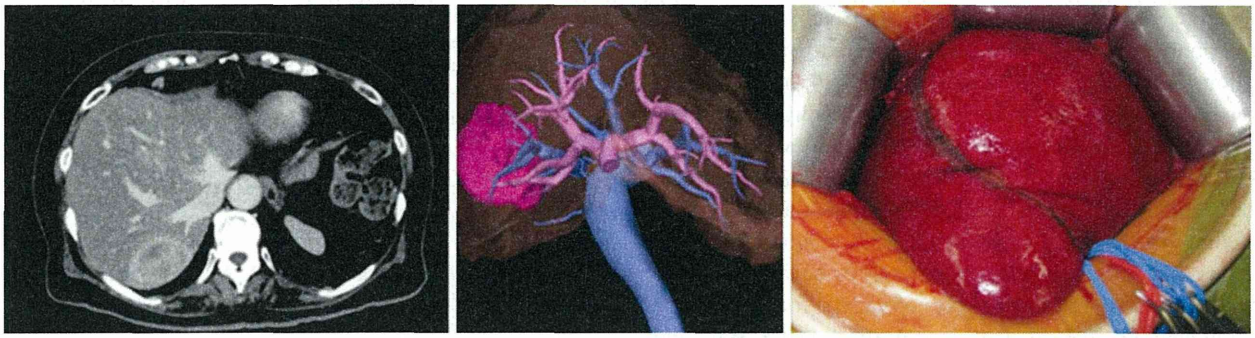

Fig. 6 Contrast-enhanced CT scan showed a mass lesion in segment 7 of the liver. The *middle panel* is a 3D reconstructed image made from CT scans obtained by a Synapse Vincent instrument (Fujifilm Medical, Tokyo, Japan). The intraoperative photograph shows the line of resection for the posterior sectionectomy

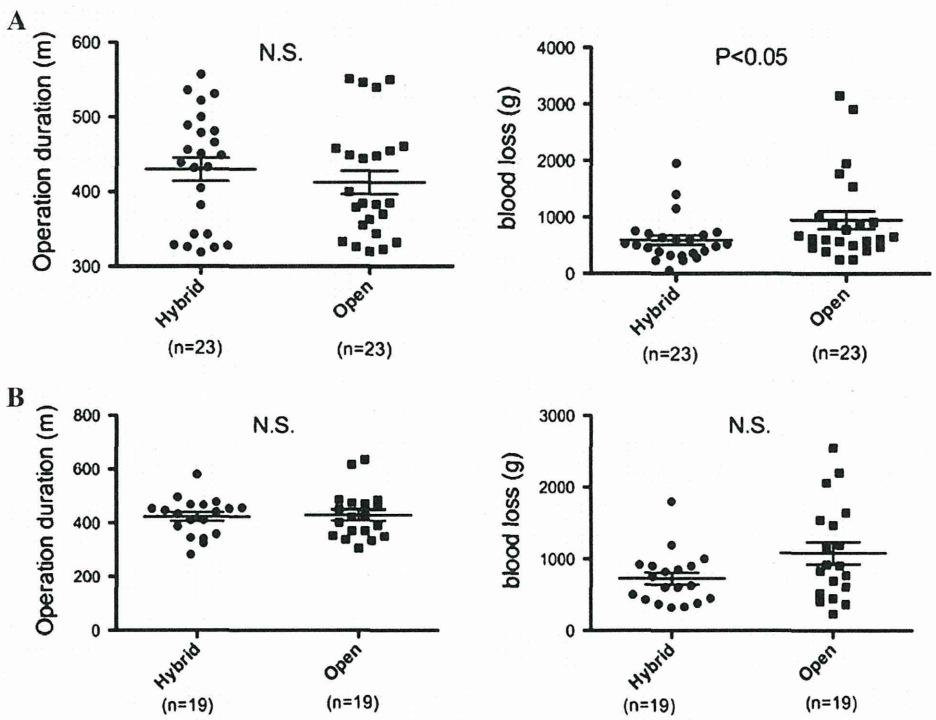

Fig. 7 Comparing the surgical outcomes of the hybrid procedure and open procedure for **A** living-donor left hemihepatectomy and **B** right-donor left hemihepatectomy

another novel device that contributes to reducing the need for ligation during liver parenchymal transections, because it can be used for tissue sealing [11]. Aloia et al. [12] introduced a two-surgeon technique for hepatectomies to resect neoplasms in adults, and demonstrated promising results. Palavecino et al. [13] demonstrated that the mean intraoperative blood loss was significantly decreased after the introduction of the two-surgeon technique compared with other techniques (stapling alone, ultrasonic dissection alone, saline-linked cautery alone, and the clamp-crush technique). We previously demonstrated that SLC could be adapted safely for living liver donor surgery without injuring either the graft or the remnant liver [14]. With the introduction of the liver hanging maneuver, which brings the transection line to just beneath the upper midline incision with pulling up of the liver [5, 15], to the hybrid method, parenchymal transection with the two-surgeon technique can be conducted as well as during open procedures. As a result, parenchymal transection can be successfully completed through the 10- to 12-cm upper midline incision without additional stress for the surgeon.

The upper midline incision that we adopted for the hybrid procedure is considered to have several advantages compared to the right subcostal incision, which was previously reported for the hybrid method. The upper midline can avoid muscle disruption and disturbing the sensory nerve dominating the abdominal wall. Jain et al. [16] reported the presence of persistent numbness of the

abdominal skin between the subcostal incision and the umbilicus in patients who had undergone liver transplantation. Surprisingly, 100 % of the patients ($n = 101$) had persistent numbness up to 9 years following liver transplantation. Five percent of these patients developed thermal injuries or blunt trauma complications. According to the results of a randomized, double-blind trial concerning midline versus transverse incisions in major abdominal surgery, although no relevant differences between midline and transverse incisions were observed for pulmonary complications, the median length of hospital stay and incidence of incisional hernias after 1 year was higher, and patients showed more wound infections, in the transverse group ($P = 0.02$) [17]. Given the development of the above-mentioned postoperative complications, the upper midline incision seems to be a more reasonable approach.

Some authors have reported previously the feasibility of major hepatectomy using a midline incision, including living donor hepatectomy [18, 19]. Lee et al. concluded that the procedure after an upper midline incision was more difficult in male donors with large fatty livers and deep truncal cavities. Without randomized, controlled trials, we presently cannot show objective data comparing our procedure and midline hepatectomy without laparoscopy. However, laparoscopic mobilization of the liver under pneumoperitoneum has been reported to be a safe and effective procedure with a good multidirectional surgical view and a wide working space [1, 20]. Hence, this virtue of laparoscopic procedure would allow for mobilization of the liver even in patients with deep truncal cavities, irrespective of the length of the midline incision. The influence of each patient's constitution on our technique seems to be smaller than that on midline major hepatectomy without a laparoscopic procedure. In this study, 17 patients (17 %) had a BMI >25, which is the cutoff value between normal and overweight. Among these patients, four had a BMI >30, which is considered obese. We therefore considered that our procedure can be applied in almost all patients, except those with morbid obesity accompanied by an extremely thick abdominal wall.

Furthermore, quick celiotomy and closure of the abdomen also were benefits of the upper midline incision [21]. The additional duration of the preparation for laparoscopic procedure was offset by the rapid opening and closing of the abdominal incision.

Although the long-term outcomes should be carefully evaluated, given the aforementioned advantages, in addition to the safety and feasibility, we consider that our technique should become more widely accepted as a standard hybrid method. Moreover, this method does not require expert laparoscopic surgical skills.

Disclosures Drs. Akihiko Soyama, Mitsuhisa Takatsuki, Tomohiko Adachi, Amane Kitasato, Yasuhiro Torashima, Koji Natsuda, Takayuki Tanaka, Izumi Yamaguchi, Shiro Tanaka, Ayaka Kinoshita, Tamotsu Kuroki, and Susumu Eguchi have no conflict of interest or financial ties to disclose.

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ORIGINAL ARTICLE

Is a fluorescence navigation system with indocyanine green effective enough to detect liver malignancies?

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Abstract
Background Although several reports have shown the efficacy of a fluorescence navigation system (FNS) with indocyanine green (ICG) to detect liver malignancies during hepatectomy, the real accuracy of this procedure is not yet clear. This study aimed to analyze the actual efficacy of ICG-FNS in cirrhotic and non-cirrhotic livers.
Methods Ten cirrhotic whole livers explanted from liver transplant recipients and 23 non-cirrhotic livers from patients who underwent hepatectomy for various kinds of liver tumors were investigated with ICG-FNS. All surgical specimens were analyzed macroscopically and pathologically.
Results In the patients with a cirrhotic liver, most nodules illuminated by ICG-FNS were diagnosed as regenerative nodules pathologically. The positive predictive value was 5.4%. There was a significant difference in positive predictive value to detect malignant liver tumors between cirrhotic liver and non-cirrhotic liver (5.4% vs 100%, $P < 0.0001$). In the non-cirrhotic livers, 11 of 33 (32.4%) tumors were not recognized by ICG-FNS through the liver surface before resection. There was a significant difference in the depth from the liver surface to tumor between illuminated nodules and non-illuminated nodules (1.5 mm vs 11.6 mm, $P < 0.01$).
Conclusions It is necessary to know the limitation of ICG-FNS when detecting liver malignancies in both cirrhotic and non-cirrhotic livers.

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Keywords Fluorescence navigation system · Indocyanine green · Liver tumor

Introduction

In liver surgery, in order to achieve absolute removal of tumors, it is essential to recognize even small tumors and to ensure the accurate surgical margin intraoperatively. Intraoperative ultrasonography has been the gold standard to detect liver tumors since it was introduced by Makuuchi et al. [1]. Recently, several reports have shown the efficacy of intraoperative navigation surgery using a fluorescence navigation system with indocyanine green (ICG-FNS) [2, 3].

Indocyanine green-fluorescence navigation system has generally been used to detect the sentinel lymph nodes in the breast [4, 5], gastric [6–8], lung [9, 10], and esophageal cancers [11] and to intraoperatively assess graft patency in vascular surgery [12, 13]. The adaption of ICG-FNS was expanded for hepatobiliary surgery [3, 14, 15]. Ishizawa et al. [2] reported that fluorescence intraoperative cholangiography with ICG is a safe and valuable procedure for a road map of biliary tract anatomy and identification of liver tumor through the visualization of the disordered biliary excretion of ICG in real time. However, the actual efficacy of ICG-FNS to detect liver tumors is not clear. For example, false positive detection might occur because regenerative nodules develop as liver damage progresses [16]. Also, small tumors located deep in the liver parenchyma might not be detected by this procedure.

Therefore, the aim of this study was to evaluate the actual efficacy of ICG-FNS in cirrhotic or non-cirrhotic liver separately.

Table 1 Characteristics of patients with a cirrhotic liver and a non-cirrhotic liver

	Cirrhotic liver for LDLT ($n = 10$)	Non-cirrhotic liver for liver resection ($n = 23$)
Gender (male : female)	6:4	16:7
Age[a]	61 (38–72)	67.5 (60–90)
liver disease	HCV:8, HBV:1, alchohol:1	HCV:5, HBV:5, NBNC:4, NL:9
liver tumor	HCC:9	HCC:12, metastases:9, CCC:1, Carcinoid:1
Total-bilirubin (mg/dl)[a]	3.2 (0.5–8.8)	0.8 (0.3–1.4)
AST (IU/l)[a]	52 (30–89)	28 (13–285)
ALT (IU/l)[a]	29 (15–75)	27 (10–100)
Albumin (g/dl)[a]	2.6 (2.1–3.8)	4.2 (2.9–5.2)
Platetlet ($\times 10^4$/mm^3)[a]	6.4 (2.3–13.4)	16.4 (10.8–27.6)
PT (INR)[a]	1.47 (1.07–1.95)	1.00 (0.87–1.13)
ICGR15 (%)[a]	44 (24–70)	11 (2–15)
Child–Pugh score[a]	9 (5–12)	5 (5–7)
MELD score[a]	14 (7–22)	–

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *CCC* cholangiocarcinoma, *HBV* Hepatitis B virus, *HCC* Hepatocellular carcinoma, *HCV* Hepatitis C virus, *ICGR15* indocyanine green retention rate at 15 minutes, *INR* international normalized ratio, *MELD score* model for endstage liver disease score, *NBNC* non-HBV non HCV, *NL* normal liver, *PT* prothorombin time

[a] Median (range)

Materials and methods

Patients

Ten cirrhotic whole livers were obtained from living donor liver transplant (LDLT) recipients. The original diseases included hepatitis C virus (HCV)-cirrhosis in eight, hepatitis B virus (HBV)-cirrhosis in one, and alcoholic cirrhosis in one. Patient characteristics are listed in Table 1. In addition, we evaluated 23 non-cirrhotic liver specimens obtained from patients who underwent hepatectomy for various kind of liver tumors (hepatocellular carcinoma [HCC] in 12, metastasis of colorectal cancer in nine, cholangiocarcinoma in one, and hepatic carcinoid in one) (Table 1).

Examination methods

As a fluorescence source, we used ICG (Diagnogreen, Daiichi Sankyo, Tokyo, Japan), which had been intravenously injected before surgery at a dose of 0.5 mg/kg as part of a routine liver function test. The intervals between the ICG injection and surgery ranged from 2 days. As a fluorescent imaging tool, we used Photo Dynamic Eye-II (PDE-II, Hamamatsu Photonics, Hamamatsu, Japan), which filtered out light with a wavelength below 820 nm, and 36 light-emitting diodes with a wavelength of 760 nm. The camera imaging head was positioned between 20 and 30 cm above the surgical specimen. The marking suture was performed near the site of the illuminated nodules during 10 min while putting the emission of light situation in the video. Any illuminated nodules clearly visualized from the liver surface regardless of signal intensity, size and illuminated pattern were marked. The recognition of the illuminated nodules was clarified by two independent surgeons (TT and IM). Surgical specimens were observed from every angle, and cut to include each tumor's maximum diameter based on gross inspection. After the operation, the number of the illuminated lesions in the video and the number of the marking sites were counted and macroscopically analyzed. In addition, the number of illuminated lesions that were detected through the liver surface and the number of identifiable tumors by pathological examination were analyzed.

Statistical analysis

Results for continuous variables are expressed as the median (range). The positive predictive value was calculated as the number of pathological tumors in illuminated nodules. Data for continuous variables were compared using the Mann–Whitney *U*-test. We set statistical significance at $P < 0.05$.

Results

Efficacy of ICG-FNS to detect HCCs in a cirrhotic liver

The median number of preoperatively detected tumors with various modalities in the 10 patients with cirrhotic liver was 2 (0–4). However, as shown in Figure 1, the median number of illuminated nodules was 20 (7–37), and the median

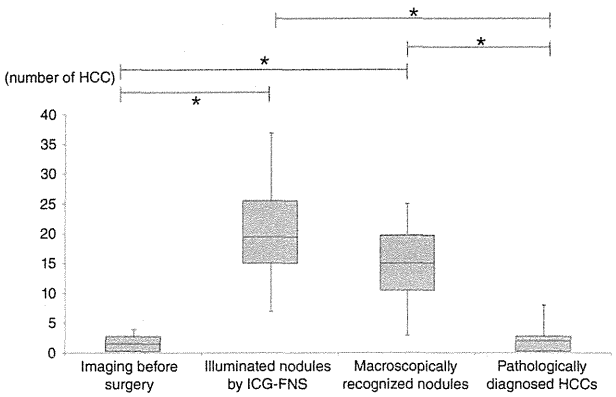

Fig. 1 The number of detected nodules in a cirrhotic liver. The median number of preoperative, illuminated, macroscopic, pathological tumors with cirrhotic liver was, in order, 2 (0–4), 20 (7–37), 16 (3–25), and 2 (0–8). There were significant differences between preoperative tumors and both illuminated nodules and macroscopic tumor ($P < 0.001$). Moreover, there were also significant differences between pathological tumor and both illuminated nodules and macroscopic tumor ($P < 0.001$). *$P < 0.05$

number of nodules to be macroscopically considered as tumors was 16 (3–25). The number of pathologically diagnosed as tumors was 2 (0–8), so the positive predictive value was only 5.4% (0–24.2%). The number of false negatives was 14 (4–17). There were significant differences between the numbers of preoperatively detected tumors and the number of both illuminated nodules and those macroscopically recognized as tumors, as shown in Figure 1 ($P < 0.001$). Moreover, there were also significant differences between the numbers of nodules pathologically diagnosed as tumors and the number of both illuminated nodules and nodules macroscopically recognized as tumors ($P < 0.001$). HCC on the liver surface with ICG-FNS revealed fluorescence in Figure 2a. The area that was actually illuminated was also macroscopically observed as HCC in Figure 2b. The same part was pathologically diagnosed as HCC in Figure 2c. However, the other areas that were illuminated and observed as nodules were diagnosed as regenerative nodules (Fig. 2d–f).

Efficacy of ICG-FNS to detect malignant tumors in a non-cirrhotic liver

The median number of preoperatively detected tumors with various modalities in 23 patients with non-cirrhotic liver was 1 (1–4). The median number of illuminated nodules and nodules macroscopically recognized as tumors was 1 (0–3) and 1 (1–4), respectively. Finally, the median number of nodules pathologically diagnosed as liver tumors was 1 (1–4), so that the positive predictive value to detect malignant tumors was 100%. There were no significant differences between the groups (Supporting information Fig. S1). There was a significant difference in positive predictive value to detect HCC or malignant tumors between the cirrhotic livers and the non-cirrhotic livers (5.4% vs 100%; $P < 0.0001$). Although some tumors could be detected from the liver surface (Fig. 3a,b) and positive predictive value to detect malignant tumors in the non-cirrhotic livers was 100%, other tumors could not be detected from the liver surface (11/34, 32.4%). These nodules were finally recognized after the liver was cut, including tumors (Fig. 3c,d) and defined as non-illuminated nodules. There was a significant difference in the median depth from the liver surface between illuminated nodules ($n = 23$) and non-illuminated nodules ($n = 11$) (1.5 mm vs 11.6 mm, $P < 0.01$) (Fig. 4a). Although there was no significant difference, non-illuminated nodules tended to be smaller than illuminated nodules (illuminated nodules, 32.4 mm vs non-illuminated nodules, 20.7 mm; $P = 0.058$) (Fig. 4b). Furthermore, the tumor size of almost all non-illuminated nodules located within 10 mm depth tended to be smaller than 10 mm (Fig. 4c).

Discussion

Indocyanine green is usually used to directly measure the actual functional state of the liver [17]. ICG is a water-soluble, inert compound that is injected intravenously. It mainly binds to plasma proteins, is taken up by hepatocytes, and is excreted unchanged into the bile. Moreover, ICG is known to absorb infrared rays [18]. Lights, and specially near-infrared light (NIR) in the biological window (700–900 nm), can be exploited for intraoperative imaging guidance. Tanaka et al. demonstrated that (1) the appearance of the surgical field is not altered, (2) it is safe, (3) wavelengths in the 800 nm range penetrate relatively deeply into living tissue, and (4) there has been a tremendous recent effort in developing general-purpose NIR fluorophores that can be conjugated to targeting or other molecules, thus creating "contrast agents" matched to any desired surgical application [19]. Therefore, ICG injection combined with FNS is widely used to detect sentinel lymph nodes [4–11], because this technique is convenient and safe for assessing lymph node status in the oncological field. Recently, adaptation of ICG-FNS was expanded further to hepatobiliary surgery and there have been some reports of its capability in detecting tumors [2, 3]. Furthermore, several reports also showed that ICG-FNS was effective to check the bile leakage and the surgical margin [2, 3, 14, 15].

Our team sectioned whole livers with HCC removed from recipients who underwent living donor liver transplantation and fully investigated them to detect small HCCs [20]. Generally, hepatocytes have the ability to reproduce by

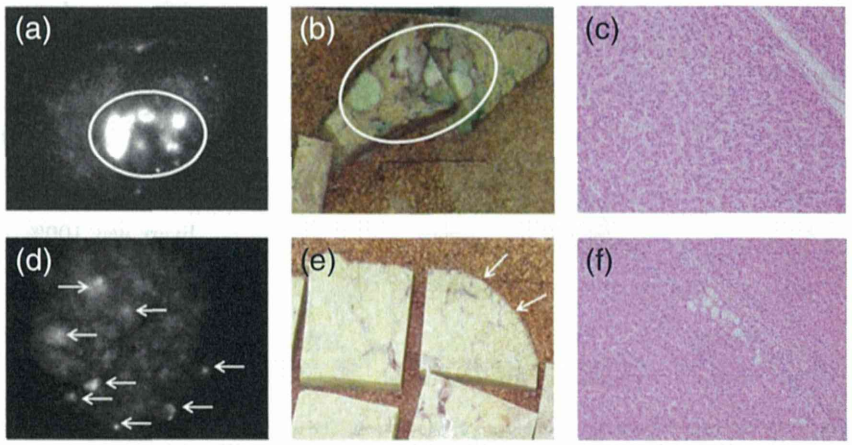

Fig. 2 Indocyanine green-fluorescence navigation system (ICG-FNS) for patients with cirrhotic liver and pathological results. (**a**) Hepatocellular carcinomas (HCCs) on the liver surface with ICG-FNS revealed the illuminated nodules. (**b**) The area that actually emitted light was also macroscopically observed as liver tumor. (**c**) The same area was pathologically diagnosed as HCC. (**d**) The other areas on the liver surface in the same case also revealed the illuminated nodules. (**e**) The areas that emitted light were macroscopically observed as nodules. (**f**) Finally, these areas were diagnosed as regenerative nodules

themselves even if the hepatocytes are exposed to disorders. However, when hepatocytes are exposed to inflammation for a long time and repeatedly, hyperplasia of the collagen fiber deposits occurs strongly and many benign nodules, such as regenerative nodules, in a cirrhotic liver appear surrounded by collagen fiber in the extracellular matrix. In addition, the structure of the hepatic lobule is disturbed and the function of hepatocytes is affected [21]. On the other hand, as described above, ICG binds to plasma proteins, one of which is ligandin. Ligandin is the binding protein of ICG and is uniformly distributed over hepatocytes in a normal liver. The expression of ligandin deviates with liver damage, and it is not expressed in areas of necrosis, fibrosis, or severe inflammation, so that the expression of ligandin becomes relatively rich in the regenerative area [22]. Finally, the coloration of ICG is thought to accumulate in the regenerative nodules [16]. In fact, in our series of cirrhotic livers, many false positive nodules were illuminated, possibly because of the severe liver function disorder and the biliary excretion disorders [2, 16, 21, 22]. However, Ishizawa et al. demonstrated that the signal intensity of the noncancerous liver parenchyma was higher in patients with an unfavorable ICG retention rate and in patients who had received the ICG injection within 24 h before surgery [2]. Although the interval longer than 2 days might be better to obtain a good lesion-to-liver contrast, especially in patients with advanced cirrhosis, we routinely perform the ICG test when the patients were hospitalized generally 2 days before the operation. Also, the optimal interval between ICG injection and surgery to detect tumors remains controversial.

Second, we attempted to evaluate liver resection cases with non-cirrhotic livers. The aim of this study was to determine whether the fluorescence navigation system was effective or not to detect tumors. Therefore, we did not

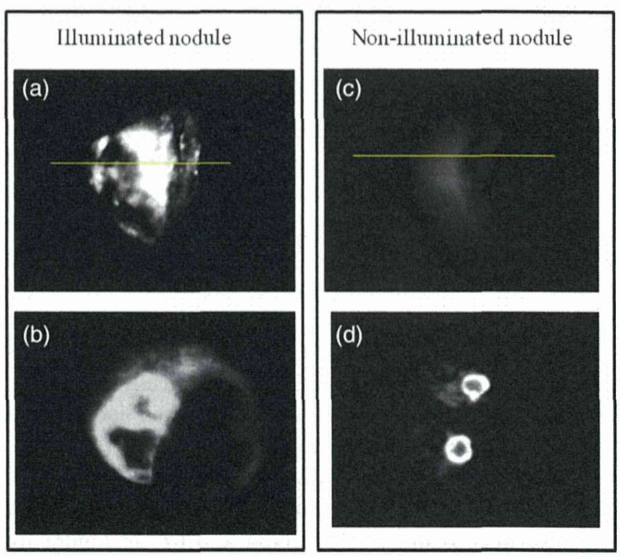

Fig. 3 Indocyanine green-fluorescence navigation system (ICG-FNS) for patients with liver resection with non-cirrhotic liver. Liver tumors in illuminated nodules could be observed as illuminated nodules on the liver surface (**a–b**); liver tumors in non-illuminated nodules could not be observed as illuminated lesions on the surface, and finally recognized after the liver was cut including the tumor under ultrasound (US) guidance (**c–d**)

evaluate the illuminated pattern of tumors. However, we got the same results as Ishizawa's report concerned with illuminated pattern of liver tumors (data not shown) [2]. It was clear that although false positive nodules were not observed in the non-cirrhotic livers, smaller tumors and tumors that were located deeper than 10 mm from the liver surface to tumor were difficult to recognize as illuminated nodules. Kim et al. have already described that near-infrared light penetrates human tissues to a depth of

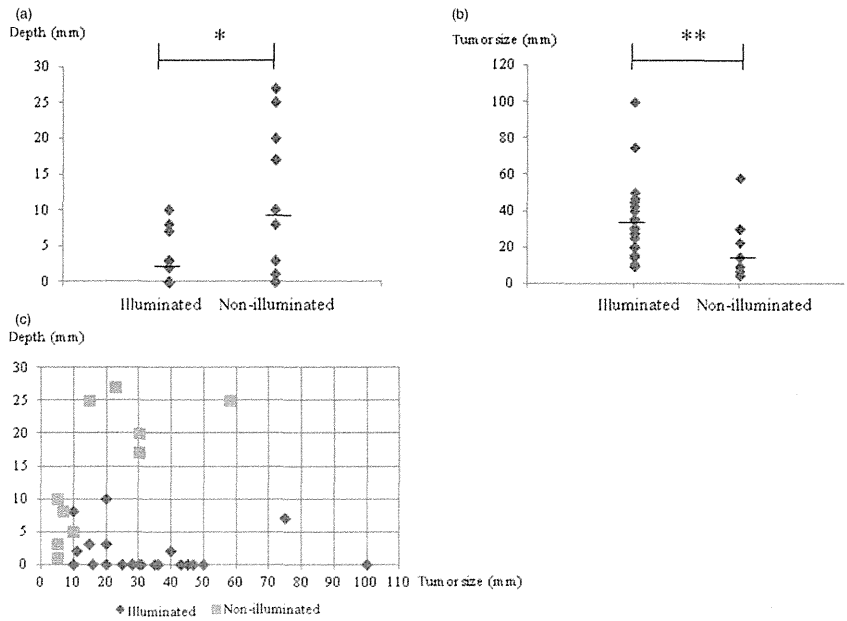

Fig. 4 Comparison between illuminated nodules and non-illuminated nodules on the liver surface in a non-cirrhotic liver. (**a**) There was a significant difference in median depth from the liver surface between illuminated nodules and non-illuminated nodules (1.5 mm vs 11.6 mm, $P < 0.01$). (**b**) Non-illuminated nodules tended to be smaller than illuminated nodules (illuminated nodules, 32.4 mm vs non-illuminated nodules, 20.7 mm; $P = 0.058$). (**c**) Correlation diagram of the depth and tumor size presented. The tumor size of almost all non-illuminated nodules located within 10 mm depth tended to be smaller than 10 mm

about 5–10 mm and Ishizawa et al. have also described that cancer detectability using the fluorescent imaging technique seems mainly to depend on the depth of the tumors from the liver surface because of the limited tissue penetration of near-infrared light [2, 23]. Therefore, in our study, ICG-FNS was not able to detect tumors located deeper than 10 mm. Possibly, 10 mm is the maximum limit of depth for ICG-FNS to detect tumors. Based on these results, we suggested that the capability of detecting tumors with ICG-FNS alone was not sufficient in both cirrhotic and non-cirrhotic livers.

We conclude that although ICG-FNS have the capability of detecting tumors and checking the bile leakage and surgical margin, it is necessary to know the limitation of ICG-FNS when searching liver malignancies in cirrhotic livers or small and deep liver malignancies in non-cirrhotic livers. Additional modalities including ultrasound should be adapted for use with this procedure to detect small liver tumors.

Author contribution Study design: Takayuki Tanaka. Acquisition of data: Takayuki Tanaka, Takanobu Hara, and Izumi Muraoka. Analysis and interpretation: Takayuki Tanaka, Mitsuhisa Takatsuki, Masaaki Hidaka, and Akihiko Soyama. Manuscript drafted by: Takayuki Tanaka and Mitsuhisa Takatsuki. Revision: Takayuki Tanaka and Susumu Eguchi. Statistical Advice: Takayuki Tanaka, Tomohiko Adachi and Tamotsu Kuroki

Conflict of interest None declared.

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Supporting information

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Fig. S1 Number of detected nodules in a non-cirrhotic liver. The median number of preoperative, illuminated, macroscopic, and pathological tumors with non-cirrhotic liver was, in order, 1 (1–4), 1 (0–3), 1 (1–4), and 1 (1–4). There were no significant differences in each group.

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Review Article

Liver transplantation for HIV/hepatitis C virus co-infected patients

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Since the introduction of antiretroviral therapy (ART) in the mid-1990s, AIDS-related death has been dramatically reduced, and hepatitis-C-virus (HCV)-related liver failure or hepatocellular carcinoma has currently become the leading cause of death in HIV/HCV co-infected patients. Liver transplantation may be one of the treatments of choices in such cases, but the indications for transplantation, perioperative management including both HIV and HCV treatments, immunosuppression and the prevention/treatment of infectious complications are all still topics of debate. With the improved understanding of the viral behaviors of both HIV and HCV and the development of novel strategies, especially to avoid drug interactions between ART and immunosuppression, liver transplantation has become a realistic treatment for HIV/HCV co-infected patients.

Key words: hepatitis C virus, HIV, liver transplantation

INTRODUCTION

IN JAPAN, IN the late 1980s, contaminated blood production of coagulation factor for hemophilia caused co-infection of HIV and hepatitis C virus (HCV). Actually, greater than 90% of HIV-infected patients have HCV as well.[1]

After antiretroviral therapy (ART) was introduced in the late 1990s, successful control of HIV was achieved in most cases and death due to AIDS was dramatically reduced, but HCV-related death due to liver failure or hepatocellular carcinoma became a serious problem, not only in Japan, but all over the world.[2–6] In such cases, liver transplantation (LT) is the only treatment option to achieve long-term survival, but several modifications of perioperative management are required. In this review, the outcome and the points of management of LT for HIV/HCV co-infected patients were reviewed.

REPORTED OUTCOME OF LT FOR HIV/HCV PATIENTS

THE REPORTED OUTCOMES of LT for HIV and HIV/HCV co-infected patients from Western countries after the introduction of ART are summarized in Table 1.[7–11] In general, most reports concluded that the results were worse than in the cases with HCV mono-infection, with a 3-year survival of approximately 60–70%. In Japan, the Tokyo group reported six cases of living donor liver transplantation (LDLT) between 2001 and 2004, of whom four died.[12] These unfavorable outcomes are likely related to the difficulties of determining the indications for LT and of perioperative management, including HIV/HCV treatment and the prevention and treatment of infectious complications. Terrault *et al.* reported that older donor age, combined kidney–liver transplantation, an anti-HCV positive donor and a body mass index of less than 21 kg/m^2 were independent predictors of graft loss.[10] After transplantation, several studies showed that acute cellular rejection was more frequent and severer in HIV/HCV co-infected patients than that in HCV mono-infected patients, possibly due to the difficulties in achieving optimal immunosuppression because of interactions between antiretroviral agents and immunosuppression.[10,11]

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Table 1 Outcome of liver transplantation for HIV/hepatitis C virus co-infection

Authors	Publication year	Country	*n*	Patient survival (%)		
				1 year	3 years	5 years
de Vera *et al.*[7]	2006	USA	27	67	56	33
Schreibman *et al.*[8]	2007	USA	15	73	73	–
Duclos-Vallee *et al.*[9]	2008	France	35	–	73	51
Terrault *et al.*[10]	2012	USA	89	76	60	–
Miro *et al.*[11]	2012	Spain	84	88	62	54

SPECIAL ISSUES REGARDING LT INDICATIONS FOR HIV/HCV CO-INFECTION

ART-related non-cirrhotic portal hypertension

IN HCV MONO-INFECTED patients, LT should be considered when the patients develop deteriorated liver function as indicated by a Child–Pugh classification of B or C. In HIV/HCV co-infected patients, liver failure due to HCV hepatitis was generally enhanced by ART-related hepatotoxicity, especially non-cirrhotic portal hypertension.[13–15] Accordingly, not only in cases with deteriorated liver function but also in class A cases, the patients can easily develop severe liver dysfunction suddenly,[16,17] so that all HIV/HCV co-infected patients should be carefully followed up so as not to miss the chance for LT. Also, Murillas *et al.* reported that Model for End-Stage Liver Disease (MELD) score is the best prognostic factor in HIV-infected patients,[18] so that HIV/HCV co-infected patients may be considered for LT before MELD score increase to achieve comparable results with HCV mono-infected patients. Several studies showed the aggressive fibrosis in HIV/HCV co-infected patients compared with HCV mono-infected patients,[19,20] but the mechanism of this aggressive fibrosis remains unclear. Recently, transient elastography or acoustic radiation force impulse imaging to check for liver stiffness has been introduced as an effective and non-invasive modality to determine patients' candidacy for LT.[21–23]

Count of CD4$^+$ T lymphocytes

Generally, the count of CD4$^+$ T lymphocytes has been required to be more than 200/μL to perform general elective surgeries in HIV-infected patients,[24] but in HIV/HCV co-infected patients, current studies show that a count of more than 100/μL is acceptable,[25,26] because patients generally have portal hypertension which can cause pancytopenia. In such patients, the ratio of CD4/CD8 is reported to be a feasible marker to predict postoperative complications including opportunistic infections. When the ratio is less than 0.15, the incidence of infectious complications is significantly higher.[27]

Preoperative infections

In regard to latent opportunistic infections that occur before LT, they are not absolute contraindications when they can be expected to be controlled.[28] Infections regarded as contraindications for LT included uncontrollable multidrug resistance HIV infection, chronic *Cryptosporidium enteritis*, progressive multifocal leukoencephalopathy and lymphoma.[29]

MANAGEMENT OF HIV/HCV IN LT

Management of HIV

THE NUMBER OF HIV RNA copies before LT is suggested as an independent risk factor of postoperative mortality, so that HIV should be controlled sufficiently before LT.[30] Accordingly, in the patients who are under consideration to receive LT, ART can be safely stopped before LT because HIV is generally well-controlled for a long period by ART. After LT, ART should be restarted as soon as possible because HIV RNA appears at 3–30 days after ART is stopped,[31] but the timing of restart of ART depends on the patient's condition, including liver function.[32] As long as the liver function has not fully recovered, or partial liver graft such as in LDLT has not sufficiently regenerated yet, ART cannot be started. Castells *et al.* reported in their case–control study that ART was started at a median of 8 days after LT (range, 4–28 days).[33] In principle, the ART administrated after LT should be the same as the pretransplant regimen, but the majority of ART drugs including protease inhibitor (PI) and non-nucleoside reverse transcriptase inhibitor (NNRTI) have interactions with calcineurin inhibitors

(CNI) or mammalian target of rapamycin (mTOR),[34] so that the monitoring of blood levels of immunosuppression is extremely important to avoid infectious complications or rejection. Currently, a novel HIV-1 integrase inhibitor, raltegravir (RAL), is expected to be a feasible drug because it has no interactions with CNI, unlike other drugs.[35,36]

Management of HCV

The treatment strategy for HCV in HIV/HCV co-infected patients is the same as in HCV mono-infected patients. Combination therapy of pegylated interferon (PEG IFN) and ribavirin is the standard treatment both before and after LT. The timing of the induction therapy after LT is controversial. A Tokyo group proposed early induction as a preemptive therapy before patients develop hepatitis,[37] while several other reports showed favorable results when the treatment was administrated only after the development of hepatitis was confirmed by liver biopsy.[38,39] Theoretically, the treatment should be started as soon as possible, because in HIV/HCV co-infected patients, HCV recurrence may be accelerated in an immunocompromised state.[30,40] The novel protease inhibitor, telaprevir, is currently introduced as an effective drug to achieve sustained viral response of 70%, even in genotype 1b, with PEG IFN/ribavirin in a non-transplant setting,[41] but this drug is metabolized via cytochrome P450 as a substrate, as are CNI and various protease inhibitors of ART for HIV. Close monitoring of the CNI trough level should be performed, and although triple therapy with telaprevir/PEG IFN/ribavirin is currently reported to be effective to prevent HCV recurrence after LT in HCV mono-infected cases, special attention should be paid when this regimen is adapted in HIV/HCV co-infected patients.

IMMUNOSUPPRESSION

AS PREVIOUSLY MENTIONED, many factors including ART, anti-HCV treatment and an HIV-related immunocompromised state make post-LT immunosuppressive treatment difficult. Many ART drugs, both PI and NNRTI, cause instability in the blood concentration of CNI through the cytochrome P3A4 (CYP3A4)-related metabolism. Most PI cause the overconcentration of CNI by inhibiting CYP3A4, while most NNRTI cause decreased levels of CNI by stimulating CYP3A4.[29,42] As mentioned earlier, RAL is introduced as a key drug in LT in HIV positive patients, because the metabolism of this drug is not related to CYP450, so it does not affect the blood concentration of CNI. Several reports have demonstrated both the *in vitro* and *in vivo* effectiveness of rapamycin in reducing HIV replication,[43–45] and Di Benedetto *et al.* found that rapamycin monotherapy was significantly beneficial in long-term immunosuppression maintenance and HIV control after LT.[46] Mycophenolate mofetil is expected to be an effective immunosuppressive drug because of its efficacy in reducing HIV infection by both virological and immunological mechanisms.[47–49] Using these drugs, a more effective regimen of immunosuppression with ART may be established.

In regard to the steroid, several studies proposed that a steroid-free regimen can be safely applied and effective in LT for HCV cirrhosis. Also, in HIV/HCV co-infected patients, steroid-free protocol may be beneficial to prevent both HIV and HCV recurrence after LT.[50,51]

CONCLUSIONS

LIVER TRANSPLANTATION FOR HIV/HCV co-infected patients remains challenging, but with recent developments in perioperative management and novel drugs for both HIV and HCV, the results are likely to be improved.

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原著

HIV/HCV 重複感染患者の肝障害病期診断における acoustic radiation force impulse（ARFI）elastography の有用性

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要旨：【背景】HIV コントロールの改善により HIV/HCV 重複感染者の死因として肝疾患の割合が増加している.【目的】重複感染者における acoustic radiation force impulse（ARFI）elastography による肝疾患進行度評価の有用性を明らかにする.【方法】肝実質硬度をせん断弾性波の速度（Vs）として定量化し，他の肝機能評価項目との相関を検討.【結果】Vs 値は血小板数，脾容積，ヒアルロン酸，IV 型コラーゲン，アシアロシンチ LHL15 値と有意な相関あり.【考察】ARFI は肝線維化・予備能評価に有用であり，HIV/HCV 重複感染者に対する非侵襲的で正確な肝疾患進行度評価に応用可能と考えられた.

索引用語：HIV/HCV 重複感染，肝移植，ARFI elastography，腹部超音波検査

はじめに

1990 年代後半の anti-retroviral therapy（ART）の登場によって human immunodeficiency virus（HIV）のコントロールは改善し，HIV 感染例の死亡数は減少するとともに死因に大きな変化が見られた．HIV 感染者における acquired immunodeficiency syndrome（AIDS）以外の死亡で最も多いのは肝疾患であり，その原因の多くは hepatitis C virus（HCV）感染症であった（Weber ら[1)]）．本邦においても平成 22 年度厚生労働省調査で，HIV/HCV 重複感染患者における死因の 1/3 は肝疾患であることが報告された[2)]．本邦における HIV 感染者の 19.2% が HCV に重複感染しており，その原因のほとんどが過去の HIV/HCV 混入血液製剤の投与であるが，血液製剤による HIV 感染者の HCV 抗体陽性率は 97% と極めて高い[3)]．このような薬害による HIV/HCV 重複感染者に対する的確な病期分類は，救済医療としての面からも今後その重要性が増すと考えられる．

重複感染者では単独感染者に比して線維化の進行が早いと報告されているが[4)]，過去の血液製剤の使用による重複感染者では血友病を有しているため，肝生検による線維化評価は困難である．近年，非侵襲的な肝線維化評価の方法として acoustic radiation force impulse（ARFI）elastography の有用性が報告されている[5)]．ARFI とは収束超音波パルスで組織に微細な変形をおこし，パルスが止んで組織が元の形に戻る際に体表に対して水平に発生するせん断弾性波の速度（velocity of shear wave；Vs）を測定し，組織の硬度を定量

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化したものである．今回われわれは，HIV/HCV重複感染者に対して，ARFI elastographyによる肝線維化評価の有用性を明らかにする目的で検討を行った．またわれわれは，重複感染者においては一般肝機能検査などが正常でも肝予備能が低下している症例が少なからず存在することを報告した（曽山ら[6]）．ARFIの測定結果と予備能の関連についても検討を行った．

I 対象と方法

2009年9月から2013年6月までに当院で精査を行ったHIV/HCV重複感染患者37名のうち，ARFI elastographyを施行したChild分類A症例23例を対象とした．37例はいずれも原疾患に血友病を有し，過去の血液製剤の使用によってHIV/HCV重複感染をきたした症例であり，全例男性，年齢の中央値は40歳（30～63歳）であった．肝機能の内訳はChild Aが34例，Bが1例，Cが2例であった．対照として同時期に当院消化器内科で加療を行ったHCV単独感染症例18例，および健常群として同時期に当院でグラフト採取術を施行した生体肝移植ドナー10例と比較を行った．まずARFIを用いて肝線維化をVsとして数値化し（右肋間より右葉のVs値を5回測定し平均値を用いた），重複感染群，HCV単独感染群，健常群でVs値の比較を行った．次にHIV/HCV重複感染群においてVs値（肝右葉・左葉それぞれでVs値を5回ずつ測定してそれぞれの平均値を算出し，左右の値の平均値を肝全体のVs値として用いた）とALT，総ビリルビン値，血小板数，CTでの肝形態，脾容積，ヒアルロン酸，IV型コラーゲン，ICG15分停滞率，アシアロシンチLHL15値との相関を検討した．脾容積はAquilion™，64列（東芝メディカルシステムズ，日本）を用いて動脈相，門脈相，平衡相の3相で造影CTを撮影し，平衡相の脾容積をSYNAPSE VINCENT（富士フイルムメディカル，日本）を用いてvolumetryを行い測定した．また肝形態の評価は当院の放射線科医師が読影し，正常肝の他に脂肪肝，慢性肝炎，肝硬変の4つに分類した．統計学的検定には，統計解析ソフトystat 2008（医学図書出版，東京）を用い，2群間比較にはMann-Whitneyの順位和検定を，相関についてはSpearmanの順位相関を行った．

II 結 果

Vs値（以下中央値と範囲）はHIV/HCV重複感染群で1.27（0.98～2.61）m/s，HCV単独感染群で1.27（0.85～3.00）m/s，健常群で1.08（0.98～1.33）m/sであり，重複感染群は健常群に比べて有意に高値であった（$p=0.010$）が，重複感染群とHCV単独感染群，およびHCV単独感染群と健常群では有意差を認めなかった（それぞれ$p=0.436$，$p=0.059$，Figure 1）．またARFI施行時の年齢については，重複感染群が46（31～63）歳に対して単独感染群が61（33～76）歳と，重複感染群は単独感染群に比べて有意に若年であった（$p=0.008$，Figure 1）．

重複感染群におけるVs値と他の肝機能検査の比較では，ALT（$p=0.358$）や総ビリルビン値（$p=0.949$）では両群に相関を認めなかったが，血小板数（$r=0.737$，$p<0.001$），脾容積（$r=0.592$，$p=0.006$），ヒアルロン酸（$r=0.637$，$p=0.003$），IV型コラーゲン（$r=0.569$，$p=0.009$）はVs値と有意な相関を認めた（Figure 2）．またCTで正常肝を示したものは23例中6例のみで，その他の内訳は脂肪肝1例，慢性肝炎8例，肝硬変8例であった．CTによる形態評価とVs値の相関については，正常肝6例で1.24（1.11～2.12）m/s，その他の17例では1.87（1.14～3.04）m/sであり，両群に有意差を認めなかった（$p=0.058$）．肝予備能評価項目との比較検討では，ICG15分停滞率（$p=0.054$）とは相関を認めなかったものの，アシアロシンチLHL15（$r=0.503$，$p=0.024$）とは有意な相関を認めた（Figure 3）．

III 考 察

HIV/HCV重複感染者のChild A症例においては，健常者と比較してVs値が有意に高値であり，ARFI elastographyの線維化測定は正しく行われていると考えられた．またVs値は，肝線維化のマーカーとして知られるヒアルロン酸やIV型コラーゲン，門脈圧亢進症の所見である血小板数，脾容積と相関を認めた．今回の検討では血友病のため肝生検を行っておらず，組織学的な線維

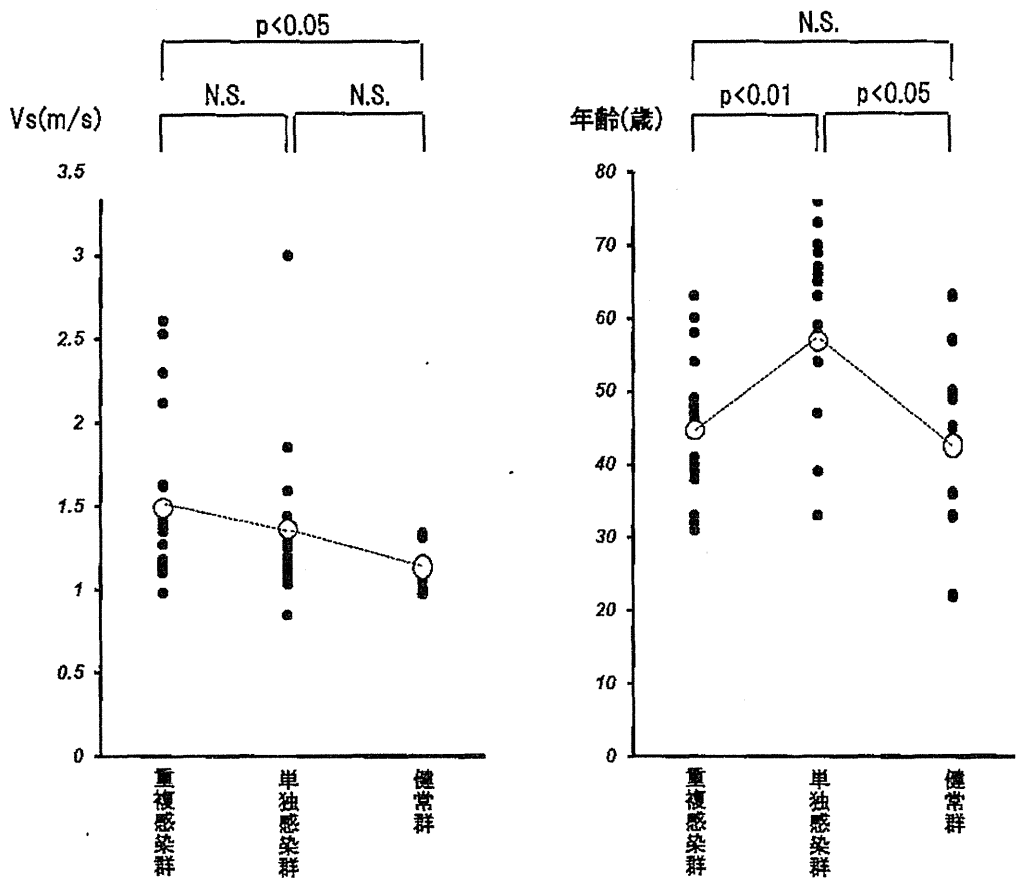

Figure 1. Comparison of Vs and age among HIV/HCV co-infected patients, HCV mono-infected patients, and healthy control (living donor liver transplantation (LDLT) donor).

化の評価はできていないが，HIV/HCV 重複感染者では Child A 症例であっても肝線維化が進行している症例を含んでいることを反映していると思われた．重複感染群と HCV 単独感染群との比較では Vs 値に有意差を認めなかったが，測定時の年齢については，重複感染群の方が有意に若年であった．視点を変えれば，重複感染においては若年者でも既に単独感染の高齢者と同等の線維化をきたしているということになる．重複感染者においては肝線維化の進行が HCV 単独感染群より早いことが諸家から報告されている[4]ことを念頭に，若年期から適切な治療のタイミングを逸しないように，綿密なフォローアップを行うことが重要である．

重複感染者においては HIV/HCV の相互作用や ART による薬剤性肝障害によって肝線維化の進行が早い事例が存在することに加えて，肝実質障害に比して門脈圧亢進症の進行が早い非硬変性門脈圧亢進症の病態を呈することもあり[7][8]，一般肝機能検査のみでは病勢を正確に評価できないケースを多数認める．今回の検討では Vs 値は ALT やビリルビン値などの一般肝機能検査とは相関を示さず，また CT で正常肝であった症例と異常を認めた症例間の Vs 値に有意差は認めなかった．ARFI により Vs 値を測定することは従来の一般肝機能検査とは違った角度から肝機能を評価することとなり，より正確な病期診断につながる可能性が示唆された．

今回の検討で興味深いのは，ARFI elastography の結果が，肝予備能評価と相関していたことである．重複感染者は現在全国各地でフォローアップされているが，肝臓病専門施設や地域中核病院から遠方に居住する患者では，定期的な肝予備能評価が行われていないことも少なくない．さ

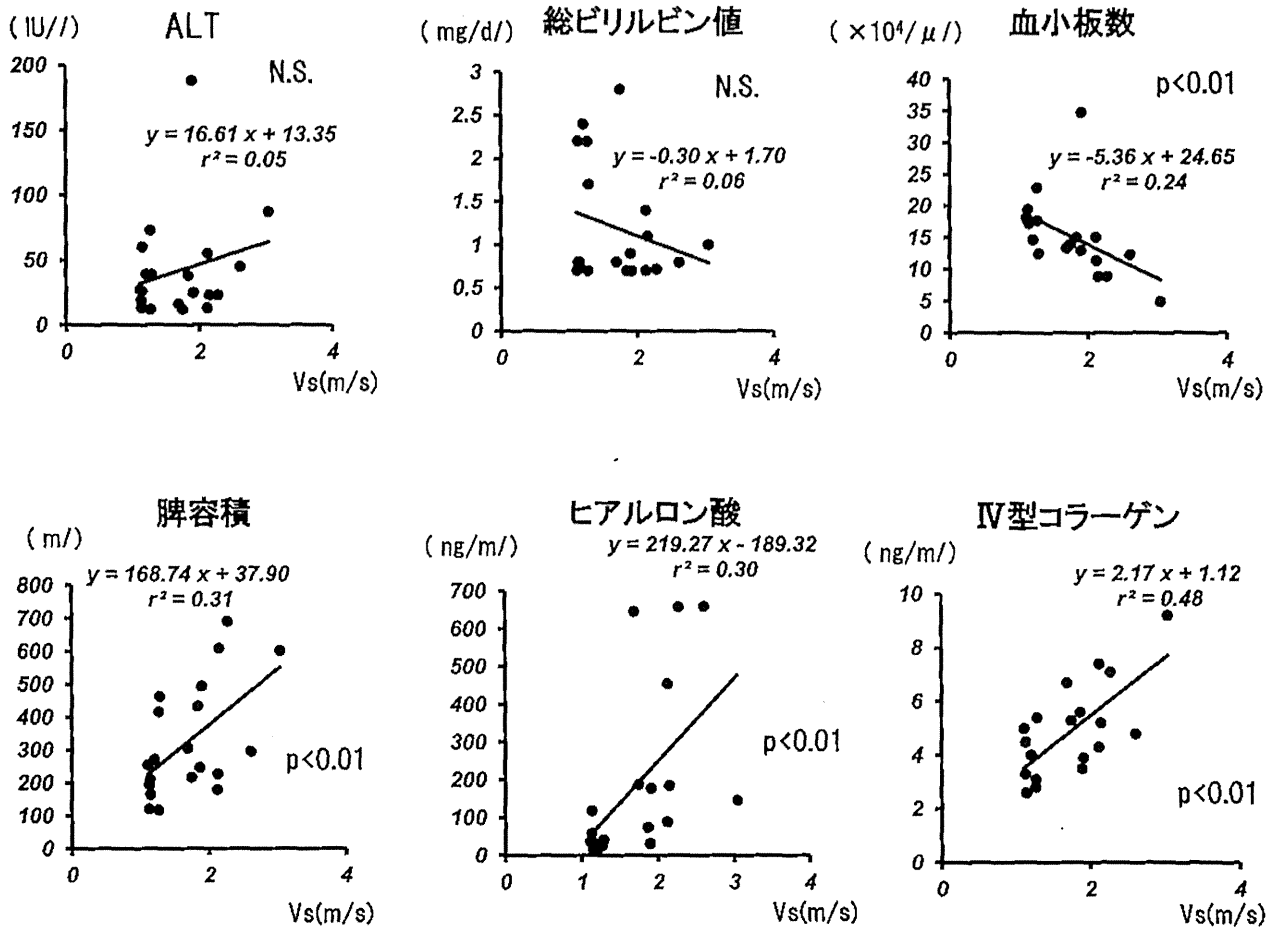

Figure 2. Correlation between Vs and other liver function (ALT, total bilirubin, and platelet counts), splenic volume, hyaluronic acid, type Ⅳ collagen.

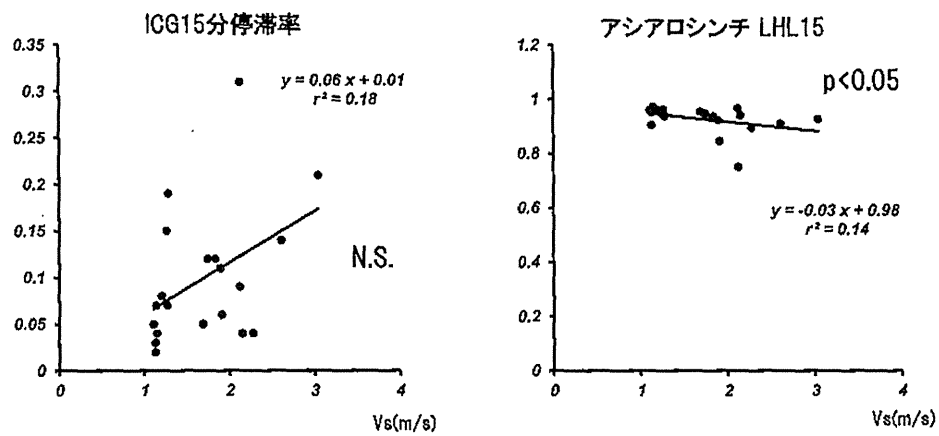

Figure 3. Correlation between Vs and hepatic functional reserve (indocyanine green retention rate and LHL15 in 99mTc-GSA scintigraphy).

らに本邦における重複感染の原因の大半は血友病に対する血液製剤使用にあり，凝固異常のため肝生検による病期診断が困難であるという点が，重複感染者の病期評価を困難にしている一因と思わ

れる．超音波検査はシンチグラムなどに比べて簡便で特別な装置を必要とせず，肝疾患専門施設以外でも導入しやすいと思われる．また凝固能の異常があっても繰り返し施行することが可能であり，病状の進行を経時的に評価することができる．このように従来の肝線維化・肝予備能の指標と有意な相関を示し，かつ非侵襲的なARFI elastographyは有用な診断ツールと考えられる．

前述の如く，HIV/HCV重複感染者における肝線維化の進行は多因子に影響される．ARFIにより肝線維化の程度を数値化し，その経時的測定値と，各種臨床データや服薬歴などとの相関を明らかにすることで，重複感染者における線維化進行のメカニズム解明につながる可能性がある．ひいては個々の症例が有する危険因子から，肝疾患の正確な予後予測へとつながることが期待される．

HIV/HCV重複感染者におけるelastographyを用いた肝線維化評価としては，transient elastography（FibroScan®，Echosens，フランス）の測定結果と組織学的な肝線維化進行度との有意な相関が報告されている[9][10]．FibroScanもARFIと同様にせん断波の速度を測定することにより肝硬度を評価する装置であるが[11]，FibroScanは低周波弾性波を用いて体表と垂直方向の弾性波伝搬速度を測定するため，腹水貯留例や高度肥満例においては測定が困難とされている[12]．またARFIではBモードも可能であり，ルーチンの観察を行った後に肝線維化を測定するといった使用法も可能であるという利点がある．ARFIとFibroScanの精度については，ウイルス性肝炎における検討ではほぼ同等とする報告が多い[5][13]．HIV/HCV重複感染者におけるARFIと組織学的線維化の相関についてはいまだ報告はなく，今後の検討が必要と思われる．

近年，HIV/HCV重複感染者に対する肝移植の成績が欧米を中心に報告されており，その成績は3年生存率が60％程度であった[14]．これはHCV単独感染よりやや不良であるが徐々に改善が見られている．近年ではRaltegravirのようにTacrolimusとの相互作用を認めない抗HIV薬も登場しており，今後移植後の免疫抑制剤の調整が容易となることでさらなる成績の改善が期待される[15][16]．

結　語

ARFI elastographyは，HIV/HCV重複感染者の肝線維化や肝予備能評価のツールとして，肝疾患病期診断に有用であり，肝移植も含めた適切な治療の選択の判断材料となると思われた．

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本論文内容に関連する著者の利益相反
：なし

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Acoustic radiation force impulse elastography for liver disease staging in human immunodeficiency virus and hepatitis C virus co-infection

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Background : Survival of human immunodeficiency virus (HIV)-infected patients has improved due to the widespread use of anti-retroviral therapy. However, mortality has increased when HIV-infected patients are co-infected with hepatitis C virus (HCV), and the liver disease in such patients is rapidly progressive compared with that in HCV monoinfected patients. Therefore, accurate staging of the liver disease is critical when determining appropriate treatment. Aim : To clarify the efficacy of acoustic radiation force impulse (ARFI) elastography for the evaluation of liver fibrosis and hepatic functional reserve in HIV/HCV co-infected patients. Methods : The correlation of shear wave velocity (Vs), measured by ARFI elastography, with liver fibrosis or hepatic functional reserve was analyzed. Results : Vs was significantly correlated with platelet count, splenic volume, hyaluronic acid, type IV collagen, and LHL15 (receptor index : uptake ratio of the liver to the liver plus heart at 15min) in 99mTc-GSA (technetium-99m-diethylenetriaminepentaacetic acid-galactosyl human serum albumin) scintigraphy. Conclusion : ARFI elastography was useful for the staging of liver disease in HIV/HCV co-infected patients and it facilitated minimally invasive and accessible evaluation of fibrosis and functional reserve.