

Table 1 Baseline patient characteristics and conditions

Patient no.	1	2	3	4	5
Sex	Male	Male	Female	Female	Female
Age at inclusion (years)	66	55	44	38	32
Age at onset (years)	35	35	25	8	7
Observation period (weeks)	104	104	104	104	104
Symptom at onset (weakness)	Lower extremities	Lower extremities	Lower extremities	Neck	Lower extremities
Ventilator since (age in years)	58	49	36	32	21
Duration of ventilator use (years)	8	7	8	6	11
Wheelchair-bound	Complete	Complete	Complete	Complete	Partial
Ventilator use (h/day)	24	10 (at night)	24	22	10 (at night)
Tracheotomy (age in years)	None	48	36	None	None
Wheelchair since (age in years)	51	48	36	36	29
Genotype	c.1585–1586TC > GT(p.S529V) homozygote	c.546 G > T(p.T182T) homozygote	c.307 T > C(p.C103R)/ c.546 G > A(p.T182T)	c.1309 C > T(p.R437C)/ c.1857 C > G(p.S619R)	c.546 G > T(p.T182T)/ c.1798 C > T(p.R600C)
Enzyme activity ^a	1.2 (M)	0.6 (M)	1.88 (M)	0.46 (F)	3.8 (M)
Complications	Diabetes mellitus	Atrial fibrillation	Interstitial pneumonia pneumothorax	Pneumothorax subcutaneous/ mediastinal emphysema	—
Pathology	Myopathic changes	Myopathic changes	Myopathic changes	Myopathic changes	Myopathic changes
AcP- and PAS-positive vacuoles	Few	Scattered	Scattered	Stained for acid phosphatase	Many

^a (M) Muscle (nmols 4MU/mg/h) (14.6±4.4), (F) fibroblast (mmol/pg protein) (161±32.4)

and occlusal force were provided by the manufacturer, and three healthy volunteers were tested as controls for pinch power [see Table in Electronic Supplementary Material (ESM)]. Blood cell counts and blood chemistry tests were conducted regularly. We interviewed patients and their families about activities of daily living (ADL). IgG antibodies to rhGAA were measured regularly by enzyme-linked immunosorbent assay (ELISA) (Kishnani et al. 2006).

Annual changes in quantitative parameters (pulmonary function tests, grip power, pinch power, and occlusal force) were calculated for the first and second years by subtracting old data from new data. Changes were analyzed with the Mann-Whitney *U* test. Statistical analyses were performed with SPSS for Macintosh (version 18, SPSS, Chicago, IL).

Results

Case presentation

Patient 1 suffered from limb muscle atrophy at age 35. He could not climb stairs and visited us at age 44. Muscle biopsy and acid maltase activity revealed Pompe disease. He lost ambulation at age 51. He experienced dyspnea, and %VC was

22.4 at age 58. Nocturnal NIV was initiated; he required continuous NIV from age 63 and was able to remove the NIV mask for <1 min before ERT. ERT was initiated at age 66. After 6 months of ERT, the patient was able to stop NIV for 9 min, allowing for a much easier transfer of the patient from car to wheelchair by the caregiver. This also provided the caregiver more than 5 min for shaving and/or cleaning the patient's face, compared to the 1-min limit before ERT.

Patient 2 had difficulty climbing stairs from age 36. He experienced dyspnea in the supine position at age 47 and visited a physician due to morning headache and severe dyspnea. He presented with pneumonia and CO₂ narcosis; nocturnal oxygen therapy was initiated after recovery. A muscle biopsy led to the diagnosis of Pompe disease. The patient lost ambulation during hospitalization. He visited us at age 50 and nocturnal NIV was initiated. The patient had difficulty lying down in the supine position without NIV before ERT. After ERT was initiated at age 55, he was able to lie down for 10 min at 24 weeks of ERT and for 60 min at 48 weeks without respiratory support. He was also less fatigued in the afternoons and able to drive alone for 2 h after 40 weeks.

Patient 3 noticed gait disturbance at age 22, visited a neurologist at age 26, and was diagnosed with limb-girdle

muscular dystrophy. At age 36, she complained of morning headache and drowsiness; she was intubated and tracheostomy was performed due to CO₂ narcosis and pneumonia. The patient lost ambulation during hospitalization and had recurrent pneumothorax and pneumonia. She visited us at age 39 and was diagnosed with Pompe disease by muscle biopsy and GAA activity. Recurrent pneumonia due to *Pseudomonas aeruginosa* required hospitalization with intravenous antibiotics once every 2 months before ERT. After ERT was initiated at age 44, she developed a mild fever of <38°C twice at 12 and 36 weeks after ERT, and recovered without antibiotics. She was able to open a plastic bottle unaided after 24 weeks of treatment, a task that could not be completed for 8 years prior to treatment. She was able to easily move from bed to wheelchair after 44 weeks. She also noticed less fatigue during meals, was able to pull up both legs unaided after 2 years of ERT, and could put on socks while sitting in the wheelchair.

Patient 4 had proximal weakness at age 15. She was referred to a neurologist and found to have high creatine kinase levels (1,256 U/L) and mild respiratory dysfunction (%VC: 77) at age 21. She was diagnosed with late-onset Pompe disease by muscle biopsy and fibroblast acid maltase activity. At age 32, she experienced dyspnea and initiated NIV during the night. At age 35, her %VC decreased to 18.9 and she required NIV all day. She began to use a wheelchair due to exertional dyspnea. At age 36, she presented with a right-sided pneumothorax, and %VC decreased to 15.8. She was able to turn off NIV only for 5 min to take a bath and could not comb her hair by herself before ERT. At 24 weeks after ERT initiation, pinch power increased from 48.4 N to 55.2 N, and she was able to stand with less effort. At 64 weeks of treatment, she was able to switch off NIV for 15 min while taking a bath and combing her hair. However, she experienced severe dyspnea and recurrent pneumothorax after 64 weeks of ERT and became fully dependent on NIV thereafter. She developed pneumothorax and emphysema at 80 weeks of ERT again and was completely bedridden and required cuirass ventilation in addition to NIV. She was also treated with parenteral hyperalimentation, including standard calorie and protein, for approximately 1 month due to inability to eat caused by dyspnea. After recovery from severe emphysema, she remained bedridden and consequently lost ambulation. Occlusal force was also lower after parenteral hyperalimentation.

Patient 5 could not stand without hand support and visited a pediatrician at age 13 and visited us and muscle biopsy and acid maltase activity. She initiated NIV at age 21 and required a wheelchair at age 29. After ERT was initiated at age 31, she found it easier to expectorate sputum through coughing than before ERT and could move her hip from floor to chair unaided after 44 weeks, which had been impossible for several years. She also noticed alleviation of

lumbago, and after three doses of ERT, she was able to discontinue non-steroidal anti-inflammatory drugs (NSAIDs) used for back pain. The patient suffered from emaciation before ERT and was advised that this could not be resolved, but she gained 3 kg of body weight after ERT. At present, she can drive 2.5 h to go to the hospital every 2 weeks, which was impossible before ERT due to fatigue and back pain.

ERT-induced changes

Table 2 lists the results of clinical and laboratory tests before and after ERT. The mean duration of follow-up was 104 weeks. Grip power (Fig. 1a) and pinch power (Fig. 1b) showed gradual improvement in all patients. In patient 4, both grip and pinch powers continued to improve until 60 weeks after ERT initiation, but deteriorated thereafter. Occlusal force improved markedly in patients 1 and 3 (Fig. 1c), but deteriorated in patient 4. No changes in MMT were noted in any of the patients. GMFM improved slightly in patients with a score of >25, while it remained unchanged in those with a score of <5. After initiation of ERT, all patients, except patient 4 who had severe emphysema and pneumothorax, showed improvement in %VC (Fig. 2a), PEF (Fig. 2b), PCF (Fig. 2c), %FVC (Fig. 2d), and/or FEV1.0 (Fig. 2e).

Creatine kinase (CK) levels decreased during treatment in patients 2, 4, and 5, and particularly in patient 4 (Table 2). CK levels were normal in patients 1 and 3 at the commencement of treatment and did not show marked changes during and after treatment. Body weight [44.4 (17.0) to 43.6 (16.1) kg, $p=0.93$] and lean body mass [25.8 (7.9) to 25.8 (10.2) kg, $p=0.99$] did not change.

Changes in the first year were greater than in the second year (Table 3). Most data were not available for patient 4 at the first year evaluation because bed rest was required for pneumothorax therapy. Changes in %VC, %FVC, PEF, PCF, pinch power, and occlusal force were greater in the first year than in the second year ($p<0.05$). While %VC, %FVC, PEF, PCF, pinch power, and occlusal force significantly changed in the first year after ERT, changes in these parameters were not significant in the second year.

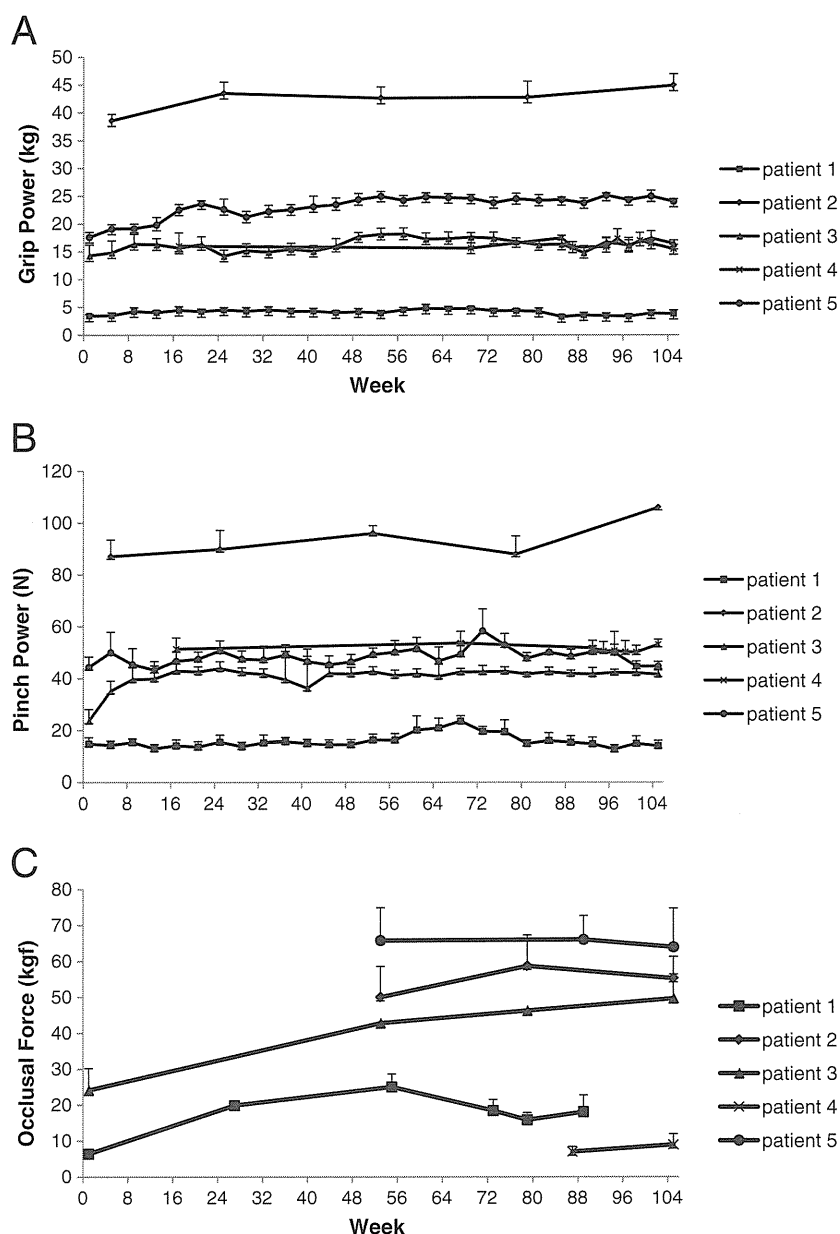
IgG antibody against Myozyme® was measured in patients 1, 3, 4, and 5 (see figure in ESM). All patients were IgG antibody positive at around weeks 12 to 16, but patients 4 and 5 became negative thereafter. Furthermore, IgG antibody titers increased to a peak level in patient 3, and increased in patient 1 to 25,600. The antibody titer of patient 2, measured once at 108 weeks after ERT, was negative. Only patient 3 developed a skin rash immediately after Myozyme® infusion at 12 weeks, but the rash disappeared completely after treatment with an antihistamine. Other patients did not experience any infusion-related reactions.

Table 2 Results of clinical and laboratory tests before and after ERT

		Patient 1			Patient 2			Patient 3			Patient 4			Patient 5		
		Pre	1 year	2 year	Pre	1 year	2 year	Pre	1 year	2 year	Pre	1 year	2 year	Pre	1 year	2 year
MMT	Neck flexion	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
	Shoulder flexion	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
	Shoulder abduction	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
	Elbow flexion	1	1	1	3	3	4	3	3	3	4	4	4	3	4	4
	Elbow extension	1	1	1	4	4	4	4	4	4	4	4	4	3	3	3
	Wrist flexion	4	4	4	5	5	5	5	5	5	4	4	4	5	5	5
	Hip flexion	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
	Knee flexion	1	1	1	2	2	2	2	2	2	3	3	3	2	2	2
	Knee extension	1	1	1	2	2	2	2	2	2	3	3	3	2	2	2
	Ankle flexion	1	1	1	5	5	5	2	2	2	4	4	4	5	5	5
Body weight (kg)		44	43	43	73.0	70	69	42	40	42	33	31	31	30	31	33
Lean body mass (kg)		23.9	22.6	22.6	39.8	39.8	39.8	23.0	24.4	24.4	21.1	NT	19.9	21.4	22.2	22.2
Pulmonary function	%VC	4.9	10.7	9.6	45.6	62.0	67.2	12.1	15.4	17.3	17.6	NT	9.2	13.1	19.5	21.4
	%FVC	0.0	26.8	7.7	46.3	51.2	66.1	9.3	12.5	16.1	14.2	NT	7.0	10.3	17.7	20.4
	FEV1.0	0.00	0.62	0.21	1.52	1.78	1.99	0.24	0.49	0.41	0.32	NT	0.14	0.29	0.50	0.55
	PEF (L/s)	0.38	0.93	0.50	3.72	6.40	5.49	0.46	0.63	0.70	0.58	NT	0.25	1.24	1.63	1.70
	PCF (L/s)	0.34	0.74	0.69	4.87	7.26	7.16	0.60	0.82	0.85	1.52	NT	0.86	1.19	1.96	2.17
Grip power (kg)		3.4	4.1	4.4	39.6	42.7	44.1	14.2	17.4	16.5	17.0	18.0	17.7	17.5	23.9	25.0
Pinch power (N)		14.7	21.1	15.5	81.9	96.1	98.8	23.6	42.4	42.5	48.3	56.3	53.0	44.3	48.5	47.3
Occlusal force (kgf)		6.4	15	15.9	NT	50.0	55.2	24.1	42.8	46.3	16.4	NT	8.4	NT	65.8	64.0
GMFM		NT	3	3	NT	25	31	NT	5	5	NT	56	59	NT	32	35
CK (IU/l)		47	36	50	238.0	132	10	166	132	100	621	NT	154	241	161	166
Barthel index		20	20	20	75.0	75	75	55	55	55	80	80	70	80	80	80

%VC Percent vital capacity, %FVC percent force vital capacity, FEV1.0 forced expiratory volume in the first second, PEF peak expiratory flow, PCF peak cough flow, GMFM gross motor function measure, CK creatine kinase, NT not tested

Fig. 1 Effects of ERT on grip power (a), pinch power (b), and occlusal force (c). Each data point represents the average of three bilateral measurements. ERT improved all of these parameters in four of five patients (with the exception of patient 4). Data are presented as mean \pm SEM

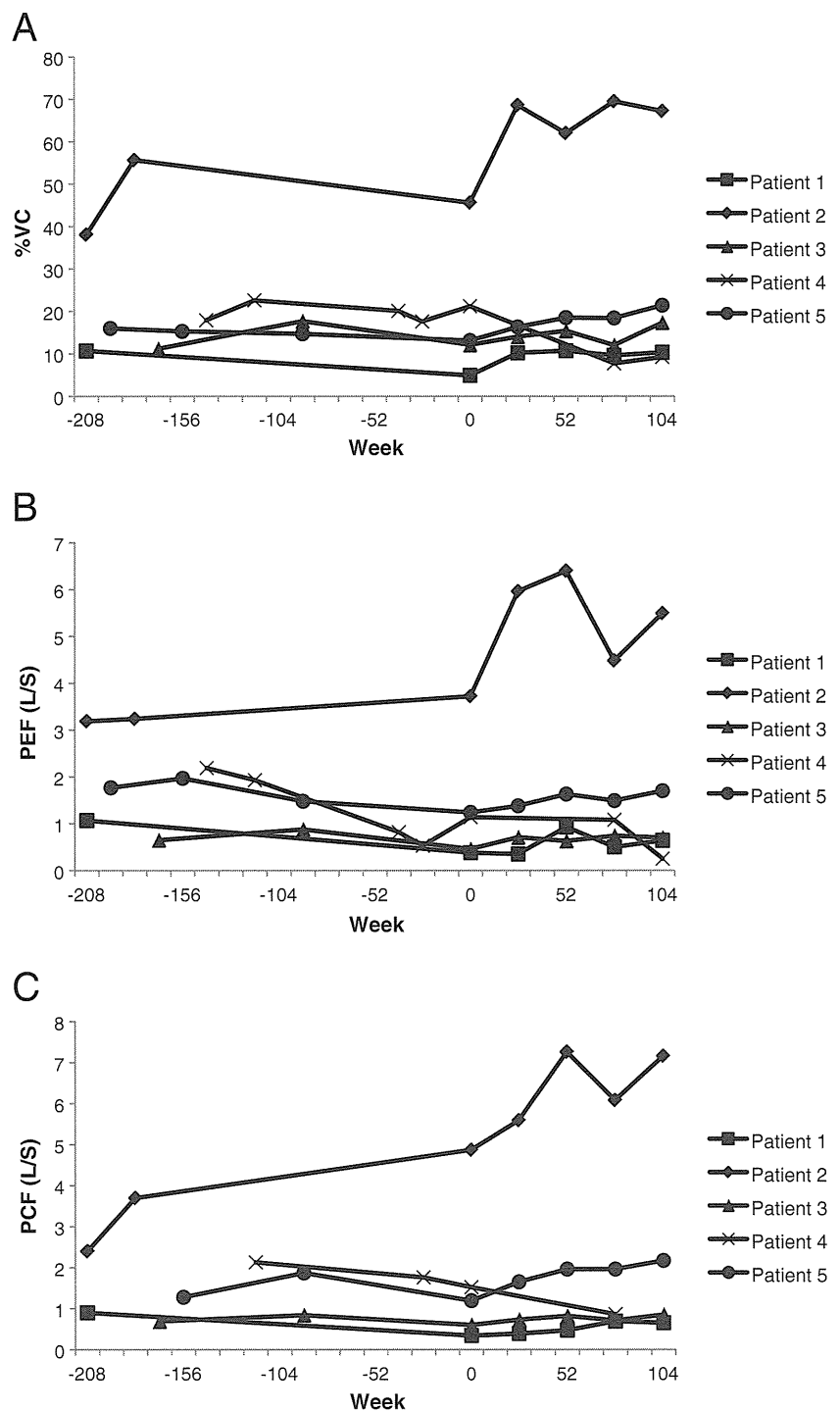


Discussion

ERT is often difficult to initiate in the early stages of subclinical GSDII or in early-stage GSDII because the disease is difficult to diagnose due to heterogeneity in clinical presentation and overlapping symptoms with other neuromuscular diseases. Accordingly, it is important to gain an understanding of ERT efficacy in patients with advanced GSDII. Our study demonstrated that ERT is effective for 2 years without severe complications in adult patients who have advanced GSDII and are dependent on ventilator and wheelchair support. During the 2 years of ERT, all patients showed some improvements in muscle and pulmonary function and ADL.

All parameters improved during the first year of treatment. While the results of various tests in the second year were lower than those recorded at the end of the first year, they were still better than before ERT initiation. Although the rate of improvement differed widely among patients, our results indicate that ERT is more effective in the first year and it maintains its efficacy for 2 years. At present, there is no explanation for the better outcome in the first year compared to the second year. Taking into consideration the muscle pathology associated with GSDII, intracellular accumulation of large amounts of glycogen may cause displacement, replacement, or compression of normal cellular organelles. Thus, ERT may normalize cell function by reducing such accumulation in surviving

Fig. 2a–d Effects of ERT on respiratory function. Percent vital capacity (a), peak expiratory flow (b), peak cough flow (c), percent force vital capacity (d), and forced expiratory volume in the first second (e). Note the low values of all parameters prior to ERT and their improvement after ERT. The improvement is more pronounced in patients with spared baseline functions

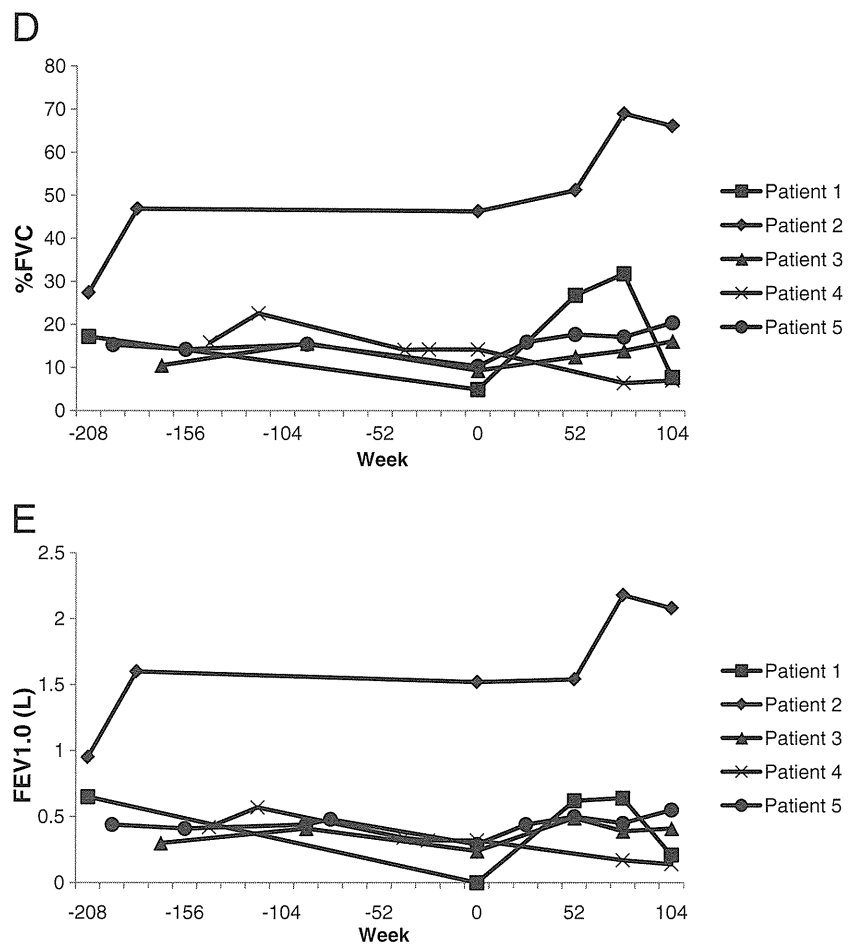


myotubes, followed by a gradual regeneration of myofibers. The observed effects of ERT may represent its acute effect on intracellular glycogen accumulation.

Younger or milder cases, including those presented in a randomized controlled study of ERT, showed a greater improvement over advanced cases (Winkel et al. 2004; Pascual-Pascual et al. 2006; van der Ploeg et al. 2010). Van der Ploeg and colleagues reported on ambulant patients

whose %VC was greater than 30 (van der Ploeg et al. 2010). In this clinical study, ERT elicited significant improvements in walking distance and stabilization of pulmonary function. On the other hand, efficacy of ERT in patients with advanced GSDII seemed to be milder or partial. A case report of a 67-year-old wheelchair-bound woman described alleviation of muscle symptoms following ERT, although pulmonary function tests showed no improve-

Fig. 2a–d (continued)



ment, suggesting cases with no respiratory recovery (Merk et al. 2007). Furthermore, one open-label observational study of ERT in 44 late-onset GSDII patients showed that both motor function tests and CK levels improved, and pulmonary function stabilized (Strothotte et al. 2010). Orlikowski et al. reported a 52-week follow-up of five patients (Orlikowski et al. 2011) with respiratory dysfunction as severe as in our patients, and respiratory and motor functions in all patients improved somewhat. Our data further these findings by suggesting that the improvements continue through the second year of ERT and that ERT is beneficial even for patients with advanced-stage Pompe disease.

Only patient 4 failed to show a clear recovery at the end of the follow-up period. However, grip and pinch powers increased in this patient at 60 weeks of ERT. Immobility and suspension of oral feeding resulted in reduction of muscle power, particularly in the masseter muscles. Pneumothorax also influenced the improvement in pulmonary function. Thus, we speculate that the small improvement was offset by the negative influence of pneumothorax. Because patients in similar condition at the beginning of the study responded to treatment (patients 3 and 5), one can rule out any effects of age, body weight, lean body mass,

and lung dysfunction on the prognosis. Variability in the response to treatment may reflect individual differences in disease severity at treatment initiation and rate of disease progression.

The benefits conferred by ERT may not be adequate when considering ERT costs, as none of the patients exhibited an improvement in Barthel index; however, observation before ERT indicated gradual deterioration before the therapeutic intervention was initiated (Table 2). In one study, dramatic changes did not occur at the advanced stage, although certain benefits were evident (Orlikowski et al. 2011). However, we speculate that patient conditions will deteriorate if ERT is terminated after the first year, a period showing the greatest improvements. Serial pulmonary function tests indicated that the respiratory function of our patients will sequentially deteriorate (Fig. 2).

Based on our assumption that therapeutic effects of ERT cannot be measured by MMT or morbidity function in 6-min walk tests, we attempted to measure muscle power in relatively spared functions. Occlusal force is known to decrease in parallel with disease progression in Duchenne muscular dystrophy (DMD) (Ueki et al. 2007). Occlusal,

Table 3 Annual changes in parameters

Years	%VC		%FVC		FEV ₁ (L)		PEF (L)		
	1	2	1	2	1	2	1	2	
Patient 1	5.8	-1.1	4.7	21.9	-19.1	2.8	0.6	-0.4	0.21
Patient 2	16.4	5.2	21.6	4.9	14.9	19.8	0.3	0.2	0.47
Patient 3	3.3	1.9	5.2	3.2	3.6	6.8	0.248	1.000	0.248
Patient 4 ^a	Not tested	Not tested	0.057	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested
Patient 5	5.4	2.9	8.3	7.4	2.7	10.1	0.21	0.05	0.26

PCF (L)	Grip power (kg)		Pinch power (N)		Occlusal force (kgf)				
	1	2	1	2	1	2			
1	2	1 + 2	P: 1 vs 2	P: 1 vs 1 + 2	P: 2 vs 1 + 2	P: 1 vs 1 + 2	P: 2 vs 1 + 2	P: 1 vs 1 + 2	P: 2 vs 1 + 2
0.4	-0.05	0.35	0.7	0.3	1.0	6.4	-5.6	0.8	8.6
2.39	-0.1	2.29	3.1	1.4	4.5	14.2	2.7	16.9	50
0.22	0.03	0.25	0.028	0.885	0.020	3.2	-0.9	2.3	18.7
Not tested	Not tested	0.7	6.4	1.1	7.5	0.083	0.905	0.142	18.7
0.77	0.21	0.98	Not tested	0.7	6.4	1.1	7.5	0.016	22.2
			0.69	0.009	0.021	0.021	0.886	0.021	0.886
			8	-3.3	4.7	Not tested	Not tested	Not tested	Not tested
			4.2	-1.2	3	65.8	-1.8	64	64

%VC Percent vital capacity, *%FVC* percent force vital capacity, *FEV₁* forced expiratory volume in the first second, *PEF* peak expiratory flow, *PCF* peak cough flow
^aPatient 4 could not be evaluated at 1 year after ERT initiation due to severe pneumothorax

grip, and pinch powers were relatively spared in all patients, except patient 1. Four of five patients could write, use utensils, fasten a button, or bite foods as efficiently as healthy people, although their data revealed some decrements compared to normal controls. Cranial muscle involvement is thought to be rare, but we found that occlusal force was mildly reduced in patients with advanced Pompe disease. This suggests that occlusal force is a sensitive parameter for assessing the response to ERT.

Conclusions

The present study showed that ERT improved respiratory function and muscle power for 2 years even in adult patients with advanced GSDII. Improved muscle strength resulted in better ADL and quality of life during the long follow-up period. Taking our results into consideration, we recommend the initiation of ERT in GSDII patients, irrespective of age and disease severity.

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Significance of Platelet Count in the Outcomes of Hepatectomized Patients with Hepatocellular Carcinoma Exceeding the Milan Criteria

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Abstract

Background The appropriate treatment strategy for advanced hepatocellular carcinoma (HCC) that does not meet the Milan criteria (MC) is unclear. The aim of this study was to determine the significance of surgical treatment for such patients.

Study design From January 1990 to December 2007, 151 patients with HCC exceeding MC who underwent curative surgical treatment were enrolled. Survival and recurrence data and clinicopathological factors were examined. Prognostic factors were analyzed to identify those that contributed to improved surgical outcomes retrospectively.

Results After the initial hepatectomy, the overall 3-, 5-, and 10-year survival rates were 73%, 55%, and 33%, respectively, for the 151 patients in this study; the corresponding disease-free survival rates were 36%, 30%, and 17%, respectively. A platelet count under $10^5/\text{mm}^3$, multiple tumors, and liver cirrhosis of noncancerous tissue were adverse survival and disease-free survival factors by univariate analysis. Platelet count was an independent prognostic factor by multivariate analysis. The 3-, 5-, and 10-year overall survival rates of HCC exceeding MC in patients whose platelet count was $10^5/\text{mm}^3$ or greater reached 76%, 65%, and 44%, respectively, and were comparable with those that met MC (86%, 68%, and 37%, respectively).

Conclusions Hepatectomy for patients with advanced HCC exceeding MC improves survival, especially for patients with a sufficiently high platelet count, although recurrence rates after initial hepatectomy are high.

Keywords Hepatocellular carcinoma · Milan criteria · Platelet count · Advanced · Hepatectomy · Prognosis

MC Milan criteria
ICGR15 Indocyanine green retention rate at 15 min
RFA Radiofrequency ablation
LDLT Living donor liver transplantation

Abbreviations

HCC Hepatocellular carcinoma
TACE Transarterial chemoembolization
OLT Orthotopic liver transplantation

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. There are various options to treat HCC, including partial hepatectomy, percutaneous ablation therapy, and transarterial chemoembolization (TACE). However, the resulting prognosis of HCC remains inadequate, despite technical refinements in these treatments, due to the high incidence of recurrence of HCC.^{1,2}

Orthotopic liver transplantation (OLT) is the preferred treatment for patients with cirrhosis and early HCC per the

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Milan criteria (MC: defined as a solitary HCC of a size <5 cm or 2 or 3 tumors <3 cm with no gross vascular invasion).³ In patients with early HCC, such as within MC, as long as liver function is preserved, liver resection effects an overall 5-year survival rate that is comparable with that of liver transplantation, with minimal morbidity and mortality.^{4–6}

The treatment strategy for advanced HCC exceeding MC has not been discussed sufficiently. Due to advanced tumor status, ablation therapy cannot be the first treatment, nor can OLT. Although hepatectomy or TACE is used to treat advanced HCC patients, the 5-year overall survival rate after curative hepatectomy for advanced HCC (tumor size, >5 cm) is 30% to 35%, and its recurrence after hepatectomy is unavoidable.^{7,8}

We retrospectively analyzed the impact of hepatectomy on tumor control in patients with HCC exceeding MC. In this study, we examine the rationale for partial hepatectomy as an initial treatment and discuss the development of other strategies for recurrent HCC.

Methods

Patient data began to be collected prospectively by our program in 1986. Between January 1990 and December 2007, 781 consecutive adult patients underwent hepatectomy for HCC at Hiroshima University Hospital. A total of 651 consecutive HCC patients underwent curative intent hepatectomy in our hospital. Curative intent hepatectomy was defined as the removal of all recognizable tumors; patients with macroscopic vascular invasion in the first portal branch, portal vein trunk or hepatic vein trunk, and/or extrahepatic metastasis were excluded due to their poor prognosis.

Data for the remaining 622 HCC patients were included in the analysis. We divided the remaining patients into two groups: transplantable (meeting MC: single lesion with a maximum diameter <5 cm or three lesions with a maximum diameter <3 cm) and advanced (exceeding MC). We focused on the advanced group.

The indications and procedure for hepatectomy have been described.^{9,10} Briefly, Child–Pugh class C was regarded as a contraindication for hepatectomy. The decision to perform hepatectomy was made based on liver function and extent of tumor. Liver function was assessed according to Child–Pugh classification and indocyanine green retention rate at 15 min (ICGR15). In patients who lacked ascites and had normal bilirubin levels, ICGR15 became the chief determinant of resectability. For example, right hemihepatectomy could be tolerated if ICGR15 was in the normal range. One third of the liver parenchyma could be resected for patients with ICGR15 of 10–19%; segmentectomy was possible for patients with ICGR15 of 20–29%;

and limited resection was possible for patients with ICGR15 of $\geq 30\%$ (9.10).

Hepatectomy was indicated when all tumors could be resected with sufficient hepatic functional reserve, as determined by preoperative imaging. However, when the HCC tumors were hypovascular—suggesting that the tumor was well-differentiated HCC—and ≤ 2 cm and when the number of tumors ≤ 3 , percutaneous ablation therapies were preferable despite hepatectomy being feasible, depending on the tumor location in the liver. Clinicopathological findings were recorded according to the criteria of the Liver Cancer Study Group in Japan.¹¹ Liver cirrhosis was confirmed by histological examination of the resected specimen.

A modified Clavien classification was used to grade the severity of postoperative complications.¹² Grade I complications were defined as deviations from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic, or radiological intervention. Grade I complications also included wound infections that opened at the bedside.

Grade II complications were defined as those that required pharmacological treatment; blood transfusion and total parenteral nutrition were also included. Grade III complications were those that required surgical, endoscopic, or radiological intervention. Grade IV complications were life-threatening complications that required intermediate care/intensive care unit management. Grade V complications resulted in death. Operative mortality was defined as death within 30 days after surgery. In-hospital mortality was defined as death within the hospitalization period.

Postoperative follow-up evaluations consisted of a clinical physical examination, blood chemistry tests, and measurements of tumor marker levels, including alpha-fetoprotein and des-gamma-carboxy prothrombin, every month for 2 years. After 2 years, patients were assessed every 3 months. Patients were examined by abdominal ultrasonography every 3 months and by computed tomography every 6 months during the follow-up periods.

Our follow-up protocol included an evaluation by hepatologists to monitor cancer recurrence and the progress of chronic hepatitis or liver cirrhosis. When recurrence was noted in any of these examinations, patients underwent hepatic angiography. The patients were followed up regularly until December 31, 2008, and every patient was followed up for at least 6 months. All patients who experienced intrahepatic recurrence were managed with ablative therapy (radiofrequency ablation (RFA) or ethanol injection), TACE, or surgery, including liver transplantation, according to the same criteria as for the initial resection.

Statistical analyses were performed using unpaired Student's *t* test and chi-square test with Fisher's exact test. Overall survival and disease-free survival rates were calculated using the Kaplan–Meier method and compared using log-rank test. Disease-free survival was calculated, considering any death or recurrence as an event. A *P* value <0.05 was considered to be statistically significant. Statistical analysis was performed using StatView for Windows (Version 5.0; SAS Institute, Cary, NC, USA).

Results

As shown in Fig. 1, there were 151 patients with initially resectable advanced HCC who did not fulfill MC (i.e., exceeding MC) and 471 patients who met MC.

In the exceeding-MC group, the mean follow-up period for all survivors was 4.1±3.1 years (range, 0.5 to 14.5 years). Table 1 shows the patients' backgrounds. Overall operative mortality and in-hospital mortality rates were the same, i.e., 0.7% (*n*=1) in both conditions. The incidence of complications that developed after hepatectomy is also shown in Table 1. Thirty of the 151 patients (20%) had postoperative complications (Table 1). Nineteen of the 151 patients (13%) were grade III or more.

Figure 2a shows the survival rates of patients who underwent curative resection of HCC (meeting MC and exceeding MC). The survival rate of the exceeding-MC group was significantly lower than that of the group that met MC (*P*=0.030). The 3-, 5-, and 10-year survival rates

Table 1 Patients' background

	Number of patients	Percent
Age (year)		
≤60	57	38.4
>60	94	61.6
Gender		
Male	127	84.1
Female	24	15.9
Type of hepatitis virus		
Non-HCV	61	40.4
HCV	90	59.6
Child–Pugh grade		
A	129	85.4
B	22	14.6
Type of hepatectomy		
Limited resection	82	54.3
Segmentectomy or more	69	45.7
Operative mortality: yes	1	0.7
In-hospital mortality: yes	1	0.7
Postoperative complications ^a : yes	30	19.9
Grade I, II	11	7.3
Grade III or more	19	12.6

^a Postoperative complications was defined as any event satisfying the criteria advocated by Dindo et al.¹²

of the exceeding-MC group were 77%, 55%, and 33% and 86%, 68%, and 37% in those that met MC, respectively. The 3-, 5-, and 10-year disease-free survival rates of the exceeding-MC group were 36%, 30%, and 17% and 47%, 30%, and 13% in those that met MC, respectively (Fig. 2b).

Table 2 summarizes the results of the univariate analysis according to clinicopathological factors. A platelet count <10⁵/mm³ (*P*<0.001), multiple tumors (*P*=0.012), and cirrhosis of noncancerous tissue (*P*=0.035) were significant adverse prognostic factors for overall survival. Similarly, a platelet count <10⁵/mm³ (*P*=0.001), multiple tumors (*P*=0.005), and cirrhosis of noncancerous tissue (*P*=0.020) were significant adverse prognostic factors for disease-free survival.

By multivariate analysis, a platelet count <10⁵/mm³ (*P*=0.007) was found to be an independent adverse prognostic factor for overall survival (Table 3), and the 3-, 5-, and 10-year overall survival rates of patients with HCC exceeding MC whose platelet count was ≥10⁵/mm³ were 76%, 65%, and 44%, respectively, comparable with the group that met MC (86%, 68%, and 37%, respectively; Fig. 2a). A platelet count <10⁵/mm³ (*P*=0.039) was also an independent adverse factor for disease-free survival (Table 3).

Out of 151, a total of 107 (71%) patients with HCC exceeding MC experienced a recurrence after the initial hepatectomy. Table 4 shows the patterns of cancer recurrence

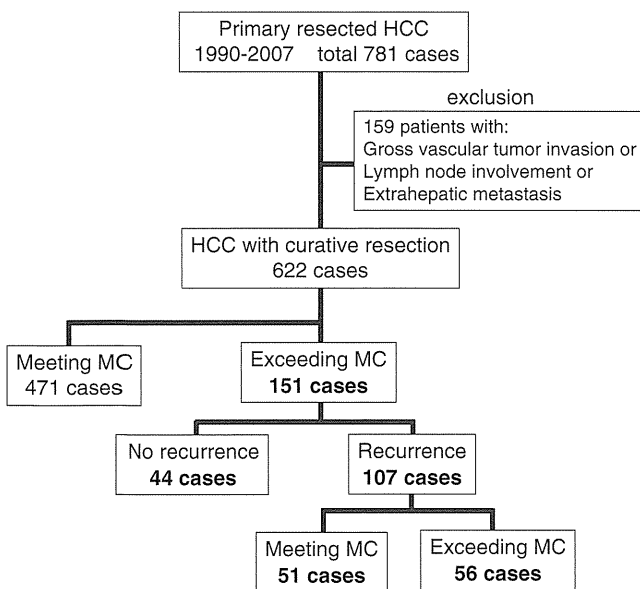


Fig. 1 Overview of outcomes of patients with primary resected hepatocellular carcinoma (HCC). The number of HCC patients who underwent curative resection was 622, subdivided by the Milan criteria (MC)

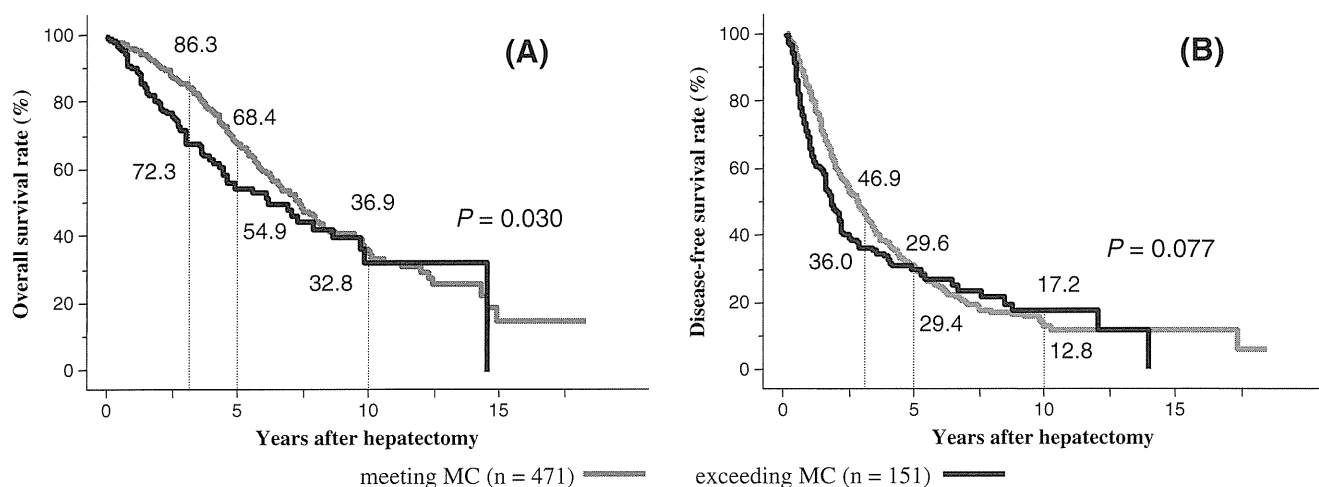


Fig. 2 Survival and disease-free survival curves of patients who received curative resection of HCC that met (471 patients) or were exceeding (151 patients) MC. (A) The 3-, 5-, and 10-y survival rates of patients exceeding MC were 72.3%, 54.9%, and 32.8%, respec-

tively, and 86.3%, 68.4%, and 36.9%, respectively, in those who met MC. (B) The 3-, 5-, and 10-y disease-free survival rates of patients exceeding MC were 36.0%, 29.4%, and 17.2%, respectively, and 46.9%, 29.6%, and 12.8%, respectively, in those who met MC

and compares the consequent treatment details between patients whose platelet counts were $\geq 10^5/\text{mm}^3$ and $< 10^5/\text{mm}^3$. The rate of HCC recurrence was significantly lower in patients whose platelet count was $\geq 10^5/\text{mm}^3$; 76 (66%) of such patients experienced a recurrence of HCC after hepatectomy, as compared to 31 (89%) patients whose platelet count was $< 10^5/\text{mm}^3$ ($P=0.009$). Further, regarding the pattern of recurrence, the proportion of patients who had a recurrence of HCC that met MC was significantly higher in patients with a platelet count $\geq 10^5/\text{mm}^3$ than those with a platelet count of $< 10^5/\text{mm}^3$ (51% vs. 39%; $P<0.001$).

The proportion of patients who received curative treatment for the first recurrence, such as repeat hepatectomy and local ablation therapy, had significantly higher platelet counts, i.e., $\geq 10^5/\text{mm}^3$ (44% vs. 23%; $P=0.047$).

Of the 107 patients who experienced a recurrence, 51 (48%) met MC and 56 (52%) were exceeding MC, including extrahepatic recurrence (Fig. 1). The 3- and 5-year survival rates after recurrence were significantly superior in patients with a recurrence that met MC (71% and 40%, respectively) than those exceeding MC (17% and 9%) ($P<0.001$; Fig. 3).

Table 5 shows the details of the treatments for recurrences after hepatectomy. The proportions of patients who received ablation therapy or repeat hepatectomy after recurrence was higher in patients with a recurrence that met MC than those exceeding MC ($P=0.001$). Two patients with a recurrence that met MC, who underwent salvage living donor liver transplantation (LDLT), did not have a recurrence after liver transplantation at the 2- and 3-year follow-up, respectively. One patient with a recurrence that was exceeding MC, and who underwent salvage LDLT, experienced a recurrence of HCC within 1.5 years.

Discussion

The ultimate goal of a treatment for HCC is to prolong survival by eradicating malignant lesions while preserving hepatic function. Surgical resection, by partial hepatectomy or total hepatectomy followed by OLT, is the standard treatment with a curative intent.¹³ The resectability and choice of procedure depend on many factors, including baseline liver function, absence of extrahepatic metastasis, size of residual liver, availability of resources (including liver grafts), and expertise of the surgical team.

Although hepatic resection, ablation therapy, and liver transplantation are accepted, effective treatments for patients with cirrhosis and early HCC, the proper strategy for advanced HCC has not been established. Therefore, we studied HCC patients who were exceeding MC—who are not eligible for OLT as the initial treatment. We investigated the impact of hepatectomy on outcomes of HCC that exceeded MC and examined the rationale of hepatectomy as an initial treatment for HCC exceeding MC.

In our series, the 5- and 10-year survival rates of patients with HCC exceeding MC were 55% and 33%, respectively, comparable with Kamiyama et al.¹⁴ We also identified significant prognostic factors of patients with HCC exceeding MC who underwent hepatectomy: platelet count, tumor number, and cirrhosis. Moreover, our multivariate analysis revealed that platelet count was the sole independent prognostic factor in these HCC patients.

The prognosis of such patients after hepatectomy was clearly stratified by platelet count, which is typically predictable by preoperative laboratory tests. The 3-, 5-, and 10-year overall survival rates of patients with HCC exceeding MC, whose platelet count was $\geq 10^5/\text{mm}^3$, were

Table 2 Overall and disease-free survival rates of patients with HCC exceeding MC according to clinicopathological factor

		Overall survival (%)				Disease-free survival (%)			
		3-year	5-year	10-year	<i>P</i> value	3-year	5-year	10-year	<i>P</i> value
All cases (<i>n</i> =151)		73	55	33		36	30	17	
Age (year)	≤60 (<i>n</i> =57)	69	58	38	0.873	35	28	17	0.977
	>60 (<i>n</i> =94)	74	53	30		37	30	18	
Gender	Male (<i>n</i> =127)	75	55	34	0.647	34	27	15	0.247
	Female (<i>n</i> =24)	61	56			45	45		
Type of hepatitis virus	Non-HCV (<i>n</i> =61)	71	65	36	0.498	46	39	25	0.054
	HCV (<i>n</i> =90)	73	50	32		29	22	12	
Total bilirubin (/mm ³)	<1.0 (<i>n</i> =125)	71	52	32	0.151	37	32	19	0.515
	≥1.0 (<i>n</i> =26)	78	72	36		30	19	9	
Platelet counts (/mm ³)	<10 ⁵ (<i>n</i> =35)	61	27		< 0.001	16	8		0.001
	≥10 ⁵ (<i>n</i> =116)	76	65	44		42	36	21	
ALT (IU/l)	<60 (<i>n</i> =106)	71	49	29	0.08	36	30	15	0.707
	≥60 (<i>n</i> =45)	77	70	45		36	27	18	
Alb (g/dL)	<3.5 (<i>n</i> =37)	73	52	41	0.995	42	31	23	0.55
	≥3.5 (<i>n</i> =114)	73	58	32		35	29	15	
ICG-R15 (%)	<20 (<i>n</i> =111)	72	57	43	0.303	40	32	20	0.467
	≥20 (<i>n</i> =39)	75	52			26	22		
Child–Pugh grade	A (<i>n</i> =129)	73	56	30	0.643	35	29	18	0.645
	B (<i>n</i> =22)	72	54	46		43	32	17	
AFP (ng/mL)	<400 (<i>n</i> =101)	77	56	31	0.905	33	26	16	0.495
	≥400 (<i>n</i> =48)	65	55	41		45	39	22	
Number of tumors	Single (<i>n</i> =60)	79	71	52	0.012	52	41	28	0.005
	Multiple (<i>n</i> =91)	68	45	23		26	22	12	
Tumor distribution	One section (<i>n</i> =77)	81	56	43	0.083	42	33	28	0.091
	more (<i>n</i> =74)	61	55	25		32	23	8	
Non-cancer tissue	Cirrhosis (<i>n</i> =52)	67	39	29	0.035	23	15	8	0.02
	Others (<i>n</i> =99)	75	65	38		42	36	23	
Preoperative TAE	Yes (<i>n</i> =102)	72	55	30	0.91	35	28	15	0.366
	No (<i>n</i> =45)	73	56	50		40	33	25	
Type of hepatectomy	Limited resection (<i>n</i> =82)	73	51	29	0.743	34	27	8	0.472
	Segmentectomy or more (<i>n</i> =69)	71	60	39		39	33	33	
Transfusion	Yes (<i>n</i> =20)	64	46	0	0.071	25	17	0	0.103
	No (<i>n</i> =131)	74	57	37		38	31	21	
Microscopic vascular invasion	Yes (<i>n</i> =74)	60	48	30	0.089	30	28	17	0.144
	No (<i>n</i> =77)	84	61	35		42	31	18	
Histologic grading	Well or moderate (<i>n</i> =122)	71	55	30	0.718	34	28	18	0.777
	poor (<i>n</i> =26)	74	52	43		42	31	12	
Diabetes mellitus	Yes (<i>n</i> =53)	73	58	34	0.929	39	30	17	0.493
	No (<i>n</i> =95)	72	52	31		33	29	17	
SF criteria	Meeting SF (<i>n</i> =59)	74	52	23	0.704	30	28	15	0.734
	Exceeding SF (<i>n</i> =92)	71	57	38		40	32	19	

HCC hepatocellular carcinoma, MC Milan criteria, ALT alanine aminotransferase, ICG-R15 indocyanine green retention rate at 15 min, AFP alpha-fetoprotein, SF San Francisco criteria (1 lesion <6.5 cm, 2–3 lesions each <4.5 cm with total diameter <8 cm)

76%, 65%, and 44%, respectively, comparable with those that met MC (86%, 68%, and 37%, respectively).

Hepatectomy should be the first-line treatment in patients with HCC exceeding MC whose platelet count is >10⁵/mm³.

Table 3 Results of Cox's proportional hazards analysis for overall and disease-free survival after hepatectomy

Variables	<i>P</i> value	Relative risk	95% CI
Overall survival			
Plt. Count: $<10^5/\text{mm}^3$	0.007	2.155	1.232–3.774
Number of tumors: multiple	0.103	1.65	0.903–3.021
Tumor distribution: more than one section	0.168	1.439	0.858–2.410
Transfusion: Yes	0.13	1.667	0.861–3.228
Microscopic vascular invasion: Yes	0.067	1.596	0.969–2.629
Non-cancer tissue: cirrhosis	0.488	1.207	0.709–2.058
Disease-free survival			
HCV infection: Yes	0.585	1.148	0.699–1.887
Plt. Count: $<10^5/\text{mm}^3$	0.039	1.653	1.025–2.667
Number of tumors: multiple	0.202	1.368	0.845–2.221
Tumor distribution: more than one section	0.098	1.412	0.939–2.123
Non cancer tissue: cirrhosis	0.274	1.277	0.824–1.979

In general, platelet count, which reflects the severity of portal hypertension, is a significant predictor of survival. Several studies have shown that platelet count is a risk factor for carcinogenesis from chronic hepatitis and for survival and recurrence of HCC after treatment, including liver resection.^{15–18} In fact, we observed that recurrence of HCC after hepatectomy decreased in patients whose platelet count was $\geq 10^5/\text{mm}^3$ and that the proportion of patients who experienced a recurrence of HCC that met MC was significantly higher in patients with a platelet count $<10^5/\text{mm}^3$. Further, the proportion of patients who underwent repeat hepatectomy or RFA as a curative treatment for a recurrence of HCC was significantly higher in patients whose platelet count was $\geq 10^5/\text{mm}^3$.

After resection with curative intent, many patients experience a recurrence, which is a significant cause of late death. In this study, the recurrence rate was high: 70.9% of patients with HCC exceeding MC were diagnosed

as having had a recurrence (mean follow-up, 4.1 years). Tumor number was an independent factor of disease-free survival, and the 3-, 5-, and 10-year disease-free survival rates were 51%, 41%, and 28%, respectively, even in patients with a single tumor.

The reported cumulative 5-year recurrence rates range from 50% to 100%.^{19–22} In our series, 107 (71%) of 151 patients with HCC exceeding MC experienced a recurrence of HCC, 51 (48%) of whom met MC. These results demonstrate that downstaging a recurrence to within MC was achieved by hepatectomy as an initial treatment for HCC exceeding MC. The proportion of patients who underwent repeat hepatectomy or local ablation therapy as a curative treatment for HCC recurrence was significantly higher in patients with a recurrence of HCC within MC versus exceeding MC. The outcomes after recurrence were significantly better in patients whose recurrence was downstaged to within MC compared with those who did

Table 4 Recurrent pattern and treatment of recurrent HCC after hepatectomy (comparison with platelet counts)

	Platelet counts $>10^5$ ($n=116$)	Platelet counts $<10^5$ ($n=35$)	<i>P</i> value
Cancer recurrence ^a : yes	76 (66)	31 (89)	0.009 ^c
Recurrent pattern ^b			$<0.001^c$
Meeting MC	39 (51)	12 (39)	
Exceeding MC or extrahepatic recurrence	37 (49)	19 (61)	
Treatments for recurrence ^b			0.047 ^c
Curative treatment	34 (44)	7 (23)	
Non-curative treatment	41 (55)	22 (70)	
Salvage liver transplantation	1 (1)	2 (6)	

Curative treatment included partial hepatectomy, local ablation therapy; non-curative treatment included transarterial chemoembolization, systemic chemotherapy, radiation therapy and conservative

HCC hepatocellular carcinoma, MC Milan criteria

^a Data are expressed as the number of patients (percentage of total patients)

^b Data are expressed as the number of patients (percentage of patients who had a recurrence)

^c Statistically significant difference

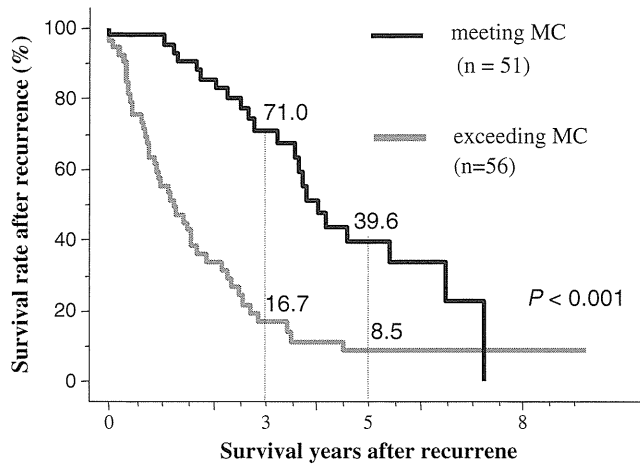


Fig. 3 Comparison of survival curves after recurrence of HCC according to recurrent pattern. The 3- and 5-year survival rates of patients with a recurrence that met MC were 71.0% and 39.6%, respectively, and 16.7% and 8.5%, respectively, in those who exceeding MC including extrahepatic recurrence

not achieve such downstaging. These results indicate that hepatectomy as an initial treatment is an important component of the treatment strategy for HCC exceeding MC.²³

With regard to the treatment of recurrent HCC patients, we reported that the more hepatectomy was repeated, the shorter the recurrence-free interval became, suggesting a limitation of repeat hepatectomy in curing recurrent HCC.²⁴ Liver transplantation has been discussed as the next strategy to treat tumor recurrences after initial hepatectomy in patients with advanced HCC. Several studies have reported salvage transplantation for recurrence after hepatectomy,^{6,25–27} suggesting that primary hepatectomy and salvage liver transplantation is a feasible and rational strategy for patients with small HCC that preserves liver function. In this series, of the patients who had recurrence

after resection for tumors exceeding MC, approximately 48% had recurrent tumors that were within MC. This result also indicates that approximately half of the patients with recurrence would be candidates for salvage liver transplantation after partial hepatectomy performed for downstaging to within MC. Salvage LDLTs were adopted for three patients, two of whom, who had a recurrence that met MC, did not experience a recurrence after salvage LDLT at the 2- and 3-year follow-up, respectively. Yao et al. and Ravaioli et al. reported that locoregional treatments, including RFA, were effective for downstaging prior to liver transplantation.^{23,28} In general, RFA was indicated for HCCs with diameters less than 3 cm. Although RFA may be effective for downstaging multiple small HCCs, its effectiveness may be limited in the case of downstaging large HCCs with diameters greater than 3 cm. Further studies are required to clarify the indications for the use of RFA and hepatectomy as downstaging modalities prior to liver transplantation.

A significant proportion of patients with HCC exceeding MC might benefit from liver transplantation. Mazzaferro et al. proposed an expansion of the indications for liver transplantation, using up to seven criteria.²⁹ Takada et al. demonstrated that LDLT could be safely extended to ≤ 10 tumors (all ≤ 5 cm in diameter and PIVA-II ≤ 400 mAU/mL) with acceptable outcomes.³⁰ Liver transplantation has been proposed as an initial treatment for patients with HCC exceeding MC whose platelet count is $< 10^5/\text{mm}^3$, although the extension of the indications of liver transplantation is restricted.

Conclusion

Hepatectomy for patients with HCC exceeding MC increases survival rates, especially for patients with sufficiently high platelet counts, although their recurrence rates

Table 5 Treatments for recurrent HCC after initial hepatectomy

Modalities	Recurrent pattern <i>N</i> (%) ^a		<i>P</i> value
	Meeting MC (<i>n</i> =51)	Exceeding MC or extrahepatic (<i>n</i> =56)	
Partial hepatectomy	10 (20)	5 (9)	<math>P < 0.001^b</math>
Salvage liver transplantation	2 (4)	1 (2)	
Resection of distant metastasis	0	3 (5)	
Percutaneous ablation therapy	18 (35)	8 (14)	
TACE	21 (41)	23 (41)	
Chemotherapy and/or radiation	0	10 (18)	
Non-treatment	0	6 (11)	

MC Milan criteria, TACE transarterial chemoembolization

^a Data are expressed as the number of patients (percentage of patients who had a recurrence of each group)

^b Statistically significant difference

after initial hepatectomy are high. Hepatectomy as an initial treatment is an important component of the treatment for HCC exceeding MC to downstage the recurrence to within MC.

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Impact of Pegylated Interferon Therapy on Outcomes of Patients with Hepatitis C Virus-Related Hepatocellular Carcinoma After Curative Hepatic Resection

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ABSTRACT

Background. Several published reports investigating the effects of interferon (IFN) therapy on survival and tumor recurrence after curative resection of hepatocellular carcinoma (HCC) have been inconclusive. The aim of this study is to investigate the efficacy of pegylated-IFN (peg-IFN) therapy after curative hepatic resection for HCC in patients infected with hepatitis C virus (HCV).

Methods. Data from 175 patients who underwent curative hepatic resection for HCC associated with HCV were retrospectively collected and analyzed; 75 patients received peg-IFN therapy after surgery, whereas 100 patients did not receive IFN therapy. To overcome biases resulting from the different distribution of covariates in the two groups, a one-to-one match was created using propensity score analysis. After matching, patient outcomes were analyzed.

Results. After one-to-one matching, patients ($n = 38$) who received peg-IFN therapy after surgery and patients ($n = 38$) who did not receive IFN therapy had the same preoperative and operative characteristics. The 3- and 5-year overall survival rates of patients who received peg-IFN therapy after hepatic resection were significantly higher than those of patients who did not receive IFN therapy ($P = 0.00135$). The 3- and 5-year overall survival rates were 100 and 91.7% and 76.6 and 50.6% in the peg-IFN group and non-IFN group, respectively. There was no significant

difference in disease-free survival between the two matched groups ($P = 0.886$).

Conclusion. Peg-IFN therapy may be effective as an adjuvant chemopreventive agent after hepatic resection in patients with HCV-related HCC.

Hepatic resection is a well-accepted therapy for hepatocellular carcinoma (HCC), but many patients show cancer recurrence and the cumulative 5-year HCC recurrence rate exceeds 70%.^{1–3} This high incidence of tumor recurrence after hepatic resection remains a major drawback. Some benefits of interferon (IFN) therapy on tumor recurrence and survival have been reported.^{4–10} IFN suppresses replication of hepatitis C virus (HCV) and exerts a tumoricidal effect on a number of tumors, including HCC.^{10,11} However, several randomized controlled trials (RCTs) have revealed inconclusive results regarding the effects of IFN on survival and tumor recurrence after curative resection or ablation of HCC, either because the effects were not statistically significant or because they were considered only with respect to defined subpopulations.^{12–15}

Recently, combination therapy consisting of pegylated interferon (peg-IFN) plus ribavirin (RBV) has been developed, and the effect of this combination has been reported to be higher than that of conventional IFN therapy.^{16,17} Peg-IFN has an extended serum half-life that provides viral suppression for 7 days, thus allowing weekly administration and enhanced clinical efficacy.¹⁷ Most Japanese patients infected with HCV are infected with HCV genotype 1b and have high viral load. Moreover, treatment with conventional IFN is complicated by a low sustained viral response (SVR) rate of 20–30%.^{18–20}

However, peg-IFN plus RBV combination therapy has good tolerability in Japanese patients with HCV and resulted in an SVR rate of approximately 40–50%.^{21–23} The impact of adjuvant immunotherapy with IFN after curative resection of HCC is debatable, and few studies have investigated the effects of peg-IFN plus RBV combination therapy on survival and recurrence after curative resection of HCC.

In the present study, we aim to investigate the impact of peg-IFN plus RBV combination therapy on survival and HCC recurrence after curative resection in patients infected with HCV.

PATIENTS AND METHODS

Patients and HCV Diagnosis

From June 2003 to June 2009, 370 HCC patients underwent hepatectomy as initial treatment at the Department of Gastroenterological Surgery, Hiroshima University Hospital, Japan. Of the 370 patients, 175 patients who were HCV RNA-positive/hepatitis B surface antigen-negative underwent curative hepatectomy. Of the 175 patients, 75 patients received IFN therapy after hepatectomy, and 100 patients did not receive any IFN therapy. Of the 75 patients who received IFN, 20 patients who received IFNs such as IFN- α or IFN- β were excluded. Of the 55 patients who received peg-IFN therapy, 43 patients who started peg-IFN within 9 months after curative resection were enrolled in this analysis. Twenty-four patients who had early recurrence of HCC within 9 months after surgery were excluded from the 100 patients who did not receive any IFN therapy, because these patients could lose the opportunity to receive IFN therapy for HCC recurrence if these patients were assigned to the peg-IFN therapy. Consequently, 119 patients were eventually enrolled in this study. Of these 119 patients, 43 received peg-IFN therapy within 9 months after hepatectomy, and 76 did not receive any IFN therapy.

Curative hepatectomy was defined as removal of all recognizable tumors. HCV RNA levels were measured by quantitative reverse-transcription polymerase chain reaction (RT-PCR; Amplicor, Roche Diagnostic Systems, CA, USA). HCV genotype was determined by PCR using a mixed primer set derived from the nucleotide sequences of the NS5 region. HCV negativity was evaluated by quantitative RT-PCR. The lower limit of the assay was 5 kIU/ml (equivalent to 5,000 copies/ml) in the quantitative method and 50 IU/ml (equivalent to 50 copies/ml) in the qualitative method. SVR was defined as undetectable HCV RNA at 24 weeks after completion of IFN therapy. The study was approved by the concerned institutional review boards. Written informed consent was obtained from all patients.

Preoperative Diagnosis and Evaluation of HCC

Hepatocellular carcinoma was diagnosed on the basis of routine imaging modalities such as Doppler ultrasonography (US), computed tomography (CT) during hepatic angiography (CTHA) and CT during arterial portography (CTAP), and magnetic resonance imaging. Tumor stage, liver damage classification, and surgical procedures were defined according to the General Rules for Clinical and Pathologic Study of Primary Liver Cancer, fifth edition, by the Liver Cancer Study Group of Japan.²⁴

Hepatectomy

The surgical procedure was determined according to tumor extent and hepatic reserve function. Liver function was assessed by liver damage classification, Child–Pugh classification, and indocyanine green retention rate at 15 min (ICGR 15).^{25,26} If permitted by liver function, anatomic resection was performed.^{27,28} In patients with insufficient hepatic reserve, limited resection was performed. We divided the liver parenchyma by using an ultrasonic dissector.²⁹ Postoperative complications were graded according to the method described by Clavien et al.³⁰

Follow-Up

Follow-up evaluation after the surgery consisted of monthly blood chemistry tests and measurements of levels of tumor markers, including alpha-fetoprotein and des-gamma-carboxy prothrombin. Patients were examined by US every 3 months and by CT every 6 months. When recurrence was indicated by any of these examinations, patients were examined by CTAP and CTHA.

Patient Selection for IFN Therapy

Patients with HCV genotype 1b in the IFN group received peg-IFN α -2b (Pegintron; Schering-Plough, NJ, USA) at weekly dosage of 1.5 μ g/kg subcutaneously for 48 weeks. Daily RBV (Rebetrol, Schering-Plough) was administered orally for 48 weeks, and the dosage was adjusted according to weight (600 mg for patients weighing \leq 60 kg, 800 mg for those weighing 60–80 kg). Patients with HCV genotype 2 received IFN monotherapy for 24 weeks. Blood samples were obtained every 4 weeks and analyzed for HCV RNA levels. All patients were informed about IFN therapy after hepatectomy, and only consenting patients received IFN therapy. The eligibility criteria for IFN therapy were as follows: (1) detectable serum HCV RNA level, (2) Eastern Cooperative Oncology

Group (ECOG) performance score of 0 or 1, (3) platelet count $\geq 70,000/\mu\text{l}$, (4) patients with no uncompensated cirrhosis (Child class C), and (5) hemoglobin concentration ≥ 10 g/dl. Peg-IFN therapy was commenced within 24 weeks of surgery or after the eligibility criteria were fulfilled.

Safety Assessments and Dose Modification of Peg-IFN Therapy

Adverse events were graded as mild, moderate, severe, or potentially life-threatening according to a modified World Health Organization grading system. The dose of peg-IFN was decreased by 50% and that of RBV was lowered to half in case of severe adverse events or when laboratory results revealed any of the following: hemoglobin concentration < 10 g/dl in patients with no cardiac disease, decrease in hemoglobin concentration > 2 g/dl in patients with cardiac disease, white blood cell count $< 3,000/\text{mm}^3$, or platelet count $< 50,000/\text{mm}^3$. Full dosage could be resumed on resolution of the adverse events. Treatment was permanently discontinued in case of life-threatening events or when laboratory results revealed hemoglobin concentration < 7.5 g/dl after 4 weeks of dose reduction, white blood cell count $< 1,500/\text{mm}^3$, or platelet count $< 30,000/\text{mm}^3$.

Treatment for Recurrence

Patients with intrahepatic HCC recurrence were managed with ablative therapies such as radiofrequency ablation (RFA), percutaneous ethanol injection therapy, transarterial chemoembolization, or surgery including living-donor liver transplantation according to the tumor characteristics (number, size, and location of the tumors) and liver function.

Statistical Analyses

Categorical variables were compared using the chi-square test, and continuous variables were compared using the Mann–Whitney *U*-test. Overall survival and disease-free survival analyses were performed using Kaplan–Meier methods; comparisons between different groups were performed using the log-rank test. *P* value of less than 0.05 was considered significant. Calculations were performed using SPSS software (version 16; SPSS Inc., IL, USA).

Propensity analysis was performed using logistic regression to create a propensity score for the IFN and non-IFN therapy groups.^{31,32} Variables entered in the propensity model were age, sex, HCV genotype, liver function test, tumor factors, and operative factors. The model was then used to provide a one-to-one match between the two groups

by using the nearest-neighbor matching method.^{33,34} Survival and disease-free survival analyses were performed in each matched subgroup to assess the impact of peg-IFN therapy on mortality after adjusting for the confounding factors.

RESULTS

Characteristics and Postoperative Course of the Entire Population

Differences in the characteristics of patients who received peg-IFN therapy after hepatic resection and those who did not receive IFN therapy after hepatic resection are presented in Table 1. Patients who received peg-IFN therapy were younger (65 vs. 71 years; *P* = 0.0003). Regarding tumor characteristics, there was no significant difference between the two groups. Operation times tended to be longer in patients who received peg-IFN therapy than in those who did not receive IFN therapy (260 vs. 242 min; *P* = 0.05). There were no hospital-related deaths in this study. Postoperative complications did not differ between the two groups. In the entire population, the 3- and 5-year overall survival rates of patients who received peg-IFN therapy after hepatic resection were significantly higher than those of patients who did not receive IFN therapy (*P* = 0.0024) (Fig. 1a). However, there was no significant difference in disease-free survival between the two groups (*P* = 0.795) (Fig. 1b).

Results After Propensity Score Matching

Characteristics of the patients after propensity score analysis are presented in Table 1. Thirty-eight of the 43 patients who received peg-IFN therapy after hepatic resection and an equal number of the 76 patients who did not receive IFN therapy were matched after covariate adjustment. The study group of 76 patients was well matched; in particular, all covariates that significantly affected recurrence and postoperative liver failure in the entire study group were equally distributed between the two matched groups. Matched patients who received peg-IFN therapy after hepatic resection had similar total bilirubin and serum albumin levels and similar platelet counts to matched patients who did not receive IFN therapy. Similarly, the tumor characteristics, the surgical procedure, operation times, and blood loss during the operation in matched patients who received peg-IFN therapy were almost similar to those in patients who did not receive IFN therapy. There were no hospital-related deaths in the matched groups. Postoperative complications also did not differ between the two groups. The median follow-up period for patients who received peg-IFN and those who