

Virological effects and safety of combined double filtration plasmapheresis (DFPP) and interferon therapy in patients with chronic hepatitis C: A preliminary study

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Short title: Combination therapy with DFPP and IFN

## **Abstract**

**Purpose:** In patients with chronic genotype 1b hepatitis C and a high viral load, the viral load was reduced by double filtration plasmapheresis (DFPP), followed by combined interferon and ribavirin therapy. The safety and virological effects of this treatment method were preliminarily investigated.

**Methods:** In 9 patients with chronic hepatitis C, DFPP was performed 3 times on days 1, 2, and 4, and the administration of interferon and ribavirin was initiated immediately after DFPP on day 1.

**Result:** The HCV RNA was undetectable in all patients after the plasma was passed through a plasma fractionator (2<sup>nd</sup> filter) in the DFPP circuit. After 2 weeks, the HCV RNA tended to decrease in the DFPP group more than in the control group ( $-2.45 \pm 1.12$  vs.  $-1.57 \pm 0.95$ ,  $p=0.073$ ). However, this decrease was not attributable to a sustained virological response (SVR) (22.2% vs. 18.2%,  $p=0.822$ ). Most of the adverse events were caused by the interferon and ribavirin combination therapy.

**Conclusion:** DFPP can be safely performed concomitantly with interferon and ribavirin combination therapy in chronic hepatitis C patients. The combination may contribute to an early virological response. The effect of DFPP on the SVR and its significance remain to be clarified.

## 1. Introduction

Hepatitis C virus (HCV) infection induces acute hepatitis, and approximately 70%–80% of these cases progress to chronic hepatitis. The course of the disease is stable in approximately 30% of chronic hepatitis cases; however, the remaining 70% of cases progress to liver cirrhosis after approximately 30–40 years. Further, the cases that progress to liver cirrhosis develop hepatocellular carcinoma at an annual rate of approximately 8% [1].

From the viewpoint of viral eradication, interferon therapy is the only radical therapy for chronic hepatitis C. Once complete viral elimination is achieved by interferon therapy, liver fibrosis improves, and the risk of liver carcinogenesis is reduced [1].

In interferon therapy for chronic hepatitis C, administration of 3 to  $6 \times 10^6$  IU interferon alone, 3 times a week for 24 and 48 weeks, has been reported to produce a sustained virological response (SVR) in approximately 6% and 16% of patients, respectively [2]. Treatment with interferon in combination with ribavirin for 24 and 48 weeks has been reported to produce an SVR in 21% [3] and 41% [2, 4, 5] of patients, respectively.

A combination of pegylated interferon (PEG-IFN)—a recently marketed long-acting interferon for once-a-week administration—and ribavirin induced an SVR in 47% of patients with chronic genotype 1b HCV infection and high viral loads in whom previous interferon

therapy failed to produce a response [6, 7].

However, even the combination therapy with PEG-IFN and ribavirin for 48 weeks, which is currently the most promising therapy, does not improve viremia in the other patients for whom there is no appropriate therapy for the eradication of HCV viremia other than time-course observation and the administration of liver-protective drugs. An NS3 protease inhibitor has been developed as a novel antiviral agent, and clinical studies have been performed using this agent; however, the clinical application of this drug will take time because of the occurrence of certain adverse events [8]. The development of an NS5 polymerase inhibitor and an internal ribosome entry site (IRES)-targeting agent is in progress [9-11].

The presence or absence of concomitant ribavirin therapy, the duration of therapy, the virus genotype, the viral load before administration, the grade of liver fibrosis, and the gender and age of the patient are all factors that influence the therapeutic effect of interferon therapy. An early virological response (EVR) is defined as a decline of more than 2 log<sub>10</sub> units in the viral load 8 or 12 weeks after the initiation of treatment; it has also been reported to be one of the important factors for an SVR [12, 13].

There have been several studies in which the HCV level was investigated in HCV-positive patients during extracorporeal circulation therapies, such as plasmapheresis and hemodialysis. In

these studies, the HCV RNA level decreased transiently by approximately 50%-90% immediately after plasmapheresis or hemodialysis; however, it returned to either the pretreatment level or to a higher level within approximately 4-6 h [14-17].

In double filtration plasmapheresis (DFPP), which is a plasmapheresis therapy, the patient's whole blood is separated into plasma and blood cell components by using a plasma separator (1<sup>st</sup> filter). The separated plasma components are further separated into high and low molecular weight components by using a plasma fractionator (2<sup>nd</sup> filter); the high molecular weight components including immunoglobulins are removed, and the low molecular weight components including albumin are returned to the body. Although this technique using 2 filters is relatively more complicated than the normal simple plasma exchange, its advantage is that supplemental plasma transfusion is not necessary.

The diameter of the HCV particle is approximately 55-65 nm [18]. In theory, these viruses are unable to pass through the 2<sup>nd</sup> filter that has a pore size smaller than the diameter of the viral particle; they are therefore eliminated from the plasma.

In this study, we attempted to reduce the viral loads of patients with chronic genotype 1b hepatitis C and a high viral load by using DFPP. We focused on HCV RNA levels both before plasmapheresis therapy and before interferon and ribavirin combination therapy, and then

investigated the early virological effects and also the safety of the treatment.

## **2. Experimental/Materials and methods**

### **2.1. Patients**

The study involved 9 patients (4 males and 5 females, mean age:  $51.7 \pm 11.3$  years) with chronic genotype 1b hepatitis C that was histologically diagnosed at our department between December 2002 and July 2004. In these patients, the HCV RNA level determined by reverse transcriptase-polymerase chain reaction (RT-PCR) was not less than 100 KIU/ml.

The inclusion criteria were as follows. (1) Minimum age: 20 years; maximum age: 70 years. (2) Blood test values before therapy: hemoglobin, 12 g/dl or higher; platelet count,  $100,000/\text{mm}^3$  or higher; white blood cell count,  $3,000/\text{mm}^3$  or higher; and neutrophil count,  $1,500/\text{mm}^3$  or higher.

The exclusion criteria were as follows. (1) Pregnancy or possible pregnancy, and lactation; (2) depression; (3) serious complications, particularly uncontrollable hypertension and impaired function of the bone marrow, kidneys, or the lungs; (4) autoimmune diseases or suspicion of the same; (5) diabetes or suspicion of the same; (6) allergic predispositions; (7) history of hypersensitivity to interferon or nucleic acid analogues; (8)

history of hypersensitivity to biological products such as vaccines; (9) suspicion of alcoholic liver injury, autoimmune hepatitis, or drug-induced liver injury; (10) multiple infections with hepatitis B virus within 48 weeks before the initiation of therapy or suspicion of the same; (11) previous hepatic encephalopathy, rupture of the esophageal varix, or ascites; (12) complications of hepatic cirrhosis or hepatocellular carcinoma on examination within 4 weeks before the initiation of therapy or ongoing treatment for the same; and (13) treatment with drugs having antiviral actions, immunoregulatory actions, or bone marrow-inhibiting actions such as interferon, Ara-A, zidopudine, glucocorticoid, interleukin 2, or Shosaikoto within 12 weeks of the initiation of therapy or administration of injections containing glycyrrhizin as the main ingredient, theophylline, antipyrine, or warfarin within 4 weeks before therapy.

## **2.2. Study design**

This study was conducted in accordance with the Good Clinical Practice guidelines, conforming to the Helsinki Declaration. It was approved by the Ethics Committee of Kanazawa University Clinical Study Center, and written informed consent was obtained from the patients before their participation in the study.

### **2.2.1. Treatment schedule**

DFPP was performed on day 1 of the therapy to decrease the viral



load, and the administration of interferon and ribavirin was initiated 1 h after the completion of DFPP. DFPP was performed 3 times, on days 1, 2, and 4, and a blood test was performed before each treatment to determine the efficacy of the treatment.

### **2.2.2. Double filtration plasmapheresis**

In order to access the blood during DFPP, a Soft-Cell double-lumen catheter (GamCath catheter N®; Gambro, Stockholm, Sweden) was inserted and indwelled in the right femoral vein for 5 days.

For DFPP, a Plasmaflo KM8800 (Kuraray Medical Inc., Tokyo, Japan) was used as the dialysis apparatus. The plasma separator and plasma fractionator (1<sup>st</sup> and 2<sup>nd</sup> filters, respectively) used were Plasmaflo OP-08W® and Cascadeflo EC-50W®, respectively (Asahi Kasei Medical Co., Ltd., Tokyo, Japan) (Fig. 1). With regard to the frequency of treatment, in a previous study it was observed that the level of fibrinogen decreased to less than 100 mg/dl when DFPP was continuously performed twice. Thus, DFPP was discontinued on day 3 to prevent fibrinogen-associated complications, such as a tendency to bleed, and the applicability of DFPP on day 4 was determined based on laboratory test results.

In order to process 50 ml/kg of blood in a single round of DFPP, DFPP was performed for approximately 3 h at a blood flow rate of approximately 80 ml/min. The potent protease inhibitor, Nafamostat

mesilate (Naotamin®; Asahi Kasei Pharma Co., Ltd., Tokyo, Japan), was used as an anticoagulant because heparin is considered to influence RT-PCR when this procedure is used for HCV RNA measurement. For fluid replacement, we used either 50 or 100 ml of 25% albumin (Kenketsu Albumin-Wf®; Mitsubishi Pharma Co., Tokyo, Japan) that was diluted with 200 ml of saline.

Blood tests were performed before each DFPP to ensure that the plasmapheresis could be performed without the occurrence of any adverse events; DFPP was not performed if the platelet count was 50,000/mm<sup>3</sup> or less, or if the fibrinogen level was 100 mg/dl or less. The DFPP was resumed after the recovery of these test values was confirmed.

### **2.2.3. Interferon therapy**

For the interferon therapy, interferon (IFN)  $\alpha$ -2b (Intron A®; Schering-Plough KK, Kenilworth, NJ) and ribavirin (Rebetol®; Schering-Plough KK) were concomitantly administered. IFN  $\alpha$ -2b was administered intramuscularly 1 h after the completion of DFPP on day 1; the oral administration of ribavirin was initiated after the completion of DFPP on day 1. IFN  $\alpha$ -2b was administered 6 times a week for 2 weeks at a daily dose of  $6 \times 10^6$  IU, followed by 3 times a week for 22 weeks or 3 times a week intermittently for 46 weeks. The ribavirin dose was determined based on the body weight measured at the time of patient registration. The dose used was 600 mg and

800 mg for body weights of less than 60 kg and 60 kg or higher, respectively. The daily dose was divided into 2 doses and administered orally for 24 weeks.

#### **2.2.4. Evaluation**

The HCV RNA was measured during and after therapy by using an RT-PCR assay (Amplicor HCV RNA Monitor®; BML, Tokyo, Japan: measurement sensitivity, 0.5 KIU or higher); the sample was diluted when the HCV RNA level was higher than the upper quantification limit (850 KIU/ml). When the HCV RNA was less than the lower quantification limit, a qualitative method was used (Amplicor HCV RNA®, BML; measurement sensitivity, 50 IU).

The HCV RNA was measured after 2, 4, 8, and 12 weeks of therapy, either before DFPP or every morning on days 1 to 6. It was measured after 24 and 48 weeks of therapy as well as at 24 weeks after the completion of the interferon therapy. Whenever possible, the HCV RNA was also measured immediately after DFPP completion. The HCV RNA in the plasma before and after the 2<sup>nd</sup> filtration in the DFPP circulation was also measured 1 h after the initiation of the DFPP and immediately before the completion of the DFPP. A negative viral detection at 24 weeks after the interferon administration was defined as an SVR.

As a control group for a comparison of the decrease in HCV RNA during the 2-week therapy period, we used the HCV RNA data of 11

patients with chronic genotype 1b hepatitis C and a high viral load who underwent IFN and ribavirin combination therapy without DFPP at our department during the same period.

In all patients, a liver biopsy was performed immediately before therapy, and fibrosis and inflammation were evaluated based on the New Inuyama classification. The inflammatory activity in the portal vein and the periportal area as well as the degrees of intralobular inflammation and hepatocyte degeneration were graded from A0 to A3 (0: none, 1: mild, 2: moderate, and 3: severe) based on the "degree of inflammatory activity." Fibrosis was also graded from F0 to F4 (0: none, 1: mild without septa, 2: moderate with few septa, 3: numerous septa without cirrhosis, and 4: cirrhosis) [19].

For the blood tests, we performed white blood cell, red blood cell, platelet, and differential leukocyte (neutrophils, eosinophils, basophils, lymphocytes, and monocytes) counts as well as the zinc sulfate turbidity test (ZTT). Further, the percentage of hemoglobin, hematocrit, and reticulocytes as well as the levels of fibrinogen, total protein, albumin,  $\gamma$ -globulin, and total cholesterol were all measured.

Adverse events were evaluated in accordance with the WHO guidelines, and these were classified into mild, moderate, severe, and life-threatening events.

The significance of the differences was analyzed using the

chi-square test, Fisher's exact test, a t-test, and logistic regression analysis.

### **3. Results**

#### **3.1. Patients' backgrounds**

The patients' backgrounds are listed in Table 1. In the DFPP group, there were 4 male and 5 female patients with a mean age of  $51.7 \pm 11.3$  years. The HCV RNA before therapy was at least 100 KIU/ml and less than 500 KIU/ml in 3 patients and 500 KIU/ml or higher in 6 patients, indicating that many of the patients had a high viral load. Three patients had previously undergone IFN therapy, which was virologically ineffective. For the remaining 6 patients, this was their first experience of IFN therapy. With regard to liver histology, the fibrosis was graded as F2 or lower in all patients, and in all patients there was no evidence of advanced chronic hepatitis. Compared with the control group, gender, age, serum HCV RNA, previous IFN treatment, liver histology, and blood biochemical data for the treatment group were statistically not significantly different.

#### **3.2. Virological changes after DFPP**

To confirm the elimination of the virus by DFPP, HCV RNA was measured before and after the 2<sup>nd</sup> filtration. One hour after the initiation of DFPP, once the DFPP exchange blood flow had stabilized,

the HCV RNA in the blood after it had passed through the 2<sup>nd</sup> filter was quantitatively undetectable in all 9 patients. This confirmed the elimination of the virus by the 2<sup>nd</sup> filter (Fig. 2). Further, at the completion of DFPP—approximately 3 h after its initiation—the HCV RNA was undetectable in all 9 patients (data not shown). These findings confirmed that DFPP is capable of eliminating the HCV particles and that the elimination efficiency does not decrease with time.

### **3.3. Early virological response**

In order to investigate the EVR, the HCV RNA was measured 2 weeks after the initiation of therapy. It was quantitatively undetectable in 4 of the 9 patients (44.4%). In the control group treated with IFN and ribavirin combination therapy without DFPP, the HCV RNA was quantitatively undetectable in 2 patients (18.2%). The number of patients with undetectable HCV RNA 2 weeks after the initiation of therapy was higher in the DFPP group, but the difference was not significant ( $P = 0.201$ ).

The EVR is defined as a viral load decline of 2 log<sub>10</sub> units or more from the baseline level at an early stage in the therapy, i.e., 2 weeks after the initiation of the therapy in the case of this study. An EVR was achieved in 6 of the 9 patients in the DFPP group (66.7%) and in 4 of the 11 patients in the control group (36.4%),

indicating that the HCV RNA tended to decrease earlier during concomitant DFPP and interferon therapy; however, the difference was not statistically significant ( $P = 0.178$ , Fig. 3A).

The change in HCV viral load also tended to decline more in the DFPP group than in the control group ( $-2.45 \pm 1.12$  vs.  $-1.57 \pm 0.95$ ;  $P = 0.073$ , Fig. 3B).

#### **3.4. Biochemical response**

The time-course changes in alanine aminotransferase (ALT) are shown in Fig. 4. The ALT level was normalized in 7 of the 9 patients (77.8%) by 4 weeks of the therapy. In the control group, it was normalized in 7 of the 11 patients (63.6%). The difference was not statistically significant ( $P = 0.845$ ).

#### **3.5. Sustained virological response**

An SVR was observed in 2 of the 9 patients (22.2%) treated with concomitant DFPP and interferon therapy (an intent-to-treat approach). Of these, 1 patient received IFN and ribavirin combination therapy for 24 weeks, and the other received IFN and ribavirin combination therapy for 24 weeks, followed by additional IFN monotherapy for 24 weeks. These patients were 2 of the 4 patients in whom the HCV RNA was quantitatively undetectable after 2 weeks of therapy. Treatment of the other 2 patients was discontinued before

24 weeks due to adverse events (skin eruption, anorexia, and general malaise). In the 6 patients for whom complete treatment was possible, the SVR rate was 33.3% (2/6) (a per-protocol approach).

In the control group, the SVR rate was 18.2% (2/11) as determined using an intent-to-treat approach and 20.0% (2/10) as determined using a per-protocol approach. The difference in the SVR rate between the 2 groups was not statistically significant ( $P = 0.822$  and  $P = 0.551$ , respectively).

The factors associated with the SVR in our patients were analyzed using uni- and multivariate analysis. Using univariate analysis, the EVR was found to be the only factor associated with SVR ( $P = 0.025$ ). Associations with other factors, such as DFPP, age, sex, pre-treatment history, and fibrosis, were not detected in our series ( $P = 0.822$ ,  $0.170$ ,  $0.822$ ,  $0.822$ , and  $0.052$ , respectively). Using multivariate analysis, the association of all of these factors was found to be statistically insignificant.

In the DFPP group, the only factor showing a relationship with the SVR was the viral negativity at 2 weeks ( $P = 0.014$ ).

### **3.6. Safety and adverse events**

In 8 of the patients, DFPP was performed 3 times on days 1, 2, and 4. It was not performed on day 4 in 1 patient because this patient's fibrinogen level was lower than 100 mg/dl before DFPP; hence, DFPP was performed only twice in this patient.



The changes in laboratory test values as a result of DFPP treatment were also investigated. After DFPP on day 1, the platelet count decreased on average by 22.5%. The decrease persisted until the completion of DFPP and then slowly recovered by 2 weeks after the initiation of the therapy. The fibrinogen level decreased by 30%-50% after DFPP on day 1. It decreased to its lowest level on day 3, slowly recovered after the completion of DFPP, and then returned to a level similar to the pre-DFPP level by 2 weeks after the initiation of the therapy. The ZTT values and the  $\gamma$ -globulin and total cholesterol levels also showed similar changes. No change was observed in the serum albumin level (Fig. 5).

The adverse events that occurred during the IFN and DFPP combination therapy are listed in Table 2. Most of these were influenza-like symptoms and digestive symptoms that were associated with the IFN and ribavirin combination therapy. The adverse events attributable to concomitant DFPP and IFN therapy were mild hypotension in 2 patients and mild transient vagal reflex in 1 patient. These events occurred during DFPP, and fluid drip injection resulted in rapid recovery from these symptoms. A blood test revealed decreases in the hemoglobin as well as in the neutrophil and platelet counts; however, these were observed during IFN therapy, suggesting that they were unrelated to the DFPP. The ribavirin dose was reduced in 3 patients due to anorexia, anemia, and skin eruption. The IFN

dose was reduced in 1 patient due to neutropenia. It was difficult to continue the IFN and ribavirin combination therapy in 3 patients due to skin eruption, anorexia, and systemic malaise; therefore, in these patients the therapy was discontinued.

#### **4. Discussion**

Currently, the most promising therapy for chronic hepatitis C is a 48-week PEG-IFN and ribavirin combination therapy. Using this approach, the SVR is increased in approximately 50% of patients [20]. However, in the other half, HCV viremia persists and, although ALT is stabilized, hepatitis activity may again increase several years later. Alternatively, chronic hepatitis persists without the normalization of ALT and may progress to liver cirrhosis resulting in hepatocellular carcinoma. To prevent these events from occurring, it is necessary to develop other novel ideas or novel drugs for therapy. With regard to ideas for therapy, long-term IFN therapy extended over 48 weeks, the use of intravenously administrable IFN- $\beta$ , and concomitant IFN and high-dose ribavirin therapy with simultaneous monitoring of the blood ribavirin concentration, have been investigated [21]. With regard to novel drugs, amantadine, IL-12, and thymosin- $\alpha$ 1 are concomitantly used with IFN therapy or with IFN and ribavirin combination therapy [22-24]. With regard to novel antiviral agents, the development of an antisense complex targeting

the viral IRES, a serine protease inhibitor targeting NS3 protease, and a polymerase inhibitor targeting NS5B have been investigated [8-11].

Previous reports have described the various factors that affect the SVR produced by IFN therapy for chronic hepatitis. The patient-related factors include gender, age, the presence or absence of concomitant ribavirin treatment, and the stage of liver fibrosis; the viral factors include the viral genotype, HCV RNA level before therapy, and the number of mutations in the NS5A interferon sensitivity-determining region (ISDR) of genotype 1b [25]. Recently, an EVR has been recognized as an important factor for achieving an SVR using IFN therapy. An EVR is defined as virus elimination at an early stage after the initiation of treatment. An EVR in 2 weeks with IFN monotherapy and 12 weeks with IFN $\alpha$ -2b and ribavirin combination therapy has been shown to be frequent in SVR cases, and the probability of achieving an SVR is high when the virus is eliminated from the circulation within these early stages [12, 13].

In this study, we focused on the HCV RNA level before therapy and attempted to reduce the viral load of chronic genotype 1b hepatitis C patients, with a high viral load of 100 KIU/ml or higher, by performing plasmapheresis therapy, DFPP, before the initiation of IFN therapy. Thus far, this is the first attempt to study the efficacy

of a combination of IFN therapy and plasmapheresis with chronic hepatitis C patients.

In previous studies, Manzin et al. measured the HCV RNA in chronic hepatitis C patients with cryoglobulinemia before and after plasma exchange; they found that the HCV RNA was reduced by 45.3%–93.3% after treatment but returned to its previous level after 4–6 h [14]. Similarly, Ramratnam et al. observed a decrease in the viral load after plasmapheresis in HIV-1- and HCV-positive patients [26]. It has also been reported that heparin-induced extracorporeal low-density lipoprotein (LDL) precipitation (HELP) apheresis decreased the HCV RNA in chronic hepatitis C patients with the complication of hypercholesterolemia [16, 17, 27]. The decrease in the HCV RNA was transient according to these reports. By fitting a mathematical model to the changes in viral load during IFN therapy, HCV production in the liver has been estimated to be  $10^{12}$  particles per day [28]. Although the virus is eliminated from the blood, virus production continues in the liver unless it is inhibited by IFN; hence, the HCV RNA level may return to the pre-therapy level after a few hours. Therefore we planned to administer IFN immediately after the first plasmapheresis.

We selected DFPP as a plasmapheresis technique because there was no necessity to exchange plasma and supplement albumin during the apheresis.

Since DFPP is based on the principle of size separation, the membrane with a mean pore size of 30 nm that was used as the 2<sup>nd</sup> filter in this study can theoretically be expected to eliminate HCV particles with a diameter of 55 to 65 nm. After the DFPP 2<sup>nd</sup> filtration, the HCV RNA was quantitatively undetectable after performing DFPP for 1 h and 3 h; this implies that the filter could eliminate HCV particles and that the efficiency did not change with time.

Other low molecular weight substances, including albumin (MW 66 kDa) and ribavirin (MW 244 kDa), passed through the 2<sup>nd</sup> membrane and were returned to patient's blood. Hence, low molecular weight substances were not eliminated by this DFPP system.

In 7 patients, in whom the serum HCV RNA level could be measured before and after DFPP on day 1, the mean rate at which the HCV RNA level decreased was 48% (18%–78%); the HCV RNA level did not increase again. This exclusion may have been due to the inhibition of virus production by the IFN therapy that was initiated immediately after DFPP.

The EVR after 2 weeks of therapy tended to be higher in the DFPP group than in the control group. The change in viral load after 2 weeks also tended to decrease more in the DFPP group than in the control group. However, both these differences were statistically insignificant. The virus negativity in the DFPP group after 4 and 8 weeks was 66.7% and 62.5%, respectively. In the control group,

the HCV RNA levels were not measured periodically and therefore no comparison was possible. However, in a double-blind, controlled study in Japan on IFN $\alpha$ -2b and ribavirin combination therapy for chronic genotype 1b hepatitis C patients with a high viral load, Iino et al. found that the HCV RNA negativity after 4 and 8 weeks after the initiation of therapy was 18.8% and 38.2%, respectively. This suggested that IFN therapy with a concomitant reduction of the viral load using DFPP may induce an early conversion to the HCV RNA-negative state.

In our study, an SVR was observed in 2 of the 9 patients (22.2%) in the DFPP group and in 2 of the 11 patients (18.2%) in the control group. These rates are comparable to the result in which an SVR was observed in 19.0% of chronic genotype 1b HCV patients after 24 weeks after the administration of an interferon and ribavirin combination therapy [30]. An SVR observed in DFPP group was not higher than that in control group although an EVR on 2 weeks in DFPP was relatively higher than that in control group. One of the possible reasons for this observation is that, in 2 of the 6 patients exhibiting an EVR, treatment was discontinued because of the adverse events. EVR was demonstrated to be the only factor related to SVR by univariate analysis in our study; this may have been because the number of patients was insufficient to conduct an accurate analysis of these factors.

With regard to the safety of the IFN and ribavirin combination

therapy with concomitant DFPP, only mild hypotension and transient vagal reflex were observed after the initiation of DFPP, and these were rapidly resolved. There were no other adverse events attributable to concomitant DFPP, and the treatment was performed safely. Since DFPP is frequently used in the treatment of severe disease conditions, such as malignant rheumatoid arthritis, thrombotic thrombopenic purpura, and multiple sclerosis, there may be no problems with the application of this procedure to chronic hepatitis patients. During the treatment, a catheter was inserted in the right femoral vein for DFPP; however, no infection or accident occurred as a consequence of its indwelling. The other adverse events were attributable to the ribavirin and IFN therapy.

With regard to results of the blood test, the platelet count and fibrinogen level slowly decreased from the initiation of therapy until the completion of DFPP; however, these gradually recovered after the completion of DFPP. On day 3, the fibrinogen level was lower than 100 mg/dl in all but 2 patients. To ensure the safety of the patients, and to prevent complications such as hemorrhage, DFPP conducted 3 times on days 1, 2, and 4 during the 1<sup>st</sup> week of therapy may be appropriate for patients with chronic hepatitis C.

In view of the fact that we found no significant difference in the EVR and SVR between the DFPP and control groups, and because the number of patients was very small, it was not possible in this

preliminary study to draw conclusions regarding the applicability of the DFPP used in conjunction with ribavirin and IFN therapy. It could be argued that a simple physical reduction in the viral load, induced by concomitant DFPP or other apheresis, is really significant for the ribavirin and IFN therapy for chronic hepatitis C patients with a high viral load. Based on our results, it may be possible to use DFPP to facilitate early viral elimination. However, the early viral elimination by DFPP was not related to an SVR. Although 5 of the 8 patients (62.5%, therapy was discontinued in 1 patient after 7 weeks) became virus-negative after 8 weeks, an SVR was observed in only 2 patients; thus, the relationship between the early virus negativity obtained by DFPP and the SVR remains unclear. Since the early conversion to the virus-negative state is a result of the reactivity of the host and virus to ribavirin and IFN, it may be possible that a simple physical reduction in the amount of the virus is not significant. However, DFPP eliminates not only the virus but also macromolecules including immunoglobulins. The elimination of some humoral factors or complement components by the dialysis membrane might be involved. The state of HCV in the blood, such as the immunoglobulin-bound state, the lipoprotein-associated state, and the non-bound (free) state, may also be important. In 2 preliminary cases, the state of HCV during the treatment was investigated using differential flotation centrifugation. In



differential floatation centrifugation, the hyperbaric fraction, including the immunoglobulin-bound HCV particles, settles to the bottom, whereas the hypobaric fraction, including free-state HCV particles, rises to the top. In the 2 examined cases, the bottom:top ratio was reduced after the first DFPP treatment; from 214 to 62 in case 1 and from 108 to 46 in case 2. Since a decline in the bottom:top ratio indicates an increased amount of free-state HCV particles compared to the amount of immunoglobulin-bound HCV particles, these results suggest that the occurrence of the free-state HCV increased after DFPP. This increase in the amount of free-state HCV may possibly involve an SVR; this supposition is based on a previous study that demonstrated that a high proportion of free-state HCV was related to an SVR [29].

In conclusion, in order to achieve a reduction in the level of HCV RNA before IFN therapy and to facilitate an early conversion to a virus-negative state, plasmapheresis therapy (DFPP) was performed on chronic genotype 1b hepatitis C patients with a high viral load. The therapy was performed without the occurrence of severe adverse events, and the results suggested the possibility that concomitant DFPP increased the rate of early conversion to a virus-negative state. Since only 9 patients were treated and IFN administration was discontinued in 3 patients, the relationship between DFPP and an EVR or SVR is unclear. If an EVR is achieved

by a combination of DFPP and interferon therapy, and this is related to an SVR, the combination therapy with DFPP will not only be of benefit to chronic hepatitis C patients with a high viral load but may also shorten the period of interferon therapy. Furthermore, the cost of interferon therapy will also be reduced, although the cost of performing DFPP 3 times will remain high at approximately 2,500 dollars. Because our study was limited by the small number of patients, further investigations using a study design that clarifies the relationship between DFPP and an EVR or SVR are necessary. It is also important to further investigate the antiviral effect of the PEG-IFN and ribavirin combination therapy with concomitant DFPP, and the effects of DFPP other than virus elimination. In order to clarify this relationship, a multi-center clinical trial is being undertaken in Japan, and the data from this study is currently being collected.

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## Tables

**Table 1.** Baseline characteristics of chronic genotype 1b hepatitis C patients with a high viral load, treated with a combination of DFPP with interferon (IFN) and ribavirin therapy (DFPP group) and IFN and ribavirin therapy without DFPP (control group).

**Table 2.** Rates of discontinuation of treatment, dose reduction, and the occurrence of adverse events during treatment.

## Figure legends

### **Fig. 1** Mechanism of double filtration plasmapheresis (DFPP)

The blood is separated into plasma and blood corpuscles by the plasma separator (1<sup>st</sup> filter), and then filtered using a plasma fractionator (2<sup>nd</sup> filter) which separates the plasma into low and high molecular weight components.

### **Fig. 2** Change in plasma HCV RNA pre- and post-plasma fractionator (2<sup>nd</sup> filter)

In all 9 cases, HCV RNA was not detected in the plasma of the post-plasma fractionator filtrate 1 h after starting the DFPP.

### **Fig. 3** Change of HCV RNA load 2 weeks after treatment

A. An EVR is defined as a viral load decline of 2 log<sub>10</sub> units or more from the baseline level after 2 weeks of treatment.

The numbers in each column indicate the ratio of EVR cases/all treatment cases.

B. Viral load change after 2 weeks. Viral load change was calculated by the formula; log<sub>10</sub> (HCV RNA load after 2 weeks/ HCV RNA load at pre-treatment).

### **Fig. 4** Change in the serum ALT level during treatment

The ALT level was normalized in 8 cases in the 2<sup>nd</sup> week of treatment.

**Fig. 5** Changes in the laboratory findings during treatment

a: Platelet count, b: Fibrinogen, c: ZTT, d:  $\gamma$ -globulin, and e: Albumin.

Table 1. Baseline characteristics of chronic genotype 1b hepatitis C patients and a high viral load treated by concomitant DFPP with interferon (IFN) and ribavirin therapy and control group

	DFPP+IFN-R	IFN-R	P-value
Gender (M/F)	4/5	7/4	0.684
Age	51.7±11.3	50.6±10.6	0.856
Serum HCV-RNA(KIU/mL)			
mean (min.-max)	2,162 (224-12,000)	818 (340-1,700)	0.254
100-500/500≤	3/6	3/8	
Previous IFN treatment			
Naive/Retreatment	6/3	5/6	0.343
Liver histology			
Stage (F0/F1/F2/F3/F4)	1/1/7/0/0	0/5/6/0/0	0.167
Grade (A0/A1/A2/A3)	0/5/3/1	1/7/3/0	0.541
ALT (IU/mL)	88.4±47.7	89.5±34.5	0.957
Hemoglobin (g/dL)	14.0±1.7	14.2±1.2	0.728
Platelet count ( × 10 <sup>4</sup> /μ L)	16.9±5.2	15.7±3.4	0.551
Fibrinogen (mg/dL)	231.0±38.9	222.7±92.3	0.804
ZTT (IU)	12.4±4.9	14.2±6.4	0.496
Total protein (mg/dL)	6.9±0.4	6.9±0.4	>0.999
Albumin (g/dL)	4.2±0.3	4.1±0.4	0.373
γ-globulin (g/dL)	1.3±0.2	1.4±0.3	0.492
Total cholesterol (mg/dL)	178.6±28.3	172.0±45.2	0.714

Table 2. Rates of discontinuation of treatment, dose reduction, and occurrence of adverse events during treatment.

Adverse events	n	(%)
Influenza-like symptoms	8	(89)
Skin eruption	1	(11)
Gastrointestinal symptoms	6	(67)
Insomnia	2	(22)
Hypotension	2	(22)
Vagal reflux	1	(11)
Depression	5	(56)
Anemia	7	(78)
Neutropenia	1	(11)
Thrombocytopenia	1	(11)
Dose reduction	4	(44)
Discontinuation of treatment	3	(33)

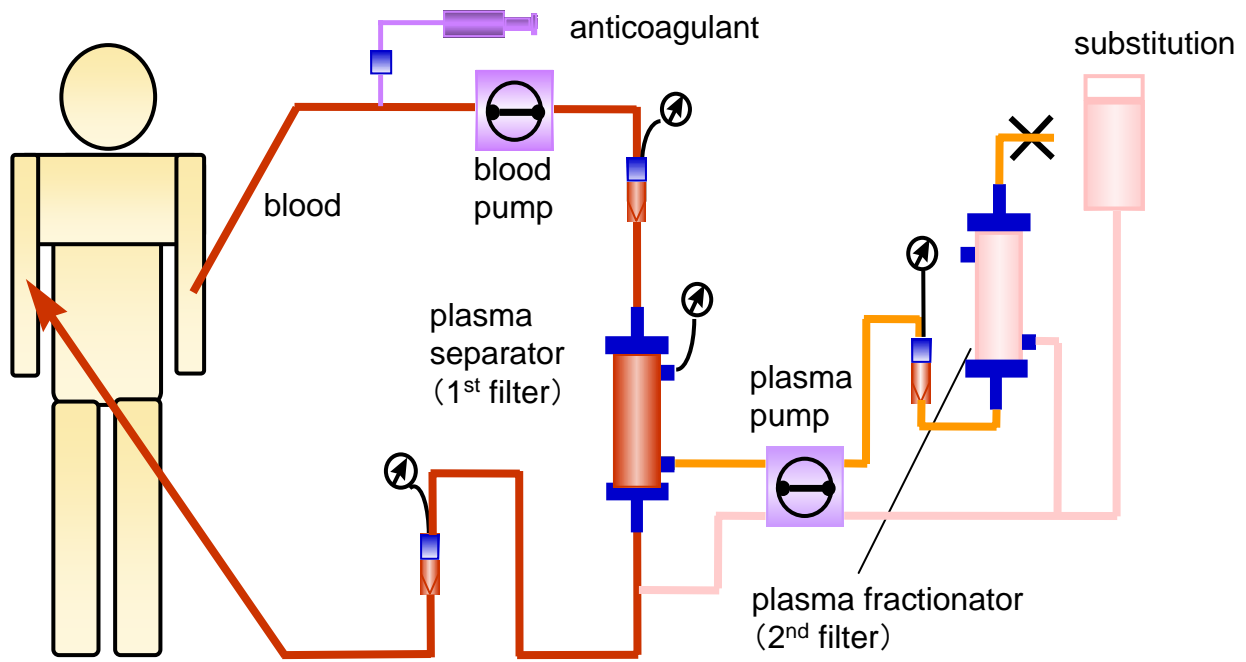
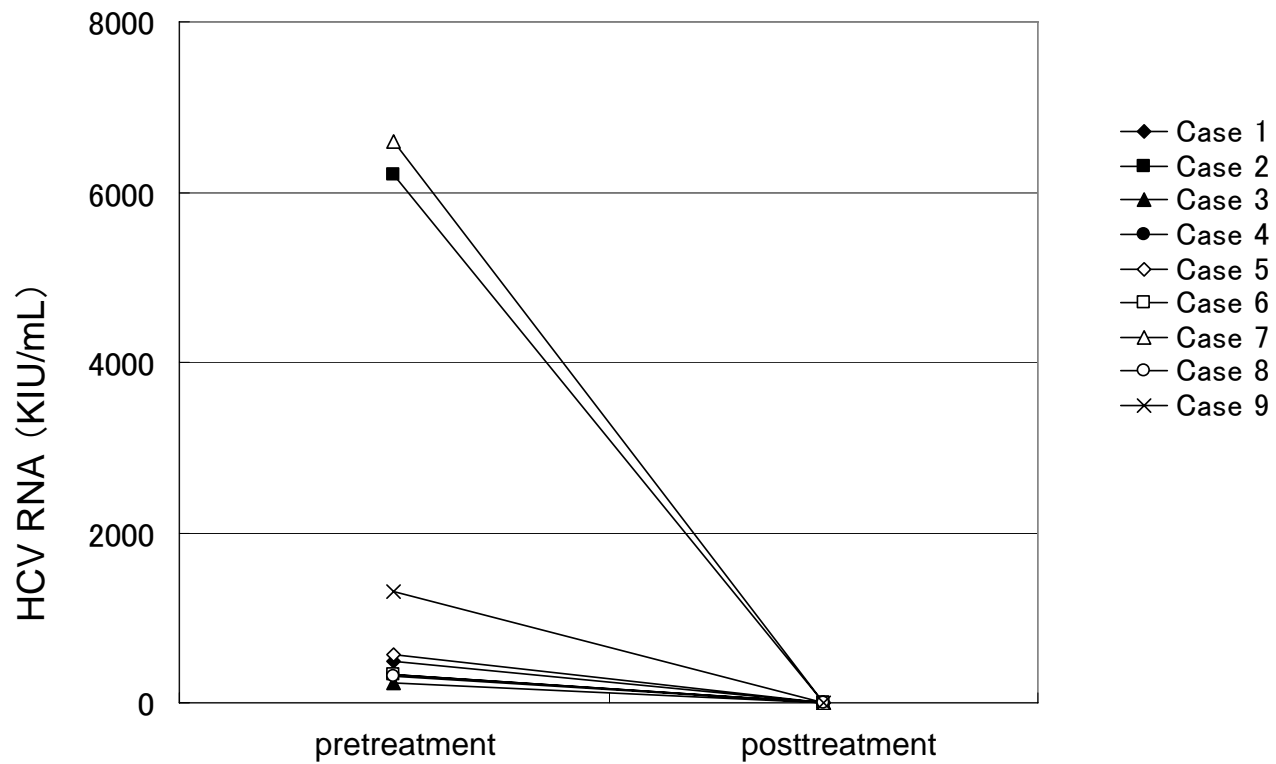
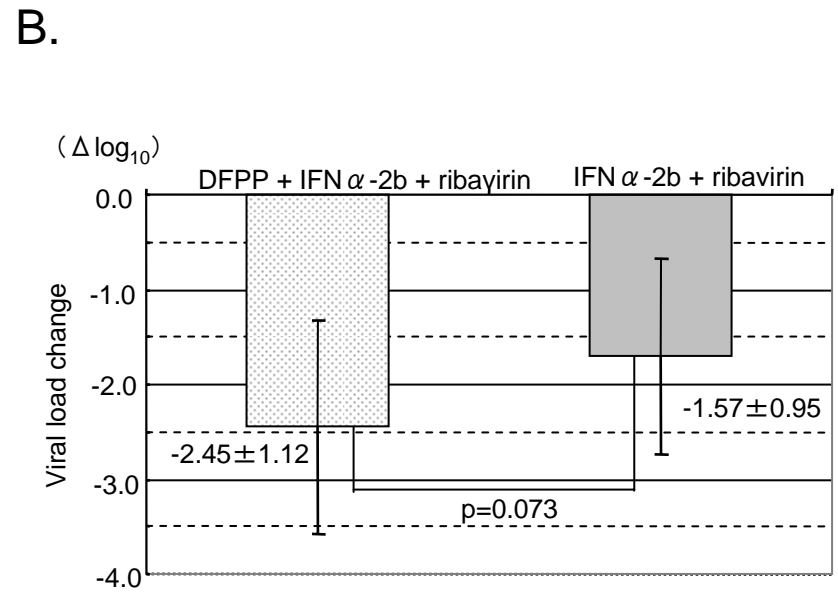
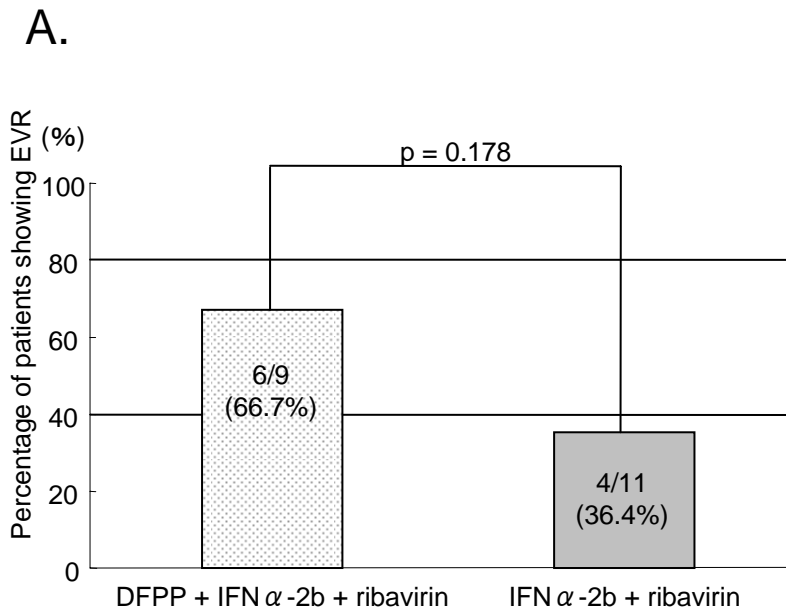


Fig.1 Mechanism of DFPP (double filtration plasma pheresis)

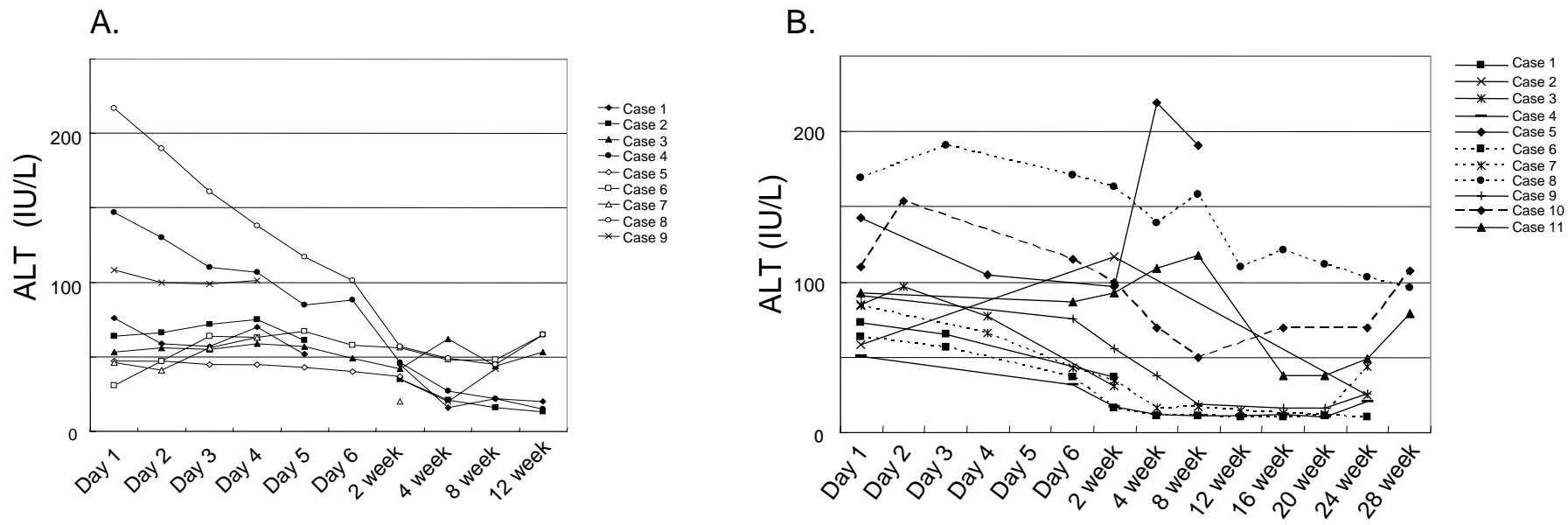


**Fig.2** Change in plasma HCV RNA pre- and post-plasma fractionator (second 2<sup>nd</sup> filter)

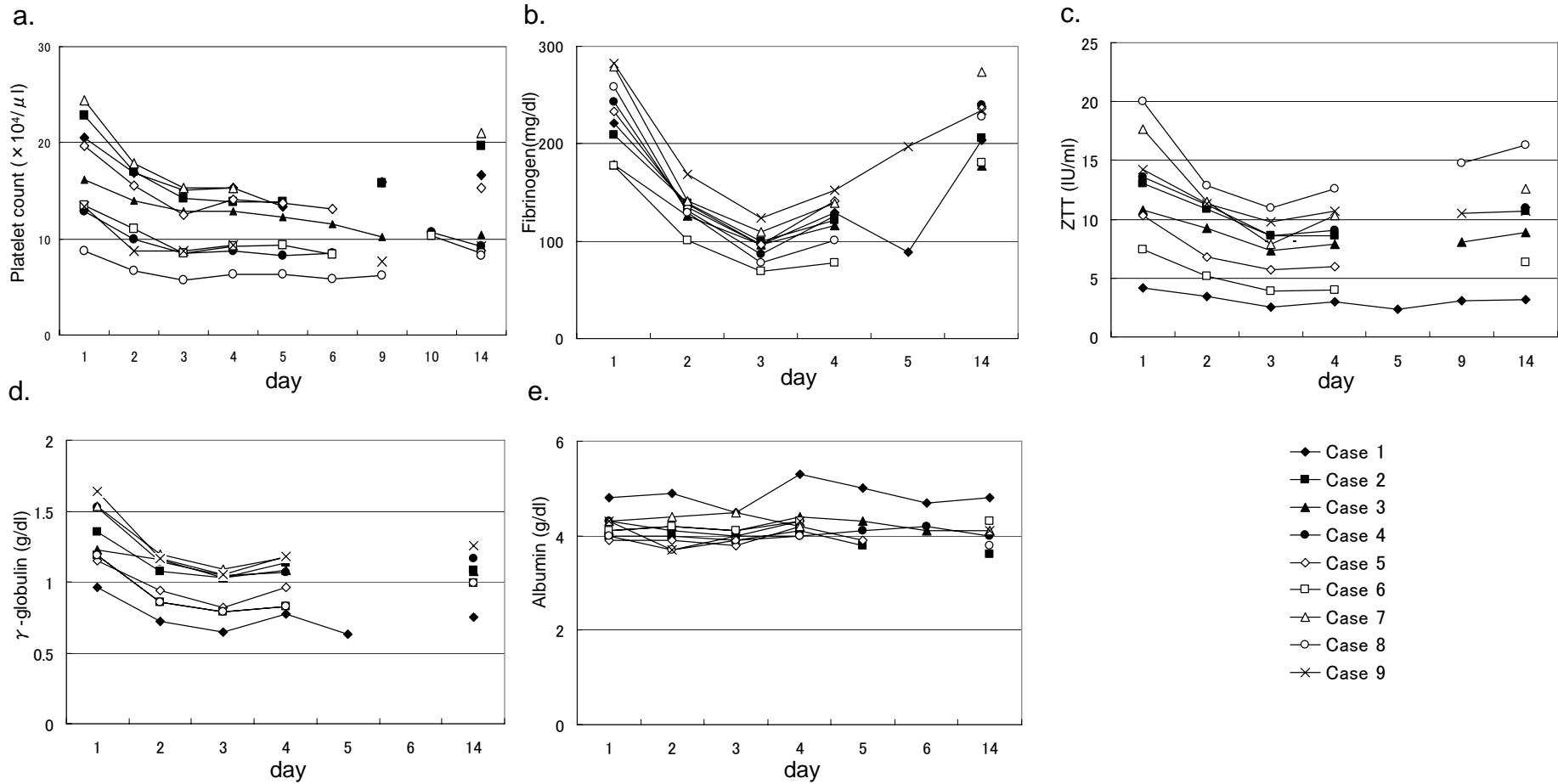




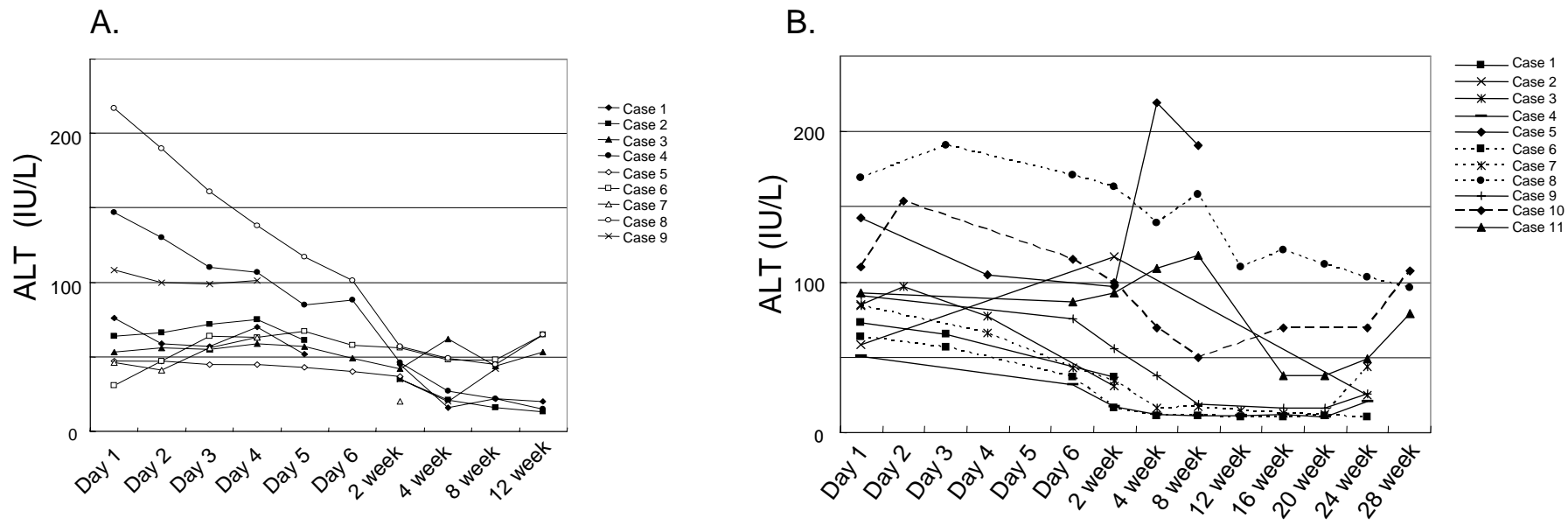
**Fig.3** Change of HCV RNA load at 2 week after treatment



**Fig.4** Change of in the serum ALT level during treatment



**Fig.5** Changes in the laboratory data findings during treatment



**Fig.4** Change of in the serum ALT level during treatment