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Research Paper

The Safety of Chemotherapy for Breast Cancer Patients with Hepatitis C Virus Infection

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Abstract

Background: Hepatitis C virus (HCV) infection is one of the major causes of chronic liver disease, and more than 880,000 people are estimated to be infected with HCV in Japan. Little information is available on the outcomes of HCV during chemotherapy for solid tumors, and the impact of HCV infection on toxicity of chemotherapy is unknown.

Materials and methods: We performed a retrospective survey of 1,110 patients diagnosed with breast cancer between January 2006 and March 2011 at our institution. All patients had been screened for hepatitis C serology at diagnosis of breast cancer. We retrospectively investigated the change in HCV load and the toxicities of chemotherapy, based on review of their medical records.

Results: 23 patients were identified as having a positive test for anti-HCV antibodies. Ten of these patients received chemotherapy. Their median age was 66 years. No patient had decompensated liver disease at baseline. Eight patients received cytotoxic agents with or without trastuzumab, and two patients received trastuzumab alone. Four of eight patients who received cytotoxic chemotherapy developed febrile neutropenia and one developed transaminases elevation. Serum HCV-ribonucleic acid (RNA) level before and after chemotherapy was evaluated in six patients. Median serum HCV-RNA level at baseline and after chemotherapy was 6.5 and 6.7 logIU/ml, respectively.

Conclusion: Chemotherapy for breast cancer patients with HCV infection is feasible, and viral load doesn't change during the chemotherapy.

Key words: HCV, HCV-RNA, febrile neutropenia, Child-Pugh criteria, liver cirrhosis, chemotherapy.

Introduction

Hepatitis C virus (HCV) infection is one of the major causes of chronic liver disease, and more than 880,000 people are estimated to be infected with HCV in Japan (1). The estimated number of HCV carriers increases with age, therefore, carriers aged from 40 to 69 years account for more than 80% of cases (1). Breast cancer is the most common cancer among Japanese women (2). Furthermore, the age-adjusted breast cancer incidence rate has been increasing since 1975, and the incidence rate of breast cancer is highest in the

age group of 40-49 years in Japan (2).

Little information is available on the status of HCV during chemotherapy for solid tumors and the influence of HCV infection on toxicity of chemotherapy is also unknown. Although there are guidelines for management of patients with Hepatitis B virus during chemotherapy, there are no data to support the use of chemotherapy to treat HCV-positive patients with solid tumors (3, 4). Some reports have noted the reactivation of HCV in patients with lymphoma who have received rituximab and combination chemotherapy (5, 6). However, there are substantial differences in immunosuppressive mechanisms between rituximab-based chemotherapy for hematologic malignancies and conventional chemotherapy for solid tumor, because rituximab, an anti-CD20 antigen, mainly inhibits B-cell function. Therefore, it may not be appropriate to use the same management during chemotherapy for HCV carrier patients with solid tumors.

The purpose of this study was to evaluate the safety profile and the change in HCV viral load during chemotherapy for HCV-carrier patients with breast cancer.

Materials and methods

Following data collection and analysis was approved by the Institutional Review Board of Toranomon hospital.

Patients

We performed a retrospective survey of 1,110 patients diagnosed with breast cancer between January 2006 and March 2011 at our institution. All patients had been screened for hepatitis C serology determined by anti-HCV antibody at diagnosis of breast cancer. Our survey identified 23 patients who were positive for anti-HCV antibodies. The incidence of HCV-positive in breast cancer patients in our institution (23/1,100 [2.1%]) is comparable to that in same age range of general women population (3,221/151,501 [2.1%]) (1). Ten of these 23 patients received cytotoxic agents and/or trastuzumab. We retrospectively investigated the baseline patient and tumor characteristics, the changes in HCV load, and the toxicities of chemotherapy for these ten patients, based on review of their medical records.

Assessment of breast cancer characteristics

We collected American Joint Commission on Cancer stage, hormone receptor (HR) status and human epidermal growth factor-2 (HER2)/neu status using immunohistochemistry (IHC) and/or fluorescent *in situ* hybridization (FISH) at breast cancer diagnosis. We classified HR-positive as estrogen receptor (ER) positive and/or progesterone receptor (PgR) positive using IHC. The cut point for ER and PgR positivity was an Allred score of 3 (7). HER2 status was defined as positive if an IHC assay demonstrated 3+ or an IHC score of 2+ with FISH demonstrated a gene copy ratio of HER2: CEP17 more than 2.2 (8).

Assessment of HCV infection status

The presence of anti-HCV antibodies was detected using chemiluminescence enzyme immunoassay (Lumipulse Ortho HCV antigen, Ortho-Clinical Diagnostics, Tokyo, Japan) at breast cancer diagnosis. HCV ribonucleic acid (RNA) in serum was detected by a TaqMan Real-Time polymerase chain reaction (PCR) assay (SRL, Tokyo, Japan). In 6 patients, serum HCV-RNA was evaluated before the initiation of chemotherapy and within two months after completing chemotherapy.

Assessment of liver function

Liver cirrhosis was assessed by clinical criteria (Child-Pugh criteria) at baseline. Data of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total bilirubin at baseline, during chemotherapy and three months after completion chemotherapy were collected and assessed by Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Assessment of other toxicities

White blood cell count, neutrophil count, platelet count at baseline and during chemotherapy were collected and assessed by CTCAE version 4.0. Events of febrile neutropenia and the use of growth factor support during chemotherapy were collected.

Results

Patients' characteristics

Ten of 23 HCV-positive patients with breast cancer received cytotoxic agents and/or trastuzumab. Among the remaining 13 patients, ten received only endocrine therapy, two had ductal carcinoma in situ and one was lost to follow-up. The reasons for receiving only hormone therapy among the ten patients included the following; five were low risk of recurrence, three were elderly, age over 75, and two had decompensated liver function.

Patients and tumor characteristics are shown in Table 1. The median age at receiving chemotherapy was 66 (range 55-77). Most patients (80%) had stage II or III disease. Tumor histology of all patients was invasive ductal carcinoma. Tumors were classified on the bases of estrogen/progesterone receptor and

HER2 status. Three tumors were HR-positive/ HER2-positive, one was HR-positive/HER2-negative, three were HR-negative/HER2-positive, and three tumors were HR-negative/ HER2-negative, respectively. Three patients had a diagnosis of liver cirrhosis. One patient was classified Child B, the remaining two patients were Child A. No patient had decompensated liver disease at baseline.

Treatment course and safety

Table 2 shows the chemotherapy regimens and their associated documented toxicities. Among the 10 patients who received chemotherapy, three patients received an anthracycline-based regimen, two received anthracycline followed by taxane, two received trastuzumab, two received combination therapy with docetaxel, cyclophosphamide and trastuzumab, and one received gemcitabine.

All patients other than the two who received trastuzumab used dexamethasone as antiemetic prophylaxis. The dose of dexamethasone followed American Society of Clinical Oncology Clinical Practice Guidelines (9, 10).

Six (75%) of eight patients who received cytotoxic chemotherapy developed grade 4 neutropenia and four (50%) developed febrile neutropenia. Transaminases elevated in one (13%) of eight patients who received cytotoxic chemotherapy. This patient had Child A liver cirrhosis and transaminase elevation at baseline. Four patients received granulocyte colony-stimulating factor (G-CSF). All received the non-pegylated formulation G-CSF. Three patients received G-CSF as a therapeutic use for neutropenia without fever. Two of these patients developed febrile neutropenia. One patient who received G-CSF as a secondary prophylaxis did not develop febrile neutropenia during G-CSF support. No patients received prophylactic antibiotics.

HCV-RNA status

Table 3 showed the change in HCV-RNA in six patients. The median HCV-RNA before the initiation of chemotherapy was 6.5 log IU/ml and after completing chemotherapy was 6.7 log IU/ml.

Table 1. Patients' characteristics.

Case	Age	Stage	Histology	Hormone receptor	HER2 status	LC	Prior HCV therapy
1	63	IIA	IDC	negative	negative	negative	positive
2	50	IIA	IDC	positive	negative	negative	negative
3	50	IIIA	IDC	positive	negative	negative	negative
4	61	IIB	IDC	negative	positive	negative	negative
5	71	IIA	IDC	negative	positive	negative	positive
6	64	IIA	IDC	positive	positive	positive	negative
7	67	IV	IDC	negative	negative	positive	positive
8	69	IIA	IDC	negative	positive	negative	negative
9	60	IIA	IDC	negative	negative	negative	negative
10	74	IV	IDC	positive	negative	positive	negative

LC: liver cirrhosis, HCV: hepatitis C virus, IDC: invasive ductal carcinoma.

Table 2. Toxicities

Case	Chemotherapy regimen	Transaminase baseline	Transaminase increase (grade)	WBC/Plt baseline	Neutropenia (grade)	Thrombocytopenia (grade)	G-CSF	FN	dose reduction (%)
1	EC	increase	0	normal	4	3	positive	positive	0
2	EC	normal	0	normal	0	0	negative	negative	0
3	EC	normal	0	normal	4	1	positive	negative	0
	DTX	normal	0	normal	4	0	positive	positive	75
4	TCH	normal	0	normal	4	1	positive	negative	0
5	H	normal	0	normal	0	0	negative	negative	0
6	H	normal	0	decrease	0	0	negative	negative	0
7	EC	increase	1	decrease	4	3	negative	positive	75
8	TCH	normal	0	normal	3	0	negative	negative	0
9	EC	normal	0	normal	4	3	positive	positive	0
	wPTX	normal	0	decrease	4	2	positive	negative	0
10	G	increase	0	decrease	4	3	negative	negative	75

WBC: white blood cell count, Plt: platelets count, G-CSF: granulocyte colony stimulating factor, FN: febrile neutropenia, EC: epirubicine + cyclophosphamide, DTX: docetaxel, TCH: docetaxel + cyclophosphamide + trastuzumab, H: trastuzumab, wPTX: weekly paclitaxel, G: gemcitabine.

Table 3. HCV viral load.

Case	HCV-RNA baseline	HCV-RNA after chemotherapy
1	NA	NA
2	6.3	6.9
3	6.7	6.7
4	NA	NA
5	6.2	6.5
6	6.5	6
7	6.7	6.7
8	6.5	6.9
9	NA	NA
10	NA	NA

NA: not assessed.

Discussion

The present study demonstrates that chemotherapy for breast cancer patients with HCV infection is feasible, and viral load does not vary during the chemotherapy.

A previous study reported that chemotherapy induced elevation of transaminases in patients with HCV infection (5, 11); however the relationship between increase in HCV-RNA and transaminase elevation is poorly investigated. Morrow et al. showed that nine of 36 (25%) HCV positive patients who received chemotherapy for breast cancer developed elevated liver enzymes, but their study did not evaluate HCV load (12). Our study demonstrated no clinically meaningful changes in HCV-RNA viral load in breast cancer patients who received cytotoxic chemotherapy and/or trastuzumab. It also showed that only one patient (13%) had transaminase elevation during chemotherapy. This patient's HCV-RNA was the same before and after chemotherapy. These findings suggest that the elevation of transaminase in our study might not be related to viral reactivation but direct liver toxicity from cytotoxic agents.

Some studies have suggested that B-cell mediated immunosuppression induced by rituximab results in the elevation of transaminase (5, 6, 13). Coppola et al. showed that rituximab-based chemotherapy resulted in an increase in HCV-RNA at least 1.5 log IU/ml (median 2.2 [range 1.5-2.6]) followed by hepatic flare (defined as ALT elevation of more than five times of upper limit of normal or more than 3.6 time of baseline ALT) among patients with lymphoma (6). However, the mechanism of liver injury in HCV infection is still unclear. Further investigations in the relationship between HCV load and liver injury are warranted.

Previous studies showed a negative impact of corticosteroids on HCV viral load (14). These studies also demonstrated that cumulative exposure to corticosteroids is associated with higher levels of HCV viremia (14). Meanwhile, our study showed that the exposure to dexamethasone use as an antiemetic might not affect HCV viral load.

Our study showed that 87.5% patients who received cytotoxic agents developed grade 3-4 neutropenia. Although the incidence of neutropenia depends on the timing and the frequency of blood tests, it is important to note that febrile neutropenia occurred in 50% of patients who received cytotoxic chemotherapy in our study. A previous study from MD Anderson Cancer Center showed that eight of 36 (22%) developed febrile neutropenia (12). Some hypotheses can be raised concerning the causes of the high incidence of febrile neutropenia in our study. The median age of 66 years in the current study was higher than that of the previous report from MD Anderson Cancer Center (48 years) and the highest incidence age of breast cancer in Japan (40-49 years) (2, 12). These findings suggest that older age might contribute to developing the greater incidence of febrile neutropenia as seen in our study. Previous studies of adjuvant chemotherapy for older patients with breast cancer showed that older patients had greater hematologic toxicity, especially neutropenia and febrile neutropenia compared to younger individuals (15, 16). Other potential causes of the high incidence of febrile neutropenia could be considered. Chronic HCV infection may lead to an immunocompromised status such as neutrophil or T-cell dysfunction. Neutropenia can be seen in patients with chronic hepatitis C infection due to cirrhosis and hypersplenism. Although four of ten patients had white blood cell counts decrease (grade 1-2) at baseline in the current study, only one of them developed febrile neutropenia (table 2). Although the relationship between HCV infection and the high incidence of febrile neutropenia is uncertain, clinicians should be concerned with the risk of high-grade neutropenia and febrile neutropenia in HCV-positive patients who receive cytotoxic agents.

Conclusion

Chemotherapy for breast cancer patients with HCV infection is feasible and clinically indicated therapy should not be withheld due to positive HCV serology. Caution regarding neutropenia/ febrile neutropenia is warranted. Use of growth factors may be considered. However, the present study is too small to draw a definite conclusion on the safety of chemotherapy for solid tumors. Further large-scaled investigations are warranted.

Abbreviations

HCV: Hepatitis C virus; RNA: ribonucleic acid; HR: hormone receptor; IHC: immunohistochemistry; HER2: human epidermal growth factor-2; FISH: fluorescent *in situ* hybridization; PCR: polymerase chain reaction; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CTCAE: Common Terminology Criteria for Adverse Events; G-CSF: granulocyte colony-stimulating factor.

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Ethics Committee Approval

The data collection and analysis of the current study was approved by the Institutional Review Board of Toranomon hospital.

Competing interests

Dr. Kumada received grant from MSD K.K., Bristol-Myers Squibb K.K., Dainippon Sumitomo Pharma, and Daiichi Sankyo. All other authors state that they have no conflicts of interest.

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ORIGINAL ARTICLE

Dermatological side-effects of telaprevir-based triple therapy for chronic hepatitis C in phase III trials in Japan

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ABSTRACT

Telaprevir-based triple therapy is highly effective for chronic hepatitis C. However, concern has been expressed over the high frequency and severity of its dermatological side-effects compared with those associated with peginterferon (PEG-IFN) and ribavirin (RBV) therapy. Thus, here, we evaluated the dermatological adverse reactions of telaprevir-based triple therapy in Japanese multicenter phase III clinical trials in an attempt to characterize the dermatological side-effects and establish appropriate management plans. In these trials, 126 treatment-naïve patients and 141 treatment-failure patients were administrated telaprevir, PEG-IFN-α-2b and RBV for 12 weeks followed by PEG-IFN-α-2b and RBV for another 12 weeks (T12/PR24 group), and 63 treatment-naïve patients were administrated PEG-IFN-α-2b and RBV for 48 weeks (PR48 group). Dermatological adverse reactions developed in over 80% patients in both groups, and most of them were grade 1 or 2. In the T12/PR24 group, there were more grade 2 or grade 3 events, and the time to onset was earlier than that in the PR48 group. Most reactions could be managed with topical corticosteroids and oral antihistamines, and the rates of discontinuation due to dermatological reactions were not high even in the T12/PR24 group. In the T12/PR24 group, however, two cases of Stevens–Johnson syndrome and one case of drug rash with eosinophilia and systemic symptoms, which corresponds to drug-induced hypersensitivity syndrome in Japan, were reported. For appropriate treatments of individual dermatological adverse reactions, the judgment of discontinuation of antiviral drugs and treatment based on the severity are extremely important in this triple therapy.

Key words: chronic hepatitis C, dermatological adverse reaction, drug rash with eosinophilia and systemic symptoms, Stevens–Johnson syndrome, telaprevir.

INTRODUCTION

Telaprevir, a novel direct-acting antiviral, inhibits the NS3-4A serine protease of hepatitis C virus (HCV) and suppresses HCV replication.[1] Triple therapy of telaprevir, peginterferon (PEG-IFN) and ribavirin (RBV) has proved to be more effective for treating chronic hepatitis C (CHC) compared to PEG-IFN and RBV combination therapy.[2–7]

In Japan, three phase III trials for telaprevir-based triple therapy were performed on treatment-naïve (TN) patients (patients who had never been treated with IFN agents), relapsers (patients who had undetectable HCV RNA during previous therapy for CHC), and non-responders (patients who never achieved undetectable HCV RNA during previous therapy for CHC). In these trials, the sustained virological response (SVR) rates of patients were as follows: TN patients, 73.0% in T12/PR24 group, 49.2% in PR48 group; relapsers, 88.1%; and non-responders, 34.4%.[6,7] The established duration of telaprevir-based triple therapy is 24 weeks, which is half of that for PEG-IFN and RBV therapy. Therefore, the higher efficacy of triple therapy achieved over a shorter period make it markedly superior to PEG-IFN and RBV therapy.

Dermatological side-effects were also observed in Japanese trials of telaprevir monotherapy that lasted for 12 or 24 weeks. The severity of the side-effects was mild to moderate, and two patients discontinued telaprevir due to development of skin disorders (pruritic rash or herpes zoster).[8,9]

Peginterferon and ribavirin combination therapy has also been known to cause cutaneous adverse reactions.[10,11] Even with IFN monotherapy, dermatological reactions were observed

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locally at injection sites or systemically on occasion, and combination therapy with RBV has been reported to cause an increased incidence of cutaneous reactions. These reactions include generalized pruritus, xerosis, erythematous papules and microvesicles mainly localized to the limbs and areas of friction.[10–14] Usually, topical corticosteroids or emollients are effective in managing the cutaneous reactions, and discontinuation of the antiviral drugs is not required.[10]

Because dermatological adverse reactions have been observed in telaprevir monotherapy and PEG-IFN and RBV combination therapy, there is a possibility that dermatological reactions develop even more frequently in the triple therapy. Phase II trials in the USA and in European countries (EU) show a higher incidence of dermatological adverse reactions in the telaprevir, PEG-IFN and RBV group than in the PEG-IFN and RBV group.[2,3] On the basis of this finding, for the Japanese phase III trials, we classified the severity in the same manner as that of the US/EU trials (Table 1) and collected detailed information. We hereby show the characteristics of the dermatological adverse reactions of telaprevir-based therapy, and consider the criteria for drug discontinuation and management plan for these reactions.

METHODS

Patients

Multicenter, randomized, phase III trials were performed at 42 Japanese medical institutions from 2008 to 2010. In these trials, 126 TN patients and 141 treatment-failure (TF) patients were administrated telaprevir, PEG-IFN and RBV for 12 weeks followed by PEG-IFN and RBV for another 12 weeks (T12/PR24 group, $n = 267$), and 63 TN patients were administrated PEG-IFN and RBV for 48 weeks (PR48 group).[6,7] The TF patients comprised 109 relapsers and 32 non-responders.[7] The principal eligibility criteria were as follows: (i) a diagnosis of CHC; (ii) infection with HCV-1; (iii) HCV RNA levels of 5.0 $\log_{10}$ IU/mL or more; (iv) age at entry, 20–65 years; and (v) bodyweight of more than 40 kg and 120 kg or less. The main exclusion criteria were as follows: (i) hemoglobin level of less than 12 g/dL, neutrophil count of less than 1500/mm^3 and platelet count of less than 100 000/mm^3; (ii) positive for antibodies against hepatitis B surface antigen or HIV; and (iii) chronic renal failure or a creatinine clearance of 50 mL/min or less.

Each patient gave a written informed consent before participating in these studies.

Study design

The clinical trial involving TN patients was a multicenter, randomized, controlled study, where 189 patients were assigned to either the T12/PR24 group or the PR48 group.[6] The trials that included TF patients (relapsers and non-responders) were open-label studies that included the T12/PR24 group alone.[7]

For patients in the T12/PR24 group, telaprevir was administrated p.o. t.i.d. every 8 h at a dose of 750 mg after meals for 12 weeks. PEG-IFN-α-2b (PegIntron; MSD, Tokyo, Japan) was administrated s.c. once a week (1.5 μg/kg; range, 1.250–1.739 μg/kg) and RBV (Rebetol; MSD) was administrated p.o.

Table 1. Severity classification of dermatological adverse reactions in phase III trials in Japan

Severity	Criteria	Management
Grade 1	Involvement of ≤50% of the body surface, localized No evidence of systemic symptoms	Consultation with a dermatologist, if needed
Grade 2	Involvement of ≤50% of the body surface, multiple or diffuse lesions Or rash with any of the following characteristics: Mild systemic symptoms Mucous membranes involved but with no ulceration/erosion	Discontinuation of the study drugs is generally not necessary, the investigators can consider the following, if needed: Discontinuation of telaprevir Discontinuation, interruption, or reduction of PEG-IFN and RBV
Grade 3	Generalized rash involving >50% of the body surface Or rash with any of the following characteristics: Appearance of significant systemic symptoms that are new and are considered to be related to the onset and/or progression of the rash Ulceration/erosion of mucous membranes Epidermal detachment (epidermal necrosis or separation of epidermis from underlying dermis) Target lesions Vesicles or bullae Palpable purpura	Consultation with a dermatologist Discontinuation of telaprevir (in principle) Consider discontinuation or reduction of PEG-IFN and RBV
Life-threatening	SJS, TEN, DRESS,† EM‡ Or other life-threatening symptoms, Or cases where features of serious disease are observed	Consultation with a dermatologist Immediate discontinuation of all drugs

†DRESS corresponds to the Japanese term for DIHS.
‡EM is not life-threatening, but in severe cases, mucous membrane lesions and systemic symptoms are encountered. In phase III trials, EM was reported in five patients and was serious in one patient. DIHS, drug-induced hypersensitivity syndrome; DRESS, drug rash with eosinophilia and systemic symptoms; EM, erythema multiforme; PEG-IFN, pegylated interferon; RBV, ribavirin; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.

b.i.d. after meals (200–600 mg/dose; daily dose, 600–1000 mg) for 24 weeks. The patients in the PR48 group received PEG-IFN-α-2b and RBV at the aforementioned doses for 48 weeks. The doses of PEG-IFN-α-2b and RBV were reduced when the patients' hemoglobin level, white blood cell count, neutrophil count or platelet count decreased, or when adverse events developed.[6,7] Patients in the T12/PR24 group and PR48 group were followed up for 24 weeks.

Assessment

Dermatological adverse reactions were investigated during the administration period of 24 weeks in the T12/PR24 group and 48 weeks in the PR48 group, and during the 24-week follow-up period in both groups. We integrated the data of the 126 TN patients and 141 TF patients (109 relapsers and 32 non-responders) who were administrated telaprevir, PEG-IFN-α-2b and RBV in three phase III trials (Table 2).

Table 1 shows the severity classification system used to evaluate dermatological adverse reactions. When the skin reactions were detected, the investigators referred the patients to a dermatologist as needed, and reported the events in reference to the diagnosis of the dermatologist. In cases where the severity was considered as grade 3, telaprevir was discontinued in principle. In addition, in cases where severe cutaneous reactions including Stevens–Johnson syndrome (SJS) and drug rash with eosinophilia and systemic symptoms (DRESS)/drug-induced hypersensitivity syndrome (DIHS) were suspected, all study drugs were immediately discontinued.

Of the dermatological adverse reactions reported by investigators, we examined 355 events in the T12/PR24 group (190 events in 111 TN patients and 165 events in 115 TF patients), and 102 events in the PR48 group (among 53 TN patients). The events such as contact dermatitis and impetigo that were unlikely to be attributable to the study drugs were excluded (Table 2).

RESULTS

Study patients

In the T12/PR24 (TN) group, the T12/PR24 (TF) group, and the PR48 group, the number of men was 66 (52.4%), 83 (58.9%), and 33 (52.4%), respectively; the median age was 53 (range: 20–65), 57 (range: 20–65), and 55 (range: 20–65) years, respectively; and the median body mass index was 22.55 (range: 16.2–31.1), 23.00 (range: 17.1–32.4), and 23.30 (range: 17.9–30.8) kg/m^2, respectively.

Incidences of dermatological adverse reactions

Table 3 summarizes the incidences of dermatological adverse reactions. The incidence of localized or systemic rash-related events, excluding reactions on the injection site only, was higher in the T12/PR24 groups than in the PR48 group, whereas events limited to the injection site were more common in the latter. The incidences of grade 2 and grade 3 rash-related events were also higher in the T12/PR24 groups than in the PR48 group. No statistical difference was observed in the incidence of rash-related events between the T12/PR24(TN) and T12/PR24(TF) groups.

Table 4 shows the rates of serious adverse events, discontinuation, interruption, and reduction of study drugs due to the dermatological events. No dermatological problem led to death. Serious adverse events requiring hospitalization or prolongation of inpatient care were observed in only the T12/PR24 (TN) and T12/PR24 (TF) groups, respectively. Two patients developed SJS and one developed DRESS/DIHS.

Because no clear differences were found in incidences, grades and features of dermatological adverse reactions between TN and TF patients in the T12/PR24 group, we integrated the data of these two patient groups to evaluate the reactions associated with triple therapy as described below.

Time to onset

Figure 1 shows the cumulative incidence of dermatological adverse reactions obtained using the Kaplan–Meier method. The incidence up to week 4 was 77.1% in the T12/PR24 groups and 55.6% in the PR48 group. Time to onset of the first reaction was earlier in the T12/PR24 group than in the PR48 group (Fig. 1a).

Most grade 1 events occurred within 2 weeks in the T12/PR24 group, and were approximately the same timing as those in the PR48 group. Grade 2 events occurred early in the T12/PR24 group, with increased incidence during weeks 4–8 after the initial administration (Fig. 1b). Regarding grade 3 events, papuloerythematous rashes affecting more than 50% of the body surface area occurred within 1 week, and rashes with

Table 2. Number of patients and dermatological adverse events in each study

		T12/PR24 group			PR48 group		
		Dermatological reactions			Dermatological reactions		
		n	No. of patients	No. of events	*n*	No. of patients	No. of events
Study on treatment-naïve patients	–	126	111	190	63	53	102
Studies on treatment-failure patients	Relapser	109	85	124	–	–	–
	Non-responder	32	30	41	–	–	–
	Total	141	115	165	–	–	–

T12/PR24: telaprevir, pegylated interferon (PEG-IFN)-α-2b and ribavirin (RBV) for 12 weeks followed by administration of PEG-IFN-α-2b and RBV for 12 weeks. PR48: PEG-IFN-α-2b and RBV for 48 weeks.

Table 3. Incidence of dermatological adverse reactions

	T12/PR24						PR48	
	Treatment-naïve *n* = 126		Treatment-failure *n* = 141		Total *n* = 267		Treatment-naïve *n* = 63	
Total	111	88.1%	115	81.6%	226	84.6%	53	84.1%
Rash-related events[†]	94	74.6%	106	75.2%	200	74.9%	37	58.7%
Grade 1	53	42.1%	63	44.7%	116	43.4%	31	49.2%
Grade 2	43	34.1%	47	33.3%	90	33.7%	12	19.0%
Grade 3[§]	15	11.9%	9	6.4%	24	9.0%	3	4.8%
Injection site-related events[‡]	60	47.6%	36	25.5%	96	36.0%	37	58.7%
Grade 1	59	46.8%	36	25.5%	95	35.6%	37	58.7%
Grade 2	1	0.8%	0	–	1	0.4%	0	–
Grade 3	0	–	0	–	0	–	0	–

[†]Localized or systemic rash-related events, excluding reactions on the injection site only. The terms reported more than 10% were rashes (T12/PR24 vs PR48: 38.6% vs 28.6%), drug eruptions (26.6% vs 3.2%) and erythema (6.0% vs 20.6%).
[‡]The terms reported more than 10% were injection site erythema (T12/PR24 vs PR48: 19.1% vs 33.3%) and injection site reaction (16.1% vs 25.4%).
[§]Including life-threatening events, two Stevens–Johnson syndrome (SJS) cases, and one drug rash with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome case. T12/PR24: telaprevir, pegylated interferon (PEG-IFN)-α-2b and ribavirin (RBV) for 12 weeks followed by administration of PEG-IFN-α-2b and RBV for 12 weeks. PR48: PEG-IFN-α-2b and RBV for 48 weeks.

Table 4. Incidence of serious events and rates of discontinuation, interruption and reduction of study drug(s) due to dermatological events

	T12/PR24						PR48	
	Treatment-naïve *n* = 126		Treatment-failure *n* = 141		Total *n* = 267		Treatment-naïve *n* = 63	
Serious adverse events	3	2.4%	5	3.5%	8	3.0%	0	–
Discontinuation of any study drug	12	9.5%	11	7.8%	23	8.6%	2	3.2%
Telaprevir only	4	3.2%	7	5.0%	11	4.1%	–	–
All study drug(s)	8	6.3%	4	2.8%	12	4.5%	2	3.2%
Interruption of PEG-IFN or RBV	0	–	2	1.4%	2	0.7%	0	–
Reduction of PEG-IFN or RBV	2	1.6%	2	1.4%	4	1.5%	0	–

T12/PR24: telaprevir, pegylated interferon (PEG-IFN)-α-2b and ribavirin (RBV) for 12 weeks followed by administration of PEG-IFN-α-2b and RBV for 12 weeks. PR48: PEG-IFN-α-2b and RBV for 48 weeks.

pyrexia and lymphadenopathy, such as SJS and DRESS/DIHS, occurred during weeks 4–8 in the T12/PR24 group.

Characteristics of dermatological adverse reactions

Table 5 shows the characteristics of the 355 events in the T12/PR24 group and 102 events in the PR48 group.

Area of dermatological adverse reactions on the body surface. In the T12/PR24 group, 27.9% of dermatological adverse reactions (multiple or diffuse reactions) affected 50% or less of the body surface, while 5.4% affected more than 50% of the body surface. Compared with the PR48 group, the involved areas tended to be large in the T12/PR24 group.

Distribution. The dermatological reactions appeared mainly in the extremities and the trunk, and sometimes on the face. In case of SJS, DRESS/DIHS and serious erythema multiforme (EM), the intraoral areas, lips and pharynx were affected.

Features of lesions. Most of the dermatological adverse reactions in the T12/PR24 groups were papuloerythematous or maculopapular rashes (Figs 2a,b), which were similarly observed in the PR48 group; a few cases were judged as EM. In some cases, florid rashes developed at the injection site (Fig. 2c). In the SJS and DRESS/DIHS cases, superficial ulceration and erosion of mucous membranes or epidermal detachment were also observed.

Pruritus. Most dermatological adverse reactions were accompanied by pruritus in both the T12/PR24 group (82.5%) and the PR48 group (82.4%) (Table 5).

Systemic symptoms. In the T12/PR24 group, 7.0% of dermatological adverse reactions were accompanied by systemic symptoms, and pyrexia occurred most frequently. In the SJS, DRESS/DIHS and serious EM cases, pyrexia of 38–39°C and lymphadenopathy were observed (Table 5).

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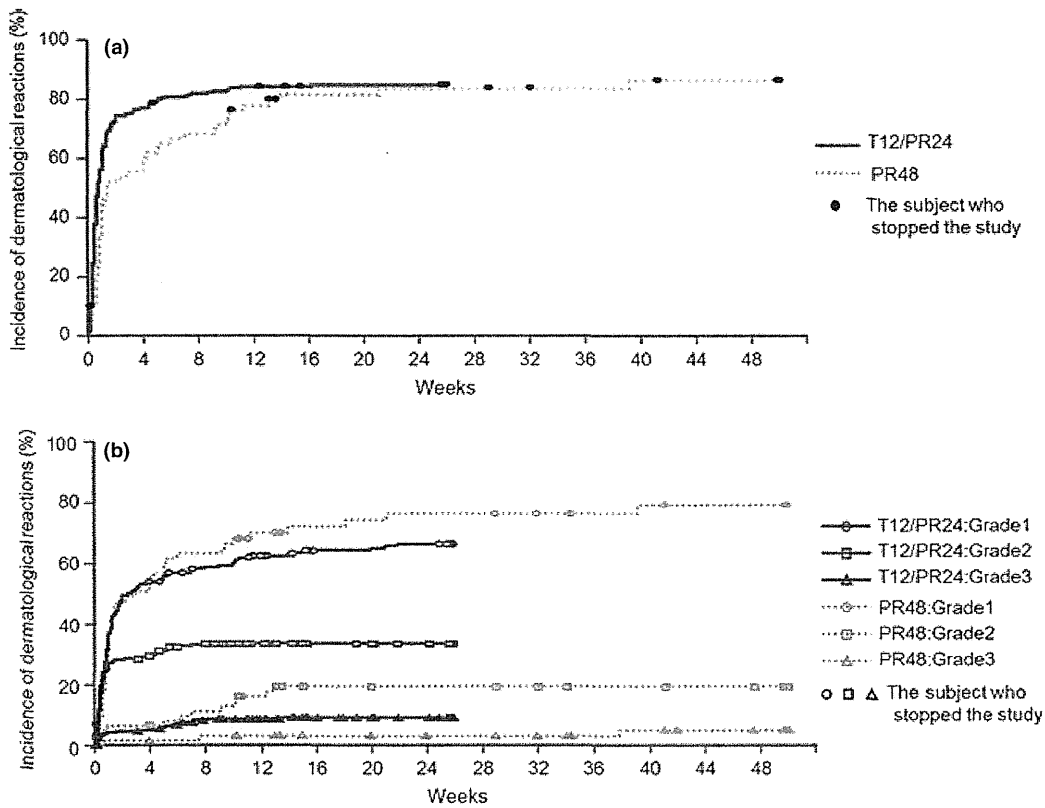

Figure 1. Time to onset of first dermatological adverse reaction. Cumulative incidence of dermatological adverse reactions (a) in total and (b) by grade in the T12/PR24 and PR48 groups obtained using the Kaplan–Meier method. T12/PR24: telaprevir, pegylated interferon (PEG-IFN)-α-2b and ribavirin (RBV) for 12 weeks followed by administration of PEG-IFN-α-2b and RBV for 12 weeks. PR48: PEG-IFN-α-2b and RBV for 48 weeks.

Serious cases

SJS. Stevens–Johnson syndrome was reported in two patients (0.7%) in the T12/PR24 group.

In one patient (female, 50 years old), a grade 1 rash was observed 8 days after the initiation of drug administration and it resolved 4 days later. On day 35, another rash appeared, and on day 44, when the patient was hospitalized, erythema affecting approximately 30% of the body surface with erosion of the intraoral mucous membrane was observed. The maximum body temperature was 39.3°C. All antiviral drugs were discontinued, and the patient was treated with systemic corticosteroids. Erythema progressed till 4 days after drug discontinuation (Fig. 2d) and resolved after 7 weeks.

In the other patient (female, 47 years old), "EM major" was diagnosed by a dermatologist but was integrated as SJS according to the clinical trial's coding rule. A localized rash was observed on day 3. Pyrexia up to 38.5°C and worsening of the rash were observed on day 25, and the patient was hospitalized due to erosion appearing on the lips and the mucous membrane of the pharynx. All study drugs were discontinued, and the patient recovered with the use of systemic corticosteroids 8 weeks after discontinuation.

DRESS/DIHS. In one patient (0.4%), a 60-year-old female, in the T12/PR24 group, DRESS/DIHS developed. Grade 1 rash was observed on day 7. On day 44, new redness appeared on the waist and legs, and on day 64 erythema worsened with persistent high fever; all study drugs were subsequently discontinued. The patient developed erythema with target lesions (Fig. 2e) and erosion of the oral mucous membranes on day 66. After discontinuation of the drugs, pyrexia over 38°C (maximum, 39.7°C) was observed. On the basis of symptom progression and laboratory test findings (increased white blood cell count [46300/μL], appearance of atypical lymphocytes [23.3%], raised eosinophil count [45.7%], high ferritin level, high lactate dehydrogenase level, lymphadenopathy and reactivation of human herpesvirus six based on a rise in titer from 1:160 [29 days after onset] to 1:2560 [57 days after onset]), a diagnosis of DRESS/DIHS was made. On administration of systemic corticosteroids, the patient recovered 11 weeks after discontinuation.

EM with mucous membrane lesions. Serious EM with mucous membrane lesions was observed in one patient (0.4%), a 58-year-old female, in the T12/PR24 group. On day 14, the patient developed a grade 1 rash, which resolved

Table 5. Characteristics of dermatological adverse reactions

	T12/PR24	PR48
No. of dermatological adverse reactions	355	102
Distribution area		
≤50% of body surface, localized	237 (66.8%)	87 (85.3%)
≤50% of body surface, multiple or diffuse	99 (27.9%)	15 (14.7%)
>50% of the body surface	19 (5.4%)	0 (0.0%)
Features of lesions[†]		
Erythema without target lesions	296 (83.4%)	89 (87.3%)
Erythema with target lesions	8 (2.3%)	0 (0.0%)
Purpura	22 (6.2%)	0 (0.0%)
Vesicles or bullae	7 (2.0%)	2 (2.0%)
Pustule	7 (2.0%)	0 (0.0%)
Ulceration or erosion of mucous membranes	4 (1.1%)	1 (1.0%)
Epidermal detachment	2 (0.6%)	1 (1.0%)
Other[‡]	85 (23.9)	22 (21.6%)
Pruritus		
Yes	293 (82.5%)	84 (82.4%)
No	62 (17.5%)	18 (17.6%)
Systemic symptoms		
No	330 (93.0%)	102 (100.0%)
Yes[†]	25 (7.0%)	0 (0.0%)
Pyrexia	23 (6.5%)	0 (0.0%)
Angioedema	2 (0.6%)	0 (0.0%)
Lymphadenopathy	6 (1.7%)	0 (0.0%)
Other[§]	2 (0.6%)	0 (0.0%)

[†]Multiple features may co-exist.
[‡]For example, redness and papules.
[§]Inflammation of lips and dry skin. T12/PR24: telaprevir, pegylated interferon (PEG-IFN)-α-2b and ribavirin (RBV) for 12 weeks followed by administration of PEG-IFN-α-2b and RBV for 12 weeks. PR48: PEG-IFN-α-2b and RBV for 48 weeks.

4 days later. The drug rash developed again on day 34, followed by pyrexia and enlargement of the rash on day 39, resulting in hospitalization of the patient. Pyrexia of up to 39.0°C was observed. Despite treatment with oral corticosteroids at 20 mg/day, the rash worsened and intraoral redness was observed 9 days after hospitalization; thus, all study drugs were discontinued. The patient's condition was diagnosed as EM, the dose of oral corticosteroids was increased to 60 mg/day and the patient recovered 11 weeks after drug discontinuation.

Treatment of dermatological adverse reactions

Figure 3 shows the medical treatments used for the dermatological events that developed during the antiviral administration period in the T12/PR24 group (347 events) and in the PR48 group (100 events). The main medical agents were topical corticosteroids and oral antihistamines in both groups. The strength of topical corticosteroids most often used were either class III (potent) or class IV (very potent).

Among the 347 dermatological events in the T12/PR24 group, 324 events did not require discontinuation of any of the study drugs. Approximately 21% events resolved without treatment. Events mainly treated with topical corticosteroids comprised 44.4%, and those treated with topical corticosteroids and oral antihistamines made up 17.9%. The frequency of treatment with systemic corticosteroids was only 4.6%. On the basis of these results, we considered almost all dermatological adverse reactions to be manageable with topical corticosteroids and oral antihistamines.

In the T12/PR24 group, 23 dermatological events required drug discontinuation. For these events, systemic corticosteroids were used more frequently (30.4%) in addition to topical corticosteroids and oral antihistamines.

Period of resolution

In the T12/PR24 group, 61.4% of the dermatological events resolved during the treatment period: 38.6% during the telaprevir treatment period and 22.8% during the PEG-IFN-α-2b and RBV treatment period. The proportion of dermatological events that resolved after completion or discontinuation of all the study drugs was 37.2% (Fig. 4).

In the PR48 group, 59.0% (59/100 events) of the dermatological events resolved by the end of the treatment period.

DISCUSSION

Compared to the dermatological adverse reactions associated with PEG-IFN and RBV therapy in phase III clinical trials, those caused by the telaprevir-based triple therapy: (i) developed early; (ii) affected large areas of the body; and (iii) were accompanied in several cases by severe rashes, mucous membrane lesions, epidermal detachment or systemic symptoms associated with high pyrexia or lymphadenopathy, including SJS and DRESS/DIHS. In addition, target lesions, purpura, and pustule were reported only in the triple therapy.

The dermatological adverse reactions associated with triple therapy were mostly of grade 1 or grade 2, and they were pruritic papuloerythematous lesions similar to the ones associated with PEG-IFN and RBV therapy. These adverse reactions could be managed with topical corticosteroids and oral antihistamines, and the associated discontinuation rate was low. Over 60% of the events resolved during the treatment phase. In view of these results and the therapeutic effect on CHC, early discontinuation of antiviral therapy due to mild or moderate dermatological adverse reactions should be avoided.

On the other hand, we cannot ignore the occurrences of SJS and DRESS/DIHS in the clinical trials. Aggravation of these serious events may lead to death and sequelae such as blindness. Therefore, on observation of clinical signs and symptoms that may lead to these serious conditions, all drugs must be discontinued immediately and appropriate treatment for these reactions should be administrated as soon as possible.

The criteria for drug discontinuation and treatment for dermatological reactions based on severity are illustrated in the algorithm in Figure 5. In addition to discontinuation of all drugs in cases of serious adverse reactions, a basic principle is to discontinue telaprevir in cases where the affected area exceeds 50% of the body surface (corresponding to grade 3) or where symptoms continued to worsen, notwithstanding a

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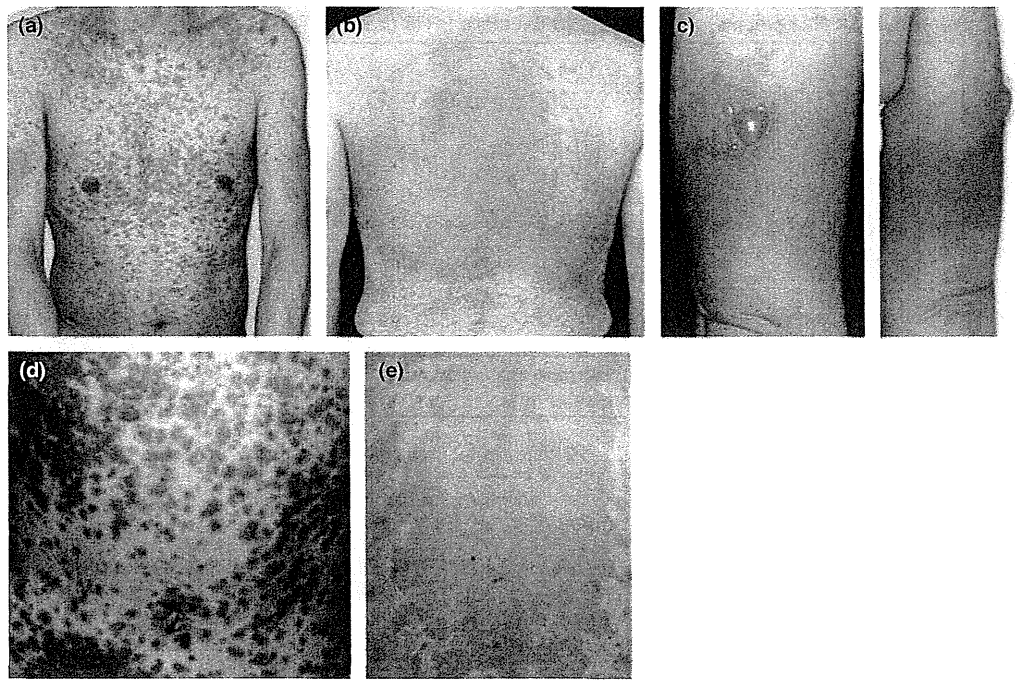

Figure 2. Examples of dermatological adverse reactions in the T12/PR24 group. (a,b) Grade 2 rashes, (c) injection site reaction, (d) Stevens–Johnson syndrome case, and (e) drug rash with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome case in the T12/PR24 group. T12/PR24: telaprevir, pegylated interferon (PEG-IFN)-α-2b and ribavirin (RBV) for 12 weeks followed by administration of PEG-IFN-α-2b and RBV for 12 weeks.

grade 2 reaction. If symptoms worsen during the remaining period when only PEG-IFN and RBV are administrated, discontinuation of these two drugs is likewise recommended. Regarding the antiviral effects observed in the patients who discontinued any of the study drugs due to dermatological reactions, seven of the 11 patients who discontinued only telaprevir achieved SVR, and eight of the 12 patients who discontinued all study drugs achieved SVR.

Grade 1 and grade 2 reactions could be managed with topical corticosteroids and oral antihistamines. These medications were used for grade 3 reactions in a similar manner without any problems, although in some cases, such as SJS, DRESS/DIHS and other severe reactions, the systemic use of corticosteroids was required. In some patients, who continued to receive the study drugs concomitant with systemic corticosteroids, further aggravation of dermatological reactions was noted when the dose of corticosteroids was reduced or when their administration was discontinued. There is a possibility that use of systemic corticosteroids masked serious problems and the tapering of steroids aggravated reactions; hence, telaprevir discontinuation should be taken into consideration when systemic use of corticosteroids is required.

The cases of SJS, DRESS/DIHS and serious EM were accompanied by pyrexia of 38–39°C, erosions affecting the mucous membranes or conjunctival lesions. It is, therefore, important to detect early signs of serious diseases, and empowering patients to identify such symptoms by informing them about the features associated with these diseases, like pyrexia and mucous membrane erosion, will enable early detection.

The mechanism of telaprevir-related dermatological adverse reactions remains unknown. Because dermatological adverse reactions were observed even in the study on telaprevir monotherapy, it can be said that telaprevir per se is a factor for dermatological reactions. The dermatological adverse reactions in telaprevir monotherapy were, however, relatively milder than those in the telaprevir-based triple therapy. Therefore, it can be suggested that the concomitant administration of telaprevir with PEG-IFN and RBV led to an additive or synergistic effect on the adverse reactions and sometimes resulted in serious cases. In addition, in the triple therapy, the dermatological adverse reactions occurred within 1 week of administration in approximately half of the cases. As the onset time of these reactions was earlier than that of the usual drug rash (i.e. drug allergy), the mechanism underlying these early occurring reactions may be different from that of allergy in general.

In the US/EU clinical trials, pharmacokinetics, human leukocyte antigen genes and multidrug resistance 1 gene were examined to elucidate risk factor(s) for telaprevir-related dermatitis. However, no specific relation was noted between these factors and the development or severity of dermatological reactions.[15]

The incidence of skin adverse reactions (rash-related events) in phase II or phase III studies performed in the US/EU was 55–56% in the triple therapy group and 33–34% in the PEG-

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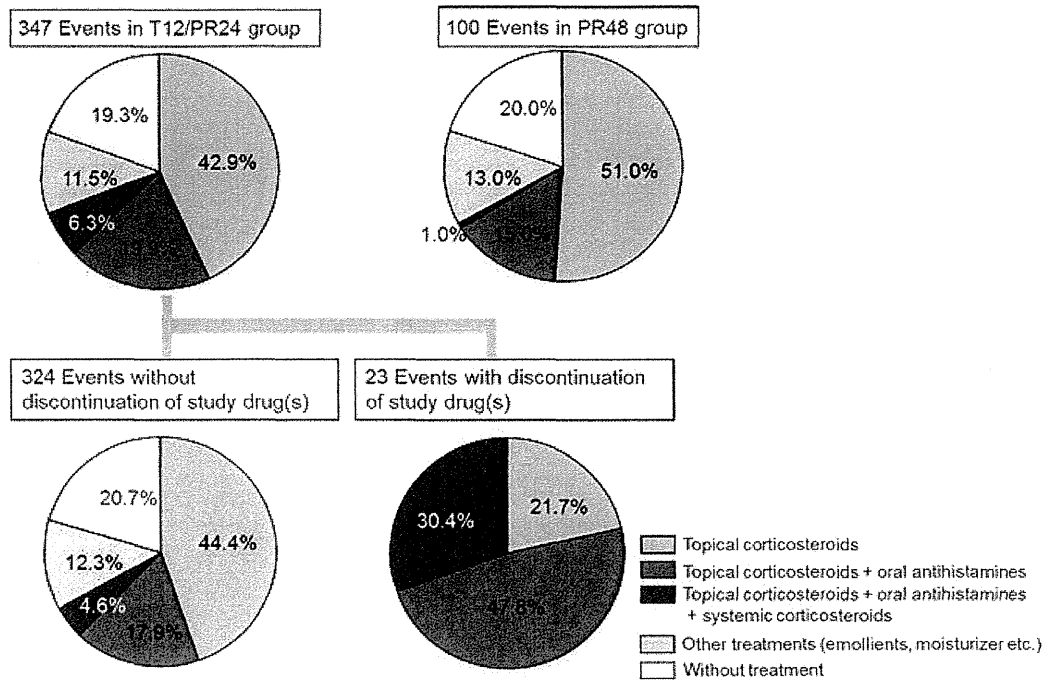

Figure 3. Treatment of dermatological adverse reactions. The medical agents shown were used for 347 events that developed during the antiviral administration period in the T12/PR24 group (324 events without drug discontinuation and 23 events with drug discontinuation) and for 100 events in the PR48 group. Treatment is classified according to the use of topical corticosteroids, oral antihistamines, systemic corticosteroids and others agents. T12/PR24: telaprevir, pegylated interferon (PEG-IFN)-α-2b and ribavirin (RBV) for 12 weeks followed by administration of PEG-IFN-α-2b and RBV for 12 weeks. PR48: PEG-IFN-α-2b and RBV for 48 weeks.

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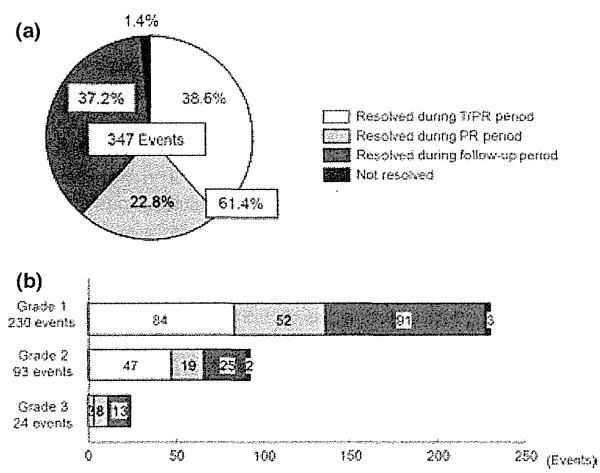

Figure 4. Period of resolution in the T12/PR24 group. (a) Of the 347 dermatological reactions that occurred during the treatment phase in the T12/PR24 group, approximately 61% resolved by the end of dosing. (b) The period of resolution of each dermatological adverse reaction is shown by grade. T12/PR24: telaprevir, pegylated interferon (PEG-IFN)-α-2b and ribavirin (RBV) for 12 weeks followed by administration of PEG-IFN-α-2b and RBV for 12 weeks.

IFN and RBV therapy group; these rates are lower than those observed in Japanese patients (74.9% and 58.7%, respectively). In addition, 12–14% and 4–5% of the subjects developed grade 2 and grade 3 reactions, respectively, in the US/EU studies, while in Japan, the respective percentages were 33.7% and 9.0%.[15,16] Although the early onset and symptom features were common between the US/EU and Japan studies, there may be differences in the rate of symptom development and in severity worsening tendency. However, a stringent comparison is difficult between the US/EU and Japan studies, because there may be differences in the interpretation of severity classification systems including definition of the involved area or in the data collection and aggregation methods between these studies.

In conclusion, the telaprevir-based triple therapy often caused dermatological adverse reactions. Most reactions were of grade 1 or grade 2 and could be managed without discontinuation of the study drugs. Some patients, however, developed serious reactions such as SJS and DRESS/DIHS. What is most important is to consider the balance between the risk and benefit for an individual patient, and address individual reactions appropriately. For appropriate treatments of individual dermatological adverse reactions, the judgment of discontinuation of antiviral drugs and treatment based on the severity are extremely important in this triple therapy.

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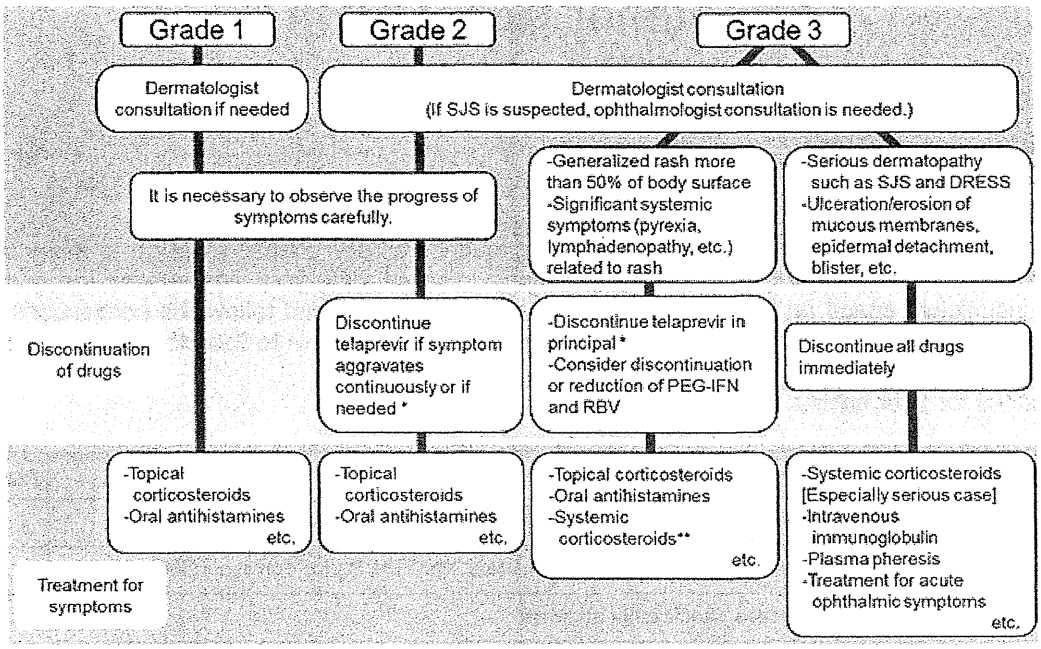

*: If the symptom worsened after discontinuation of telaprevir, it is recommended to discontinue PEG-IFN/RBV.
**: When systemic corticosteroids are needed for treatment, it is recommended to discontinue telaprevir at least.

Figure 5. Algorithm for the discontinuation of drugs and treatment for dermatological adverse reactions.

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Three-dimensional magnetic resonance imaging for stringent diagnosis of advanced fibrosis associated with nonalcoholic steatohepatitis

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Abstract
Background The definitive diagnosis of nonalcoholic steatohepatitis (NASH) is currently based on histopathological assessment. This study aimed to elucidate the utility of a novel noninvasive method, three-dimensional magnetic resonance imaging (3D-MRI), for diagnosing advanced fibrosis in patients with NASH, using histopathological diagnosis as the reference standard.
Methods This retrospective study included 30 consecutive patients who had been diagnosed with NASH by histopathology and had undergone 3D-MRI before biopsy. 3D-MRI provided a three-dimensional reconstruction of the liver from contrast-enhanced hepatobiliary phase MR images. In the present study, histopathological advanced fibrosis was defined as stage 3 and 4 NASH. Advanced fibrosis, diagnosed by 3D-MRI, was considered to be diffuse irregularity of the entire surface of the liver. The diagnostic features of 3D-MRI and the noninvasive evaluation systems (APRI, FIB-4 index, and BARD score) for identifying advanced and nonadvanced fibrosis of NASH were determined and compared.
Results Nine (30 %) of the 30 study patients were diagnosed histopathologically with advanced fibrosis, and 11 (37 %) of 30 patients were diagnosed with advanced fibrosis using 3D-MRI. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 3D-MRI for diagnosing advanced fibrosis were 100, 90, 82, and 100 %, respectively. The sensitivities of APRI, the FIB-4 index, and BARD score ranged from 78 to 89 %, the specificities from 71 to 90 %, the PPVs from 54 to 78 %, and the NPVs from 88 to 94 %.
Conclusion Compared with the common noninvasive methods for diagnosing advanced fibrosis associated with NASH, 3D-MRI was more accurate.

Keywords Nonalcoholic fatty liver disease · Nonalcoholic steatohepatitis · Advanced fibrosis · 3D-MRI · Virtual MR-laparoscopy

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease in Western countries [1–4], and recently it has become common in many Asian nations [5, 6]. In particular, patients with nonalcoholic steatohepatitis (NASH), a subcategory of NAFLD, are at an increased risk for developing hepatocellular carcinoma [7]. Like patients with viral hepatitis, NAFLD patients with advanced fibrosis have an increased risk of developing hepatocellular carcinoma [8–10]. Currently, NASH can be diagnosed only by histopathology. Usually, chronic liver

depression), (2) partially irregular (several interconnected depressions on the surface, mainly in the left lobe of the liver, with rippled or speckled appearance), and (3) diffusely irregular (including diffuse small irregularities or large irregularities with areas of nodularity).

MR image acquisition and 3D reconstruction of the liver were performed by three expert radiologic technologists. The 3D-MR images were evaluated for degree of fibrosis (nonadvanced or advanced fibrosis) by a conference of three expert hepatologists who were blinded to the pathological results. Each hepatologist had 10 or more years of experience performing conventional diagnostic laparoscopy to assess chronic liver disease.

Definition of advanced fibrosis according to APRI, FIB-4 index, BARD score, and 3D-MRI

Advanced fibrosis was defined as follows: (1) APRI >0.98, (2) FIB-4 index >2.67, (3) BARD score = 2–4, and (4) image from 3D-MRI showing diffuse irregularity of the surface of the liver (including diffuse small irregularities or large irregularities with areas of nodularity).

Statistical analysis

Differences in demographic features, laboratory data, and the features of liver biopsy specimens between patients with advanced fibrosis versus patients with nonadvanced fibrosis were analyzed by the Fisher's exact test and Mann-Whitney *U* test. The sensitivity, specificity, PPV, and NPV for identifying advanced and nonadvanced fibrosis were determined for each evaluation system (APRI, FIB-4 index, and BARD score) and 3D-MRI. A p value of <0.05 was considered statistically significant. Data analysis was performed using the Statistical Package for Social Sciences, version 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Clinical and demographic features of patients

Table 1 summarizes the demographic and clinical profiles of the 30 study patients. The patients with advanced

Table 1 Clinical and demographic features of patients with nonalcoholic steatohepatitis who underwent three-dimensional magnetic resonance imaging

	All patients	Nonadvanced fibrosis (stage 1–2) $n = 21$	Advanced fibrosis (stage 3–4) $n = 9$	p value
Gender (M:F)	22:8	17:4	5:4	0.195
Age (years)[a]	59.5 (29–80)	47 (29–73)	63 (51–80)	0.032
Body mass index (kg/m^2)[a]	25.8 (20.8–37.9)	25.4 (20.9–35.1)	26.2 (20.8–37.9)	0.533
Albumin (g/dl)[a]	4.2 (3.6–4.7)	4.2 (3.8–4.7)	3.9 (3.6–4.4)	0.086
Total bilirubin (mg/dl)[a]	0.9 (0.4–1.5)	0.8 (0.4–1.2)	0.9 (0.5–1.5)	0.150
AST (IU/l)[a]	48 (18–198)	41 (18–198)	48 (29–150)	0.422
ALT (IU/l)[a]	76.5 (22–275)	83 (22–275)	45 (22–194)	0.077
γ-GTP (IU/l)[a]	57.5 (15–502)	67 (15–502)	54 (34–125)	0.929
Platelet count (× 10^3/μl)[a]	196 (65–318)	215 (104–318)	181 (65–207)	0.002
Hyaluronic acid (μg/l)	28 (4–196)	21 (4–83)	127 (47–196)	<0.001
Diabetes mellitus (yes/no)	6:24	4:17	2:7	1.000
Uric acid (mg/dl)[a]	6.4 (3.6–9.6)	6.7 (4.7–8.9)	5.3 (3.6–9.6)	0.104
Total cholesterol (mg/dl)[a]	193.5 (94–265)	206 (94–265)	172 (107–223)	0.070
Triglyceride (mg/dl)[a]	144.5 (38–355)	157 (38–276)	140 (40–355)	0.965
LDL-cholesterol (mg/dl)[a]	104.5 (24–177)	113 (24–177)	85 (28–124)	0.025
HDL-cholesterol (mg/dl)[a]	45 (22–76)	45 (28–76)	41 (22–59)	0.304
Needle biopsy specimens of the liver ($n = 29$)[b]				
		$n = 21$	$n = 8$	
Length of specimens (mm)	15 (9–27)	15 (9–27)	20.5 (13–26)	0.024
Number of portal areas	6 (2–21)	5 (2–21)	9.5 (6–12)	0.006

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *γ-GTP* gamma-glutamyl transpeptidase, *HDL* high-density lipoprotein, *LDH* lactate dehydrogenase, *LDL* low-density lipoprotein

[a] Expressed as median (minimum, maximum)

[b] One patient who underwent surgical resection for hepatocellular carcinoma was excluded from quality evaluation of the needle biopsy specimens

fibrosis were significantly older, and they had significantly lower platelet counts, lower low-density lipoprotein-cholesterol levels, and higher hyaluronic acid levels compared with the patients with nonadvanced fibrosis. With regard to the characterization of the needle biopsy specimens (one patient undergoing surgical resection for hepatocellular carcinoma was excluded from quality evaluation of the needle biopsy specimens), the patients with advanced fibrosis had significantly larger specimens and higher numbers of portal areas.

3D-MRI and histological NASH stage

Figure 1a–d shows 3D-MRI figures that corresponded to the different histological NASH stages. These 3D-MRI figures demonstrate that in addition to altered shape of the liver, the irregularities on the surface of the liver gradually extended from the lateral segment to the right lobe. There were nine patients with the Fig. 1a pattern, and all (100 %) were histopathologically diagnosed with NASH stage 1. Of ten patients with the Fig. 1b pattern, five (50 %) were diagnosed with NASH stage 1 and five with NASH stage 2. Of 9 patients with the Fig. 1c pattern, 5 (56 %) were diagnosed with NASH stage 3, 2 (22 %) with NASH stage 4, and 2 with NASH stage 1. Two patients (7 %) had the Fig. 1d pattern, and both were diagnosed with NASH stage 4. Diffuse irregularities of the entire surface of the liver were characteristic of patients with NASH stage 3 and 4; therefore, we designated 3D-MR images showing irregularities of the entire surface of the liver as advanced fibrosis (Fig. 1c, d). A total of 11 (37 %) of 30 patients were diagnosed with advanced fibrosis from the figures obtained by 3D-MRI.

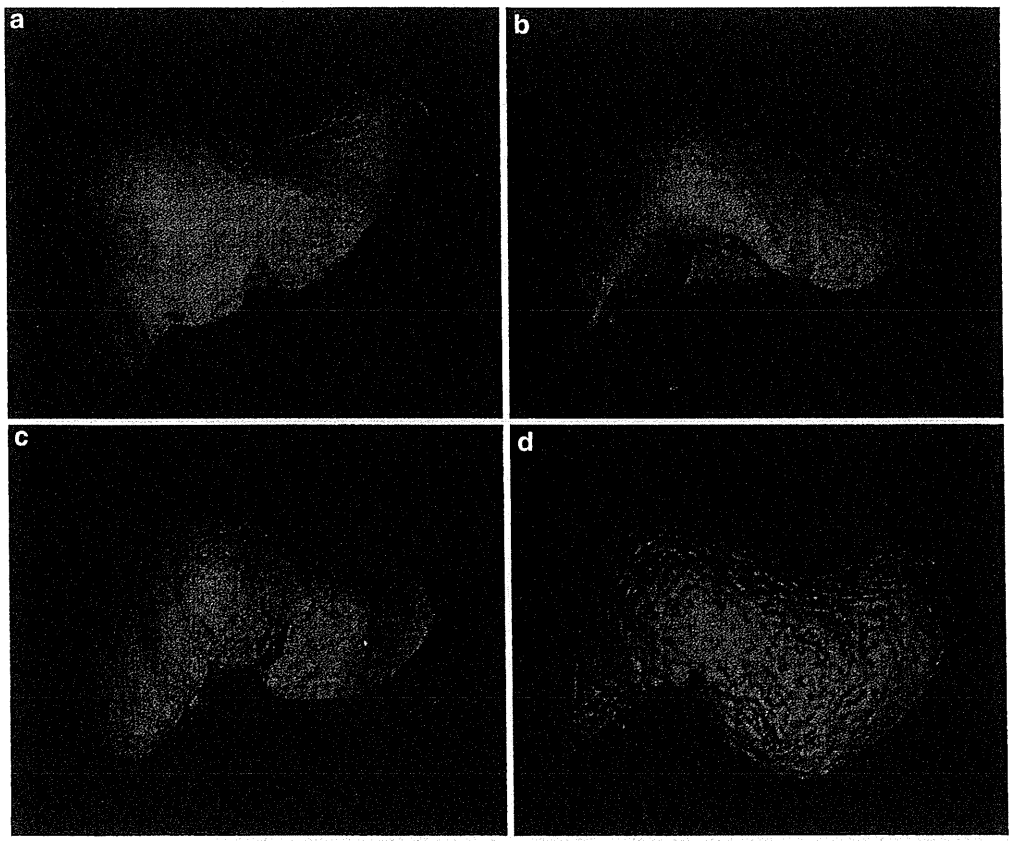

Fig. 1 **a** Three-dimensional magnetic resonance image of nonadvanced liver fibrosis in nonalcoholic steatohepatitis (NASH), stage 1, showing a smooth liver surface and enlarged lateral segment. **b** Image of nonadvanced fibrosis, NASH stage 2, showing localized small irregularities of the surface of the liver and an enlarged lateral segment. **c** Image of advanced fibrosis, NASH stage 3, showing diffuse small irregularities of the surface of the liver and enlarged lateral segment. **d** Image of advanced fibrosis, NASH stage 4, showing diffuse large irregularities of the surface of the liver, an enlarged lateral segment, and atrophic right lobe

Figures 2 and 3 are conventional laparoscopic images of livers of patients with advanced fibrosis (NASH stage 3 and 4, respectively). Figure 2a, b shows diffuse small irregularities of the entire surface of the liver, and the surface of the left lobe (Fig. 2b) has diffuse small irregularities and a large nodular area. The histological diagnosis of this patient was NASH stage 3, and the 3D-MR image of this patient is also shown in Fig. 1c. Figure 3a, b shows diffuse bilobular large irregularities and nodular areas of the surface of the liver. The histological diagnosis of this patient was NASH stage 4, and the 3D-MR image of this patient is also shown in Fig. 1d.

In the present study, one patient was found to have hepatocellular carcinoma on MRI. However, the tumor was small (26 × 22 mm) and occupied a small proportion of the area of the entire surface of the liver; therefore, the diagnostic evaluation was not affected.

Diagnostic features of 3D-MRI and the APRI, FIB-4 index, and BARD scoring systems for advanced fibrosis of the liver

Table 2 summarizes the diagnostic features of 3D-MRI and each scoring system for advanced fibrosis. For the scoring systems (APRI, FIB-4 index, and BARD), the sensitivity ranged from 78 to 89 %, specificity from 71 to 90 %, PPV from 54 to 78 %, and NPV from 88 to 94 %. For 3D-MRI, the sensitivity was 100 %, specificity 90 %, PPV 82 %, and NPV 100 %.

Distributions of patients with advanced fibrosis of the liver according to 3D-MRI, APRI, FIB-4 index, and BARD, and histopathology

Figure 4 shows the distribution of patients predicted to have advanced fibrosis by 3D-MRI and each scoring system, along with the distribution of patients diagnosed with advanced fibrosis by histopathological evaluation. The scoring systems showed more varied distributions compared with 3D-MRI, which showed a uniform distribution that was similar to the histopathological distribution.

Discussion

Up to now, the definitive diagnosis of NASH has been based on histopathological evaluation. However, in Japan, many patients with NAFLD are diagnosed with NASH using US only, because liver biopsies have a risk of major complications such as intraperitoneal bleeding. However, some noninvasive scoring systems (APRI, BARD, and the FIB-4 index) for predicting fibrosis have become available [17]. In addition, the usefulness of other noninvasive strategies for predicting fibrosis in patients with NAFLD has been reported, including transient sonoelastography [21, 22], ARFI [23], and MR elastography [24]. However, these diagnostic methods may lack sensitivity for identifying advanced fibrosis. In addition, for the majority of patients with NAFLD, these methods of prediction usually have weak objectivity and persuasive power. Therefore, a more accurate noninvasive evaluation method is needed. In the present study, we described and reported on the use of "virtual MR-laparoscopy" for 3D imaging of the liver, or 3D-MRI. Although 3D-MRI has qualitative and subjective features, we found that it accurately predicted advanced fibrosis, because it could easily provide visualization of the entire surface of the liver, and evaluation was comparatively easy for physicians experienced with conventional diagnostic laparoscopy for chronic liver disease or treatment of hepatobiliary diseases such as cholecystolithiasis or hepatic tumor. In the present study, 3D-MRI demonstrated a high positive and negative predictive value for

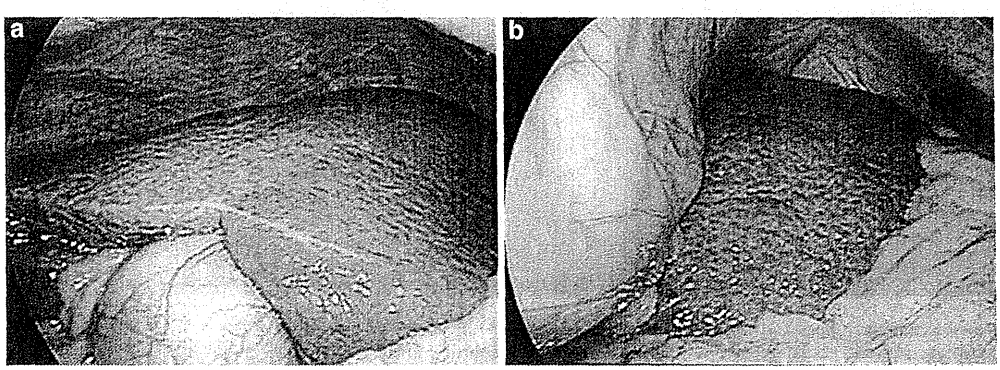

Fig. 2 Conventional laparoscopic image of the liver of the same patient in Fig. 1c. **a** View of the right lobe of the liver. The surface of the liver has diffuse small irregularities. **b** View of the left lobe of the liver. The surface of the liver has diffuse small irregularities and a large nodular area

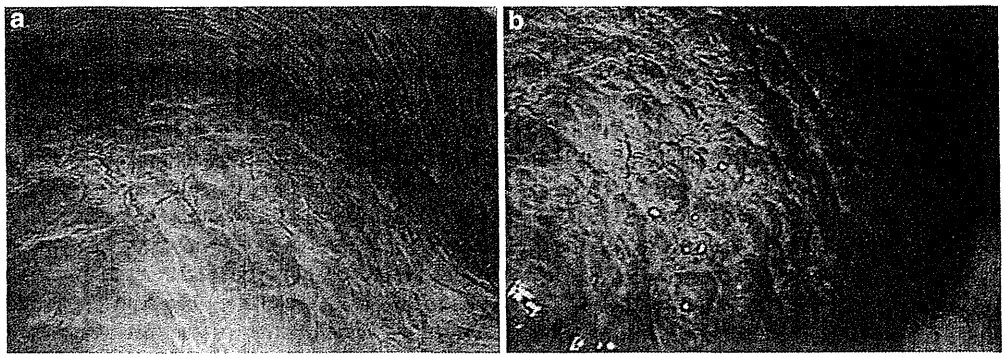

Fig. 3 Conventional laparoscopic image of the liver of the same patient in Fig. 1d. **a** View of the right lobe of the liver. The surface of the liver has diffuse large irregularities with nodular areas. **b** View of the left lobe of the liver. The surface of the liver has diffuse large irregularities with nodular areas

Table 2 Diagnostic features of three-dimensional magnetic resonance imaging and the APRI, FIB-4 index, and BARD scoring systems used to predict advanced liver fibrosis

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
3D-MRI (virtual MR-laparoscopy)	100	90	82	100
APRI	78	71	54	88
FIB-4 index	78	90	78	90
BARD score	89	81	67	94

NPV negative predictive value, *PPV* positive predictive value

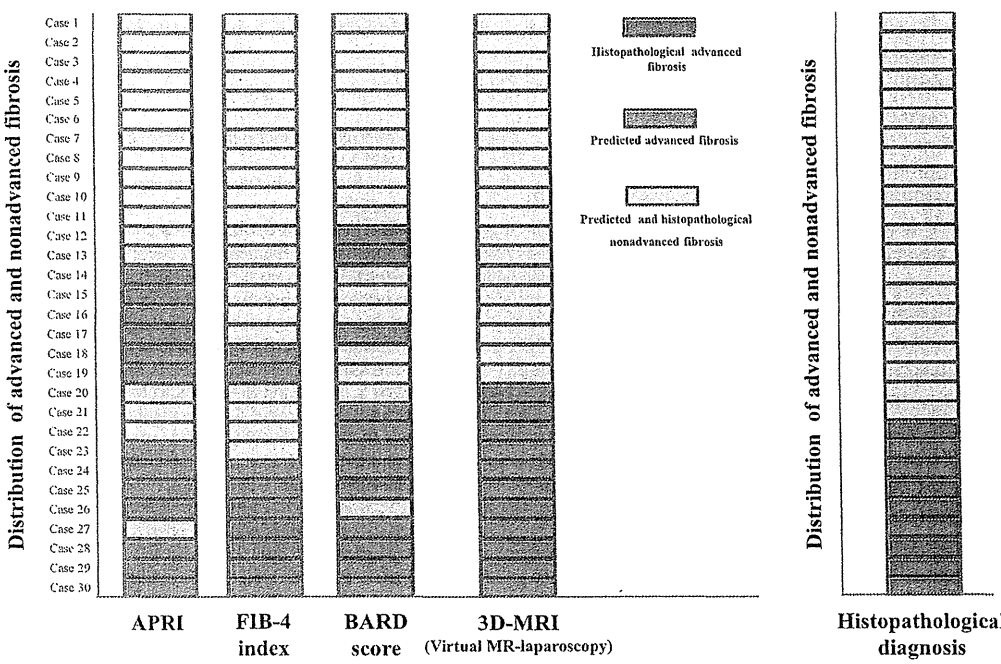

Fig. 4 Distribution of patients predicted to have advanced fibrosis by 3D-MRI and the APRI, FIB-4 index, and BARD scoring systems, along with the distribution of patients diagnosed with advanced fibrosis by histopathological evaluation

advanced fibrosis (82 and 100 %, respectively) and a sensitivity and specificity of 100 and 90 %, respectively.

We also evaluated the APRI, FIB-4 index, and BARD scoring systems, which are easy to calculate using three or four parameters that are routinely measured in outpatient medical practice. All these systems were found to have high predictive values for advanced fibrosis of the liver in this cohort. Figure 4 suggests that the combined use of 3D-MRI and a scoring system may be more advantageous for routine medical care than a single evaluation system. For example, it may be assumed that a double positive for advanced fibrosis provided by 3D-MRI and any one of the scoring systems would lead to a PPV increase from 82 to 90 %, while the NPV would remain 100 %.

However, there are some technical problems with 3D-MRI that still need to be resolved. Inadequate breath holding during the hepatobiliary phase leads to distorted hepatobiliary phase images and inaccurate findings. The 1.5-T MR-imaging system (Avanto) used for the patients in the present study required a 25-s breath hold; therefore, a patient with pulmonary emphysema might not be able to undergo this procedure. There is also the problem of motion artifact; the patients were all found to have linear surface irregularities where the superior border of the liver is near the inferior border of the heart. At present, the heart-beat-motion artifact is difficult to remove. Therefore, evaluation of liver surface irregularities seen on 3D-MRI should take into consideration the effect of the heart on the area of the liver below it. Both these problems may be resolved by increased high-speed image acquisition, which is based on the improvement of the signal-to-noise ratio resulting from the introduction of a powerful magnetic-field imaging system such as a 3.0-T MRI system and multichannel coil.

Despite the current technical problems of 3D-MRI, the large surface irregularities of liver cirrhosis associated with NASH (Fig. 1d) are easy to observe with this modality. However, the advanced fibrosis of NASH stage 3 usually manifests with small irregularities of the surface of the liver (Fig. 1c). Therefore, in patients with NASH stage 3, it is important to carefully examine the images for small irregularities, looking closely at the edge of the liver where the surface irregularities are most clearly depicted by 3D-MRI and clearly seen during conventional laparoscopy.

In the present study has some limitations. This was a retrospective cohort trial evaluating a small number of patients. There were a small number of patients because of the enrollment requirement that patients had to undergo 3D-MRI within 1 year before biopsy and histopathological evaluation. In addition, with regard to the liver biopsy specimens, there were significant differences in the length and number of portal areas of the specimens, and these differences may have led to underestimation of the extent of liver fibrosis in patients with nonadvanced fibrosis. Although in the present study there were no discrepancies among the three experienced hepatologists regarding the diagnosis of advanced fibrosis, 3D-MRI has qualitative and subjective features that might be affected by the different clinical experiences of physicians assessing the images derived from 3D-MRI. Because there were no methods for quantitative assessment of the surface irregularities of the liver seen on 3D-MRI, it was impossible to compare 3D-MRI with the other scoring systems by means of receiver-operating characteristic curve analysis. As stated above, the number of patients in the present study is too small, and some limitations have to be solved. In the near future, further additional large studies that include quantitative evaluation of the surface of the liver are needed.

However, we believe that the impact of the present study on the routine clinical care of patients with NAFLD, especially NASH patients, will be enormous. We also think that the progression of many high-risk patients to advanced liver disease, including decompensated liver cirrhosis and hepatocellular carcinoma, will be prevented by early detection of advanced fibrosis using 3D-MRI.

In conclusion, the diagnostic features of 3D-MRI for predicting advanced fibrosis associated with NASH were superior to those of other previously reported diagnostic methods.

Conflict of interest The authors state that they have no conflicts of interest regarding the content of the article.

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