate every 2 min and it was found to reach a maximum plateau 8–10 min after the start of HBSS administration containing oxidized cytochrome C (solution A). This finding has already been published elsewhere.[9] The difference between the peak absorbance and the absorbance of solution A was designated as ΔABS. It was significantly higher in the rats given LPS than in those given saline. When superoxide dismutase was simultaneously given in the liver perfusate, it was significantly suppressed and thus, the value of ΔABS was concluded to represent the level of superoxide anion formation in this model.[9]

In the present study, we could reproduce the phenomenon whereby LPS increased the superoxide anion release into the hepatic sinusoids. The time-course of the superoxide anion level in the hepatic sinusoids was studied up to 24 h after the LPS challenge. Superoxide anions were not detectable 5 min after the LPS

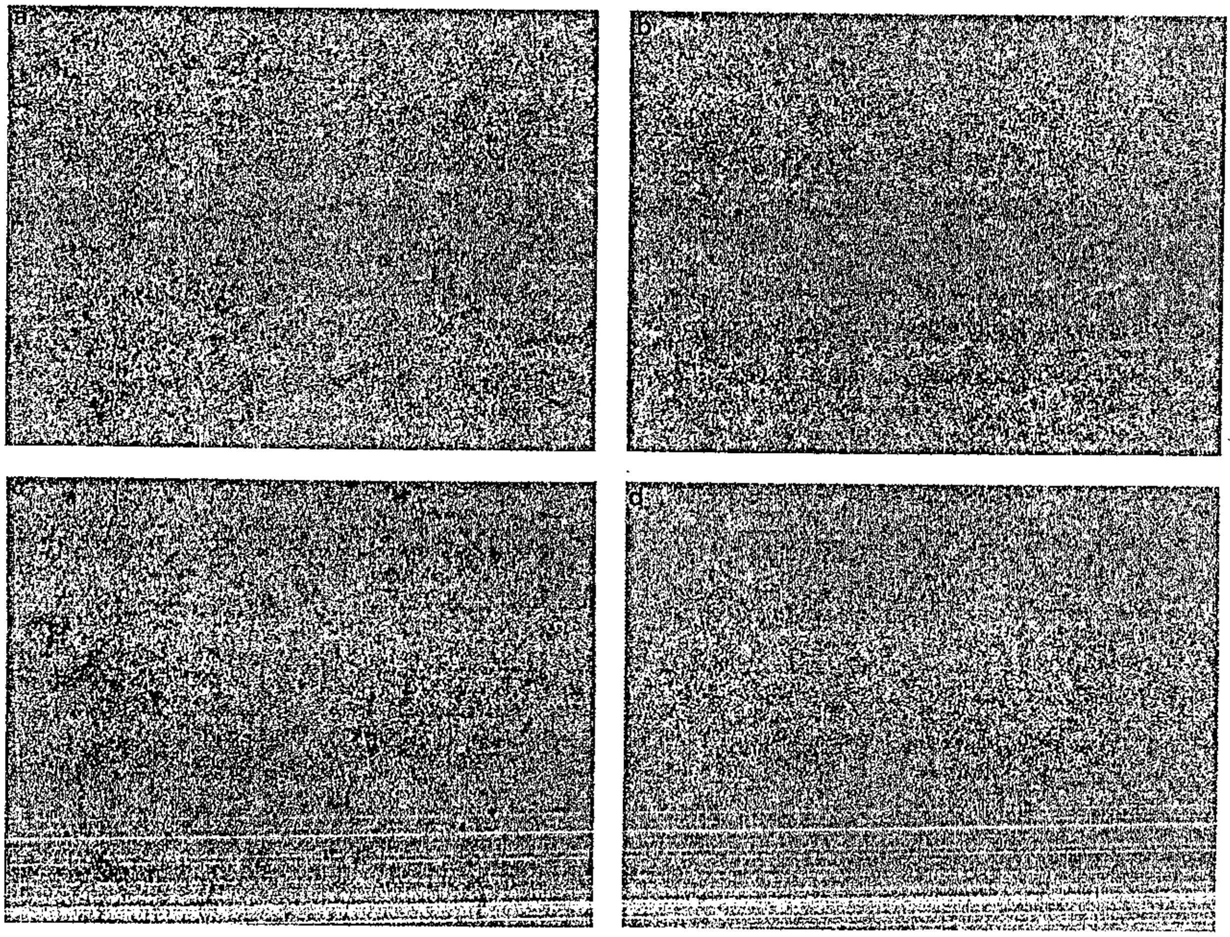

**Figure 1** Effect of gadolinium chloride on the population of ED2-positive cells after lipopolysaccharide (LPS) challenge in the liver. The population of ED2-positive cells in the liver was immunohistochemically studied. (a) Rats given saline instead of gadolinium chloride and saline instead of LPS. (b) Those given gadolinium chloride and saline instead of LPS. (c) Those given saline instead of gadolinium chloride and LPS. (d) Those given gadolinium chloride and LPS (immunohistochemistry ×20).

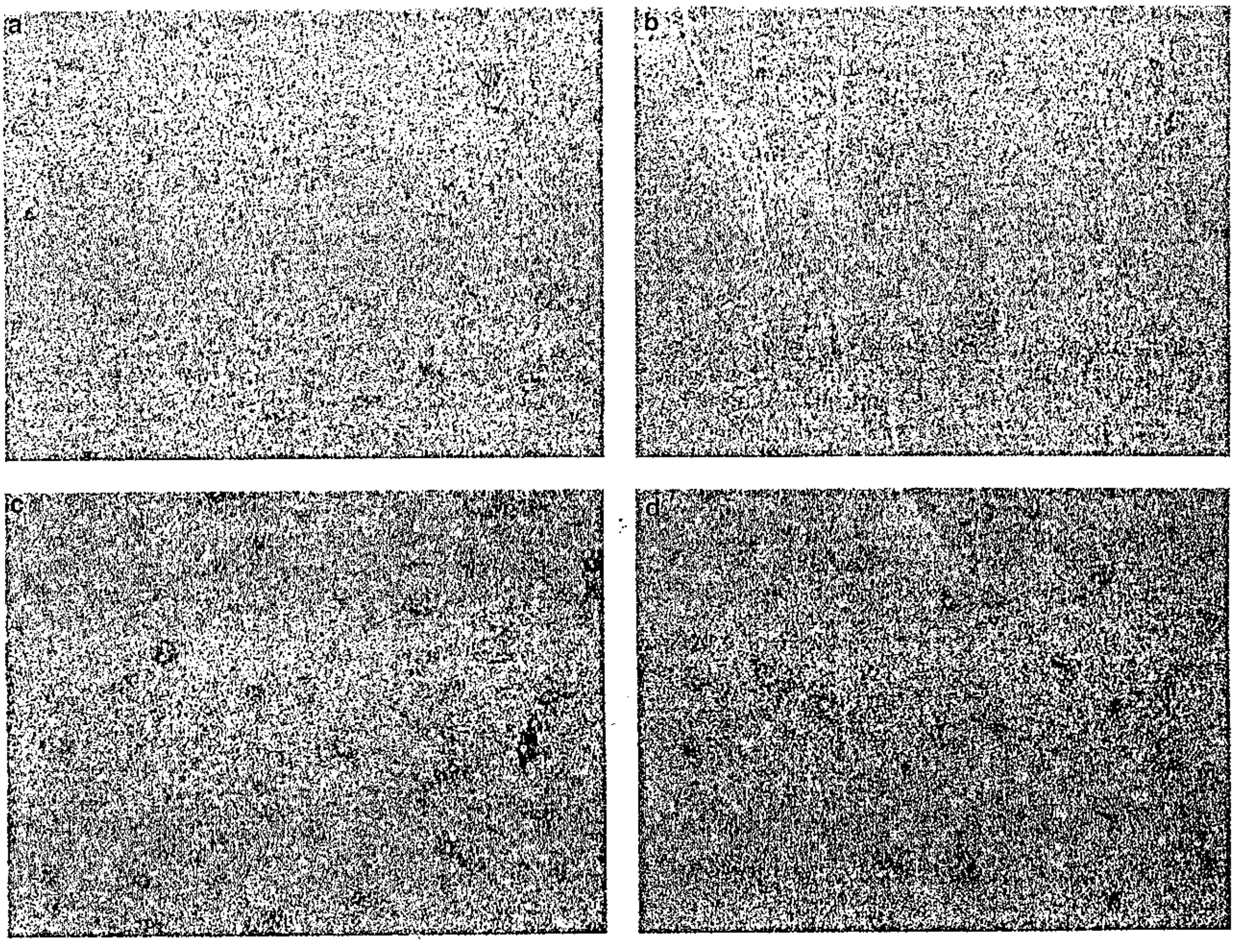

**Figure 2** Effect of gadolinium chloride on the population of macrophages after lipopolysaccharide (LPS) challenge in the liver. The population of macrophages in the liver was immunohistochemically studied with an antibody to macrophage. (a) Rats given saline instead of gadolinium chloride and saline instead of LPS. (b) Those given gadolinium chloride and saline instead of LPS. (c) Those given saline instead of gadolinium chloride and LPS. (d) Those given gadolinium chloride and LPS (immunohistochemistry ×20).

**Table 1** Effect of gadolinium chloride on the populations of ED2-positive cells, macrophages, and neutrophils in the liver 3 h after lipopolysaccharide (LPS) treatment

| | Saline + saline ($n$ = 5) | Saline + $GdCl_3$ ($n$ = 5) | LPS + saline ($n$ = 5) | LPS + $GdCl_3$ ($n$ = 5) |
|---|---|---|---|---|
| No. ED2-positive cells | 42 ± 3 | ND | 65 ± 8* | ND |
| No. macrophages | 73 ± 5 | 25 ± 4* | 127 ± 19** | 38 ± 3*** |
| No. neutrophils | 0.2 ± 0.4 | 0.2 ± 0.4 | 6.6 ± 1.5** | 1.4 ± 0.8*,**** |

*$P < 0.05$ compared with saline + saline; **$P < 0.01$ compared with saline + saline; ***$P < 0.01$ compared with LPS + saline; ****$P < 0.01$ compared with LPS + saline. ND, not detected.

treatment and the level reached its maximum 3 h after the LPS challenge (Fig. 4). Moreover, the level was significantly attenuated in the Kupffer cell-depleted rats compared to the non-depleted rats, although it was still higher than that in rats given saline plus saline (control) 3 h after the LPS challenge (Table 2).

Changes in the superoxide anion level in the hepatic sinusoids after TNF-α infusion for 2 h were also studied. The TNF-α challenge resulted in superoxide anion formation. Similarly to the LPS challenge model, gadolinium chloride significantly attenuated the superoxide anion formation after TNF-α challenge (Table 3), although the level was still higher than in controls.

We also previously reported that LPS treatment increased the PNP/GPT ratio in the liver perfusate, a marker of damage to sinusoidal epithelial cells in the

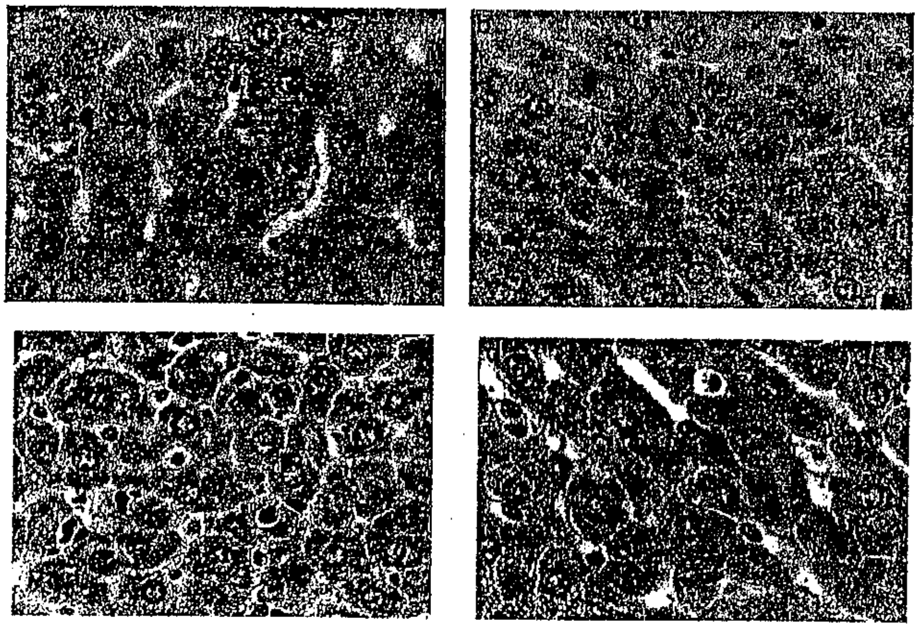

**Figure 3** Effect of gadolinium chloride on the population of neutrophils after lipopolysaccharide (LPS) challenge in the liver. The population of neutrophils in the liver was studied. (a) Rats given saline instead of gadolinium chloride and saline instead of LPS. (b) Those given gadolinium chloride and saline instead of LPS. (c) Those given saline instead of gadolinium chloride and LPS. (d) Those given gadolinium chloride and LPS (HE ×40).

**Table 2** Effect of gadolinium chloride on the formation of the superoxide anion that is released into the hepatic sinusoids 3 h after lipopolysaccharide (LPS) treatment

| | Saline + saline ($n = 3$) | Saline + $GdCl_3$ ($n = 5$) | LPS + saline ($n = 6$) | LPS + $GdCl_3$ ($n = 3$) |
|---|---|---|---|---|
| Superoxide anion formation (ΔABS) | 0.12 ± 0.01 | 0.12 ± 0.01 | 0.25 ± 0.01* | 0.17 ± 0.01**,† |

*$P < 0.01$ compared with saline + saline; **$P < 0.01$ compared with LPS + saline; †$P < 0.05$ compared with saline + saline. ΔABS, difference between the absorbance of the original perfusate and the highest absorbance in the sample perfusate.

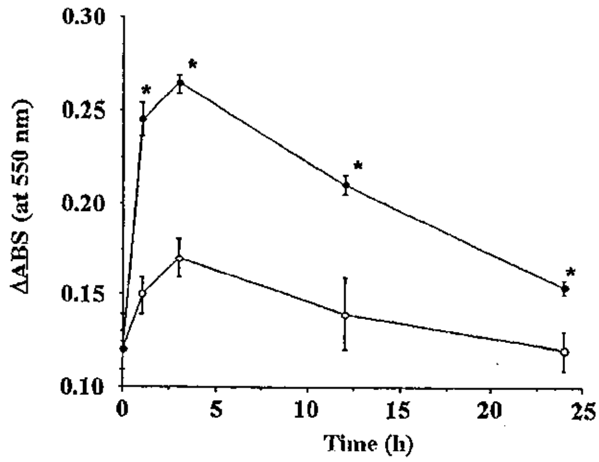


**Figure 4** Time-course of the superoxide anion level in the hepatic sinusoids after the lipopolysaccharide (LPS) challenge. The superoxide anion was not detectable 5 min after the LPS treatment and the level was maximal 3 h after the LPS challenge. (O) $GdCl_3$ + LPS; (●) LPS. ΔABS, highest absorbance among the samples. *$P < 0.01$ compared with $GdCl_3$ + LPS.

**Table 3** Effect of gadolinium chloride on the formation of the superoxide anion released into the hepatic sinusoids after the tumor necrosis factor (TNF)-α treatment

| | TNF-α ± saline ($n = 4$) | TNF-α ± $GdCl_3$ ($n = 4$) |
|---|---|---|
| Superoxide anion formation (ΔABS) | 0.25 ± 0.01 | 0.17 ± 0.01* |

*$P < 0.05$ compared with TNF-α ± saline. ΔABS, difference between the absorbance of the original perfusate and the highest absorbance in the sample perfusate.

liver, compared with controls.[9] This finding was also reproducible, and the present results showed that the increased PNP/GPT ratio was significantly attenuated by gadolinium chloride treatment (Table 4).

The time-course of the serum TNF-α level in the inferior vena cava was examined in the Kupffer cell-depleted and non-depleted rats up to 24 h after the LPS challenge. The level reached its maximum 1 h after the LPS treatment. There was no significant difference in the TNF-α levels between the two groups (Fig. 5).

Table 4 Effect of gadolinium chloride on the PNP/GPT ratio 3 h after lipopolysaccharide (LPS) treatment

| | Saline + saline ($n = 3$) | Saline + $GdCl_3$ ($n = 5$) | LPS + saline ($n = 6$) | LPS + $GdCl_3$ ($n = 3$) |
|---|---|---|---|---|
| PNP/GPT ratio (×10⁴) | 16 ± 5.5 | 18 ± 2.4 | 54 ± 2.4* | 40 ± 7.0** |

*$P < 0.01$ compared with saline + saline; **$P < 0.01$ compared with LPS ± saline. PNP/GPT, purine nucleoside phosphorylase/glutamic–pyruvic transaminase.

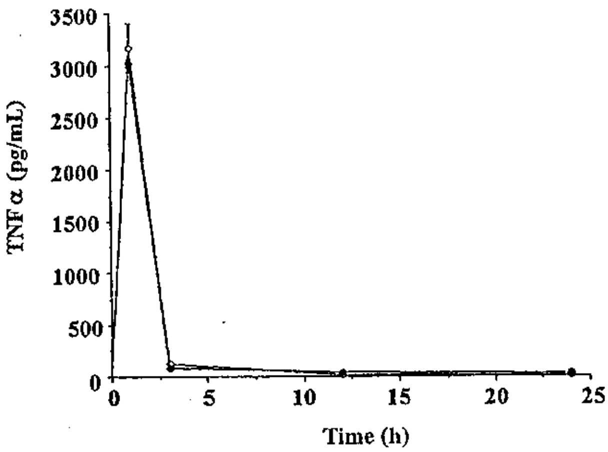


**Figure 5** Time-course of the serum tumor necrosis factor (TNF)-α level in the inferior vena cava after the lipopolysaccharide (LPS) challenge. The level was maximal 1 h after the LPS treatment. There was no significant difference in the TNF-α levels between the two groups. (O) $GdCl_3$ + LPS, (●) LPS.

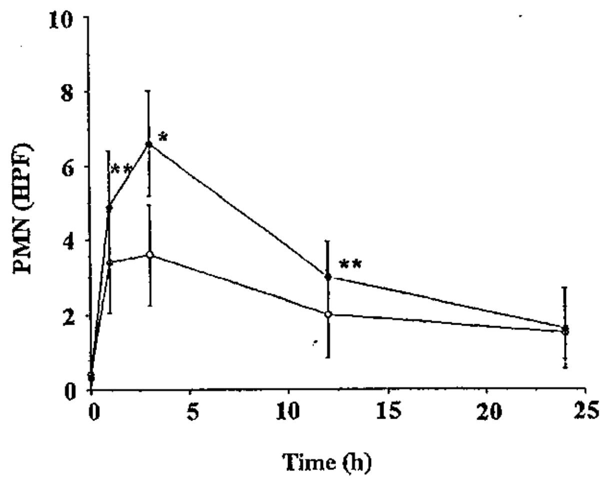


**Figure 6** Time-course of the neutrophil population in the liver specimens after the lipopolysaccharide (LPS) challenge. The population was maximal 3 h after LPS challenge in both groups and was significantly lower in the Kupffer cell-depleted rats than in the non-depleted rats. (O) $GdCl_3$ + LPS; (●) LPS. *$P < 0.01$ compared with $GdCl_3$ + LPS; **$P < 0.05$ compared with $GdCl_3$ + LPS.

The time-course of the neutrophil population after the LPS challenge was studied in the Kupffer cell-depleted rats as well as in the non-depleted rats. The population peaked 3 h after the LPS challenge in both groups and was significantly lower in the Kupffer cell-depleted rats than in the non-depleted rats (Fig. 6).

The time-course of the IL-8 level in the inferior vena cava was also studied in the both groups. Concomitantly with the neutrophil population in the liver, the level reached its maximum 3 h after the LPS challenge in both groups and was significantly lower in the Kupffer cell-depleted rats than in the non-depleted rats (Fig. 7).

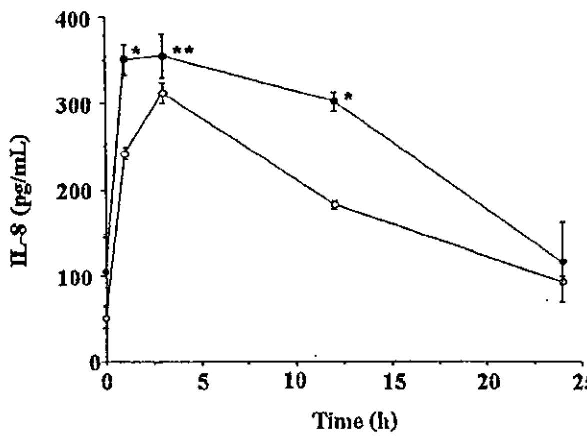


**Figure 7** Time-course of the interleukin (IL)-8 level in the inferior vena cava after the lipopolysaccharide (LPS) challenge. The level was maximal 3 h after the LPS challenge in both groups and was significantly lower in the Kupffer cell-depleted rats than in the non-depleted rats. (O) $GdCl_3$ + LPS; (●) LPS. *$P < 0.01$ compared with $GdCl_3$ + LPS; **$P < 0.05$ compared with $GdCl_3$ + LPS.

## DISCUSSION

There are several lines of evidence indicating that gadolinium chloride attenuates the progression of endotoxin-related liver damage. The present study aimed to determine the mechanisms behind this. To that end, we studied oxidative stress in the hepatic sinusoids in acute endotoxemia and the effect of gadolinium chloride on it. The oxidative stress was assessed by detection of superoxide anions in the hepatic sinusoids using the methods reported by Bautista *et al.*

The present time-course study showed that the superoxide anion level reached its maximum at 3 h after LPS treatment. This observation agrees with the previous report by Bautista and Spitzer.[7] Moreover, the time-course of the serum TNF-α level was examined, because TNF-α has been suggested to be an important factor in stimulating superoxide anion formation in this model.[8] We found that there was a time lag between the peak level of superoxide anions and the maximum serum TNF-α level; the former was 3 h after the LPS challenge whereas the latter was 1 h. However, in view of the time required to prime the Kupffer cells, this time lag is not surprising.

Gadolinium chloride can phagocytically deplete active Kupffer cells from the liver.[11] The present immunohistochemical analysis showed that there were few ED2-positive cells in the hepatic lobules of the gadolinium chloride-treated rats, indicating that Kupffer cell depletion in the present study was successful. The new finding in the present study was that gadolinium chloride treatment significantly reduced superoxide anion release into the hepatic sinusoids after the LPS challenge. This observation is reasonable because gadolinium chloride has been reported to reduce superoxide anion release after the LPS challenge from isolated liver macrophages,[17] which are expected to be the main sources of superoxide anions in this model.[10] We assume that the reduction of superoxide anions in the hepatic sinusoids may be related to a beneficial effect of gadolinium chloride against the progression of LPS-induced liver damage.

In contrast to the level of superoxide anions, there was no significant difference in that of serum TNF-α between the Kupffer cell-depleted rats and non-depleted rats. Because Kupffer cells are one of the sources of TNF-α *in vivo*, this finding was not convincing. Iimuro *et al.* reported that there was no significant difference in levels of serum TNF-α between the Kupffer cell-depleted rats and non-depleted rats,[17] and we could reproduce that observation in the present study. However, because Kupffer cells are one of the sources of TNF-α *in vivo*, this finding was not convincing. We cannot give a reasonable explanation for this. The interpretation for this fact by Iimuro *et al.* was that TNF secretion by liver macrophages would be a small part of the whole and may not affect the TNF level in peripheral blood. Having this view, we could only say that attenuation of the superoxide anion level in the gadolinium chloride-treated rats is at least due to a decrease in the number (or function) of superoxide anion sources. This view is supported by the present observation that the superoxide anion level was attenuated in the gadolinium chloride-treated rats compared to the non-depleted rats after equal doses of TNF were challenged.

It is well known that neutrophils are responsible for the deterioration of various liver injuries including that caused by LPS. Therefore, we examined the time-courses of the neutrophil population and serum IL-8 level, which is an important factor in regulating neutrophil migration *in vivo*, after LPS treatment. We found that the peaks of both factors were at least 3 h (or more) after LPS administration. Moreover, another finding that the time-courses of the two factors were parallel suggests that IL-8 also participates in neutrophil recruitment in this model.

The most interesting finding in the present study was that gadolinium chloride attenuated the serum IL-8 level and neutrophil population in the liver, although it did not affect the level of serum TNF-α, which regulates IL-8 production from various cells[18] after LPS treatment. It is therefore conceivable that factors other than TNF-α are responsible for the reduction of the serum IL-8 level and subsequent neutrophil migration into the liver. Recently, either extracellular or intracellular oxidative stress has been shown to activate IL-8 production and the expression of various adhesion molecules, resulting in an increase in the migration of neutrophils.[19] It is likely that superoxide anions in the hepatic sinusoids promote IL-8 production. In consideration of this view, it is not surprising that gadolinium chloride, which reduces the level of superoxide anions in the hepatic sinusoids, also reduces the serum IL-8 level and subsequent neutrophil migration into the liver.

We have reported that the LPS challenge increased the PNP/GPT ratio in the liver perfusate, a marker of damage to endothelial cells in the hepatic sinusoids, and the superoxide anions in the hepatic sinusoids may, at least in part, have caused this damage.[9] This phenomenon was reproduced in the present study. An important finding in the present study was that the increase in the PNP/GPT ratio, a marker of damage to the hepatic sinusoidal endothelial cells in response to LPS treatments, was significantly attenuated by gadolinium chloride treatment. It can easily be deduced that a direct effect of TNF-α is its involvement in the mechanisms causing damage to hepatic sinusoidal endothelial cells in this model. However, gadolinium chloride reduced the superoxide anion level in the hepatic sinusoids after the LPS challenge and this was associated with a reduction of the damage to the hepatic sinusoidal endothelial cells without any change in the serum TNF-α level. This indicates that superoxide anions released into the hepatic sinusoids per se damage the hepatic sinusoidal endothelial cells after the LPS challenge. As stated here, because gadolinium chloride reduced neutrophil migration into the liver after LPS treatment, the endothelial cell damage is decreased. Inversely, because damage to endothelial cells in the hepatic sinusoids leads to neutrophils sticking to them,[20] the reduction of endothelial cell damage causes a reduction in the liver neutrophil population after LPS treatment in gadolinium chloride-treated rats. In either case, the present finding is in keeping with the fact that Kupffer cell depletion has a protective effect against the progression of LPS-related liver injury.[5,6]

In the present study, the level of superoxide anions after the LPS or TNF-α challenge in the gadolinium chloride-treated rats was significantly higher than in controls, although ED2-positive cells were fully depleted from their livers. This suggests that superoxide anions are also derived from cells other than ED2-positive cells in this model. Because neutrophils and circulating macrophages produce superoxide anions upon various stimuli,[4,18] we assume that these cells could produce superoxide anions in this model. This view is supported by the present findings that a considerable number of neutrophils and macrophages were still observed in the liver specimens of the gadolinium chloride-treated rats.

Recently, we reported that an ethanol challenge also caused superoxide anion release into the hepatic sinusoids, which was attenuated by Kupffer cell depletion.[21] However, the levels of superoxide anions and subsequent damage to hepatic sinusoidal endothelial cell were much less compared to those in the LPS challenge model. Given that the involvement of neutrophils was minimum in the ethanol challenge model,[21] we assume that superoxide anions only in the hepatic sinusoid cannot activate either IL-8 production or neutrophil migration into the liver, causing further deterioration to the injured liver. Because TNF-α was not detectable in the model,[21] TNF-α could be one of the essential factors to activate IL-8 and neutrophil migration in the liver.

In conclusion, LPS causes the release of TNF-α and superoxide anions into the hepatic sinusoids, which augments IL-8 release as well as endothelial cell damage. These factors contribute to increased neutrophil migration into the liver, resulting in the progression of liver damage. This process may be the reason why alcoholic liver damage progresses when it is concomitant with endotoxemia.

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# Efficacy of non-invasive elastometry on staging of hepatic fibrosis

Hidetsugu Saito [a,*], Shinichiro Tada [a], Nobuhiro Nakamoto [a], Kumi Kitamura [a], Hitomi Horikawa [a], Satoshi Kurita [a], Yoshimasa Saito [a], Hirokata Iwai [b], Hiromasa Ishii [a]

[a] *Department of Internal Medicine, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan*
[b] *Society of Highly Advanced Medical Technology, 4-1 Kioi-cho, Chiyoda-ku, Tokyo 102-0094, Japan*

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**Abstract**

To assess the efficacy of elastmetry in the determination of fibrotic stage in the liver, we investigated correlation between liver histology and the elastometry using a device equipped with a vibrator and an ultrasound system (Echosens, Paris, France) in patients with chronic hepatitis C. Totally 75 patients, 24 in F1 stage, 17 in F2 stage, 18 in F3 stage, and 16 in F4 stage according to the new Inuyama classification without fatty change were investigated. Correlations between the staging of liver fibrosis and elastometry, serum fibrosis makers and platelet counts were investigated. The elastometry was absolutely non-invasive. Serum fibrosis markers did not well correlate with the stage of liver fibrosis. Platelet counts significantly ($P < 0.0001$) correlated with the fibrotic stage. Median platelet counts in each stage was; F1, 191.5; F2, 172.0; F3, 132.0; F4, 77.5 ($\times 10^3\ \mu l^{-1}$). However, the deviation was comparatively broad. On the contrary, the elastometry correlated well to the stage of fibrosis and the deviation was small. Median elastometric levels in each stage were; F1, 6.25; F2, 7.80; F3, 13.85; F4, 34.00 (kPa). These results suggest that elastometry is significantly useful for evaluating fibrotic staging of the liver without any invasiveness.


*Keywords:* Fibrosis; Elasticity; Staging; Chronic hepatitis C; Non-invasive

## 1. Introduction

Fibrosis of the liver is a process of hepatic wound heeling after continuous destruction of hepatocytes [1]. The major source of fibrogenesis is hepatic stellate cells (Ito cells), which transform from fat storing cells to fibroblasts and proliferate by some cytokines, such as transforming growth factor (TGF)-β [2], interleukin-13 [3], hepatocyte growth factor or angiotensin II [4,5]. Accumulation of collagen fibers and other extracellular matrix and regeneration of hepatocytes, that is, the scaring and regeneration, result in the formation of tissue change, liver cirrhosis, which progressively decreases hepatic function into chronic liver failure [6]. The progression of fibrotic change is always found in chronic viral infection and alcoholic liver diseases, but these produce different patterns of fibrosis as the disease progresses. These can be divided into those diseases that are portal-based and those that are acinar zone 3-based, and the portal-based diseases leading to cirrhosis include chronic viral hepatitis and zone 3-based diseases include alcoholic or non-alcoholic steatohepatitis.

Cirrhosis is not a static lesion and it may worsen, improve, or stay on, probably according to underlying disease activity. Recent studies revealed that the stage of fibrosis in chronic viral hepatitis C correlates to increased risk of hepatocarcinogenesis [7–11], and a success of anti-viral therapy results in decrease in both hepatic fibrotic stage and occurrence rate of hepatocellular carcinoma [11–13]. Thus, the regulation of hepatic fibrogenesis is important in the management of chronic liver diseases and evaluation of the stage of fibrosis in chronic hepatitis C is necessary for both diagnosis and treatment. Staging of the disease process in chronic hepatitis C indicates how far the disease has progressed in the course of its natural history, and at the same time how far the risk for developing liver cancer has impended. At the end stage, the disease has run its course and the patient dies

* Corresponding author. Tel.: +81-3-3353-1211; fax: +81-3-3351-8705.
*E-mail address:* hsaito@sc.itc.keio.ac.jp (H. Saito).

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by liver failure or hepatocellular carcinoma. The establishment of the staging of liver diseases is always determined by liver biopsy.

Although the complete diagnosis of the stages of liver fibrosis can be determined in autopsy specimens, where entire liver can be carefully examined, needle biopsies are the main procedure for histologic evaluation in living patients. The needle biopsy is sometimes subject to sampling error, with small amount of samples [14,15]. Moreover, although needle biopsy is safe procedure, it sometimes makes complications such as pain, intra-abdominal bleeding, sampling from other organs, or penumothorax [16–18]. These conditions lead a new technology to make a non-invasive apparatus that can evaluate the soft tissue elasticity, which reflects fibrosis in the tissue, using a low-frequency shear wave and an echogram. The apparatus named Fibroscan® (Echosens Co., Paris, France) measures the elasticity of organs by measuring the velocity of a low-frequency shear wave going through the liver using transient elastography [19–21]. The speed of the wave was determined in any 2 cm in the liver from 2.5 to 6.5 cm depth from the surface. The elasticity of the liver is determined by the accumulation of fibrosis, so that the measurement of elasticity correlates to the degree of hepatic fibrosis. The aim of this study is to evaluate the usefulness of this apparatus, Fibroscan® (version 1), in the determination of hepatic fibrotic stage. To that effect, we examined the patients with chronic hepatitis C who had been biopsied within 3 month before and after the measurement with Fibroscan®, and we investigated the correlation between the elastometry and the staging of hepatic fibrosis according to the new Inuyama classification [22] that is the standard evaluating system in Japan.

## 2. Patients and methods

Patients with chronic hepatitis C and liver cirrhosis type C, who visited and received liver needle biopsy in our department in 2003 were included in this study after giving a well documentation of the purpose and methods of this study and having their acceptance for enrolling in this study. The patient received elastometry with Fibroscan® version 1 within 3 months before or after liver biopsy. The case in which fatty deposit of hepatocytes was found in more than 10% of whole hepatocytes in the sample was eliminated in this study because it has not been determined that fatty change might affect the result of elastometry.

The patient was laid on a bed on their backs and the location of measurement was determined with B-mode ultrasound. Elastometry was performed by contacting the probe of apparatus with a range of pressure at an intercostal area on the liver, and the measurement was done within a second after pushing the switch. The elasticity was automatically calculated in the apparatus and the data were shown as kilo Pascal (kPa). The elasticity of a patient was measured 10 times and the median measured level was automatically shown at the window and the result was printed after the measurement. On the same date, we measured serum fibrosis makers, the levels of type IV collagen 7S, type III procollagen-*N*-peptide (P-III-P) and hyarulonic acid, and also platelet counts. We investigated the correlation between the levels of elastometry, the stage of liver fibrosis, platelet counts, and serum fibrosis markers. The stage of hepatic fibrosis was determined by two experts of liver pathology without information of any clinical data according to the new Inuyama clacification [21]. Liver biopsy was performed with 16-gauge needle biopsy apparatus (TopNotch, Boston Scientific Japan Co., Tokyo, Japan).

## 3. Statistic analysis

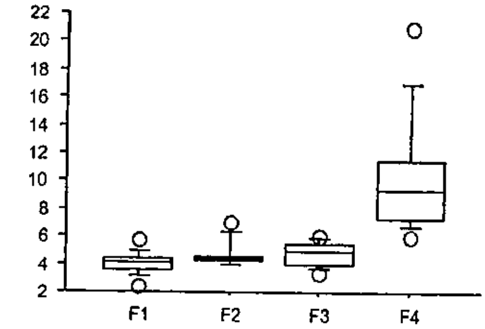
The data of elastometry was automatically shown in the central value. The data of each fibrosis stage were calculated and indicated by mean ± standard deviation. The correlation between the data and the fibrosis stage was determined by Kruskal–Wallis analysis. The correlation between the data was determined by Mann–Whitney test.

## 4. Results

Totally 75 cases of chronic hepatitis C and liver cirrhosis type C were enrolled in this study. The age distributed from 18 to 74 and the central level was 50 year-old. The number of patients in each fibrotic stage was 24 in F1 stage, 17 in F2 stage, 18 in F3 stage, and 16 in F4 stage (liver cirrhosis).

The elastometry was entirely non-invasive and no one complained anything during and after the examination. The measurement was successful in almost all patients, but it did not work in patients with ascites, with narrow intercostal spaces, and with excess obesity. We tried to measure the elasticity of ex vivo liver from pig, but we could never measure it, suggesting that the chest wall is necessary for the measurement of hepatic elasticity with this apparatus.

The correlation between the elastometry and serum fibrosis makers was not found in this study. The median level (and 50% levels) of type IV collagen 7S in each fibrotic stage was F1, 4.1 ng/ml (3.5–4.1); F2, 4.4 ng/ml (4.2–4.5); F3, 4.9 ng/ml (4.0–5.4); F4, 9.4 ng/ml (7.3–22.5) (Fig. 1). Those of P-III-P were; F1, 0.60 U/ml (0.54–0.73); F2, 0.87 U/ml (0.74–0.91); F3, 0.75 U/ml (0.59–0.91); F4, 1.30 U/ml (0.98–1.60) (Fig. 2). Those of hyaluronic acid were F1, 37.2 ng/ml (20.9–51.5); F2, 48.3 ng/ml (38.1–59.0); F3, 62.3 ng/ml (55.9–83.5); F4, 412.0 ng/ml (317.3–1110.0) (Fig. 3). The statistical differences of type IV collagen 7S between F1 and F2, F2 and F3, F3 and F4, were $P = 0.135$, 0.972, and <0.0001, respectively. Those of P-III-P were $P = 0.068$, 0.401 and 0.0215, respectively. Those of hyaluronic acid were $P = 0.0545$, 0.051 and 0.0004, respectively. Thus, these serum fibrosis makers

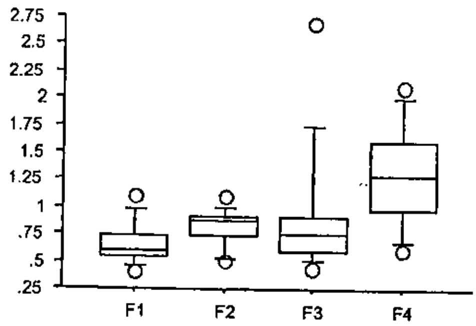



| | Median level | P value |
|---|---|---|
| F1 | 4.10 | P=0.135 (F1 vs. F2) |
| F2 | 4.40 | P=0.972 (F2 vs. F3) |
| F3 | 4.90 | P<0.0001 (F3 vs. F4) |
| F4 | 9.40 | |

Fig. 1. The median and 50% of the levels of serum type IV collagen 7S in each fibrotic stage of the New Inuyama Classification of chronic hepatitis C. The *P*-value in the table means the difference of levels between F1 vs. F2, F2 vs. F3, and F3 vs. F4, respectively, from the top. The significant difference was found in only between the levels of F3 and that of F4 ($P < 0.0001$).

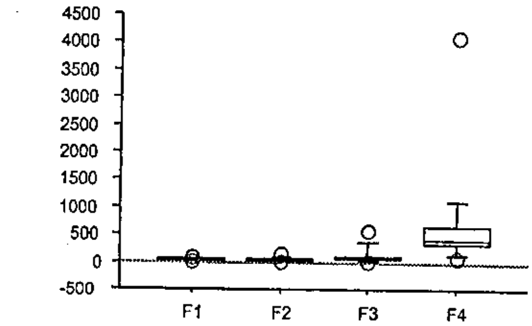
significantly increased in the fibrotic stage between F3 and F4, but they could not distinguish between F1 to F3.

On the other hand, platelet counts significantly ($P < 0.0001$) correlated with the fibrotic stage (Fig. 4). The median level of platelet counts (and 50% levels) in each stage was; F1, $191.5 \times 10^3\,\mu l^{-1}$ (173.0–235.5); F2, $172.0 \times 10^3\,\mu l^{-1}$ (145.0–203.0); F3, $132.0 \times 10^3\,\mu l^{-1}$ (104.5–163.0); F4, $77.5 \times 10^3\,\mu l^{-1}$ (67.0–107.0). However, the deviation was comparatively broad even the patients



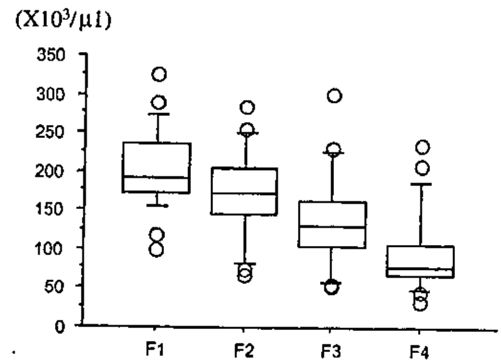

| | Median level | P value |
|---|---|---|
| F1 | 0.60 | P=0.068 (F1 vs. F2) |
| F2 | 0.87 | P=0.401 (F2 vs. F3) |
| F3 | 0.75 | P=0.022 (F3 vs. F4) |
| F4 | 1.30 | |

Fig. 2. The median and 50% of the levels of serum type III procollagen-*N*-peptide (P-III-P) in each fibrotic stage of the New Inuyama Classification of chronic hepatitis C. The *P*-value in the table means the difference of levels between F1 vs. F2, F2 vs. F3, and F3 vs. F4, respectively, from the top. The significant difference was found in only between the levels of F3 and that of F4 ($P = 0.022$).



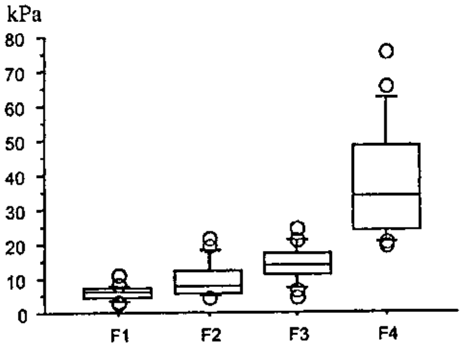

| | Median level | P value |
|---|---|---|
| F1 | 37.2 | P=0.055 (F1 vs. F2) |
| F2 | 48.3 | P=0.051 (F2 vs. F3) |
| F3 | 62.3 | P=0.0004 (F3 vs. F4) |
| F4 | 412.0 | |

Fig. 3. The median and fifty percent of the levels of serum hyaluronic acid in each fibrotic stage of the New Inuyama Classification of chronic hepatitis C. The *P*-value in the table means the difference of levels between F1 vs. F2, F2 vs. F3, and F3 vs. F4, respectively, from the top. The significant difference was found in only between the levels of F3 and that of F4 ($P = 0.0004$).




| | Median level | P value |
|---|---|---|
| F1 | 191.5 | P=0.068 (F1 vs. F2) |
| F2 | 172.0 | P=0.048 (F2 vs. F3) |
| F3 | 132.0 | P=0.020 (F3 vs. F4) |
| F4 | 77.5 | |

Fig. 4. The median and fifty percent of the levels of platelet counts in each fibrotic stage of the New Inuyama Classification of chronic hepatitis C. The *P*-value in the table means the difference of levels between F1 vs. F2, F2 vs. F3, and F3 vs. F4, respectively, from the top. The significant difference was found in between the levels of F2 and that of F3 ($P = 0.048$), and between F3 and F4 ($P = 0.020$).




| | Median level | P value |
|---|---|---|
| F1 | 6.25 | P=0.009 (F1 vs. F2) |
| F2 | 7.80 | P=0.018 (F2 vs. F3) |
| F3 | 13.7 | P<0.0001 (F3 vs. F4) |
| F4 | 34.0 | |

Fig. 5. The median and 50% of the levels of elastomtric measurement in each fibrotic stage of the New Inuyama Classification of chronic hepatitis C. The *P*-value in the table means the difference of levels between F1 vs. F2, F2 vs. F3, and F3 vs. F4, respectively, from the top. The significant difference was found between all the groups.

suffered from uniformed etiology, chronic hepatitis C virus infection. The statistical difference between each group was; F1 versus F2 ($P = 0.068$); F2 versus F3 ($P = 0.048$); F3 versus F4 ($P = 0.020$). Thus, this marker also distinguishes between F3 and F4, but differences among from F1 to F3 were not statistically significant.

On the contrary, the elastometry correlated well to the stage of fibrosis ($P < 0.0001$) and the deviation was not so broad (Fig. 5). The actual measurement levels in each stage (median and 50%) were F1, 6.25 kPa (4.60–7.80); F2, 7.80 kPa (5.70–12.48); F3, 13.85 kPa (11.20–17.40); F4, 34.00 kPa (24.00–48.20). The data of F4 were rather significantly large than other stages, but the data of F1–F3 were differentiated well. This condition was quite different from that in serum fibrosis markers. The other series of examination revealed that the elastometry of the livers from 20 healthy subjects all showed less than 5 kPa (median 4.31 kPa; 25–75%, 4.03–4.90 kPa). The statistic difference between the groups was; F1 versus F2 ($P = 0.009$); F2 versus F3 ($P = 0.018$); F3 versus F4 ($P < 0.0001$). These differences were much superior than those in platelet counts. There was no correlation between platelet counts and elasticity measurement. Examples of pathologic findings and the elastometric measurements were shown in Fig. 6. Fibrosis extends from the portal area in the upper photograph of Fig. 6 but bridging fibrosis is not clearly found. The elastometric measurement showed that the elasticity of this liver was 7.9 kPa. On the other hand, the lower photograph shows histology of a case with liver cirrhosis and the measurement result showed that the elasticity of this liver was 20.7 kPa. These data were quite compatible for the aspects of the liver histology.

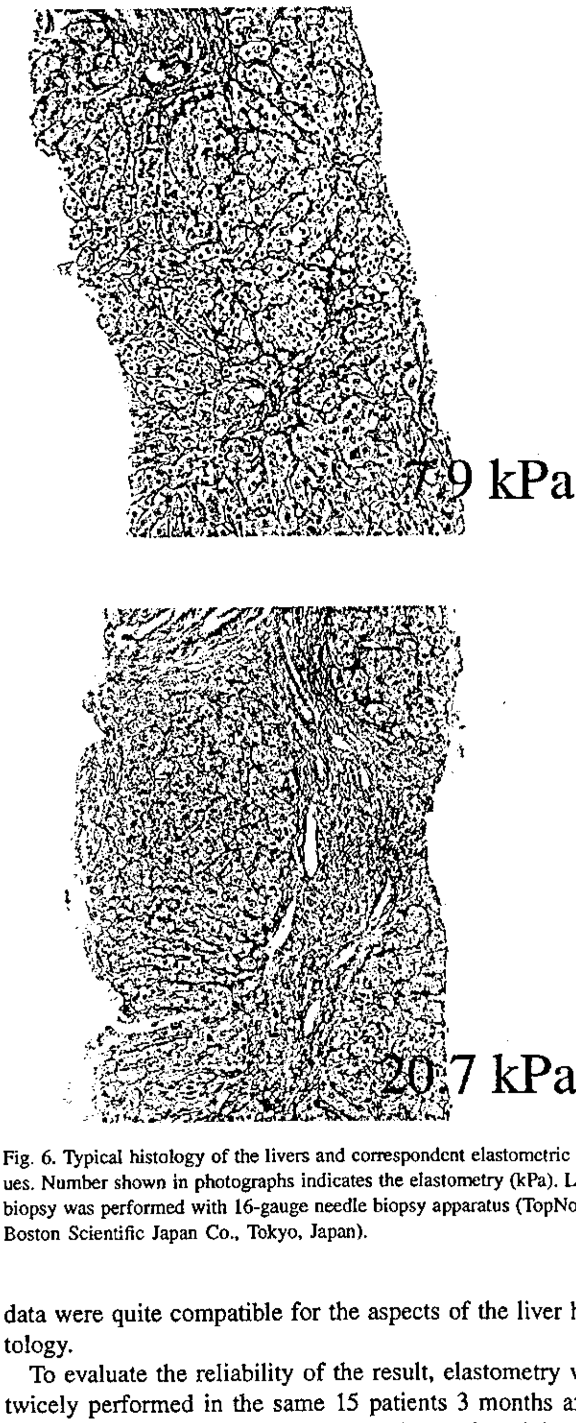



Fig. 6. Typical histology of the livers and correspondent elastometric values. Number shown in photographs indicates the elastometry (kPa). Liver biopsy was performed with 16-gauge needle biopsy apparatus (TopNotch, Boston Scientific Japan Co., Tokyo, Japan).

To evaluate the reliability of the result, elastometry was twicely performed in the same 15 patients 3 months after the initial examination. Table 1 shows the result and the two data obtained from the same patient were around the same suggesting that this elastometry is reliable.

## 5. Discussion

Liver biopsy may be necessary for confirming the diagnosis, evaluating disease severity, and ruling out other