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ORIGINAL ARTICLE

RASSF1A methylation indicates a poor prognosis in hepatoblastoma patients

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Abstract
Purpose The RAS association domain family protein 1 (RASSF1A) is known to be frequently inactivated by promoter hypermethylation in cancers. This study investigated the association of *RASSF1A* methylation with clinical outcomes in hepatoblastoma patients and whether it is correlated with the histological phenotype of hepatoblastoma tumors.
Methods Seventy-four hepatoblastoma tumors were obtained from patients enrolled in the Japanese study group for pediatric liver tumor protocol-2. From nine formalin-fixed, paraffin-embedded specimens, we extracted DNA by dissection under a light microscope. We examined the methylation status of the *RASSF1A* promoter region by bisulfite pyrosequencing.
Results Twenty-five (33.8 %) hepatoblastoma tumors were classified as having methylated *RASSF1A*. The *RASSF1A* methylation was significantly associated with metastatic tumors and a poor prognosis. Despite the complete resection, five pretreatment extent of disease II tumors showed recurrence or distant metastasis postoperatively. Among these cases, four tumors were found to show *RASSF1A* methylation. When compared to histologically different types of cell, *RASSF1A* methylation values in samples of the normal liver, fetal type, and embryonal type, were significantly elevated in ascending order.
Conclusions We confirmed that *RASSF1A* methylation is a significant prognostic indicator in hepatoblastomas, and it may become a promising molecular marker to stratify patients into appropriate risk groups.

Keywords Hepatoblastoma · *RASSF1A* methylation · Prognostic marker

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Introduction

Hepatoblastoma is the most common malignant neoplasm of the liver in children. Despite the progress of therapy, the mortality rate remains at 35–50 % in high-risk patients, such as those with extrahepatic tumors, macroscopic invasion of large vessels, or distant or lymph node metastases [1]. Complete surgical resection or liver transplantation and mainstream treatment with cytotoxic drugs are essential for achieving a favorable long-term outcome. To

improve the mortality of hepatoblastoma patients in advanced stages, innovative treatment and potent prognostic markers for better therapy planning are needed.

Histologically, hepatoblastoma tumors are classified as wholly epithelial, or mixed epithelial and mesenchymal types. In the wholly epithelial type, there are two major subtypes, the fetal subtype and the mixed fetal and embryonal subtype [2]. Fetal and embryonal components often develop in combination, that is, heterogeneity is present. The RAS association domain family protein 1 (RASSF1A) is known to be frequently inactivated by promoter hypermethylation in many adult and childhood cancers [3]. We previously reported that *RASSF1A* methylation was correlated with a poor outcome by multivariate analysis, and suggested that *RASSF1A* may be a promising molecular–genetic marker predicting the treatment outcome in hepatoblastoma patients [4]. The association between the histological type and *RASSF1A* methylation is ambiguous despite the fact that the histologic features are associated with different prognoses; a pure fetal histology is favorable and small cell undifferentiated and macrotrabecular histologies are unfavorable [1]. Therefore, the current study was undertaken to determine the association with histological types by examining each type of hepatoblastoma cell dissected separately.

In this study, we investigated the methylation status of *RASSF1A* in hepatoblastoma tumors by bisulfite pyrosequencing, which is a rapid and accurate method to quantify DNA methylation. We analyzed the results with regard to patients' clinicopathological characteristics and prognosis, and evaluated its association with the histological phenotype on the basis of the epigenetic alteration of hepatoblastomas.

Methods

Patients and samples

Seventy-four hepatoblastoma patients with a median age of 18 months underwent tumor resection and partial hepatectomy between December 1999 and December 2008 at the institutions of the Japanese Study Group for Pediatric liver Tumors (JPLT). All patients were treated in the JPLT-2 study [5]. The extent of disease was determined at the time of initial biopsy or resection according to the classification of the pretreatment extent of disease (PRETEXT) staging system [6]. Metastatic tumors were found in 15 % of the patients (Table 1). The 5-year overall survival and event-free survival rates were 86.7 and 73.4 % for the 74 patients, respectively.

The DNA samples of the 74 hepatoblastoma tumors were supplied by JPLT, and they were extracted from fresh-frozen specimens. Furthermore, formalin-fixed, paraffin-embedded (FFPE) specimens were obtained from nine patients referred to our institution for surgical treatment between 1995 and 2011. We extracted DNA from different types of cell: fetal type, embryonal type, and normal liver, by dissection under a light microscope in order to avoid contamination with normal tissues and mesenchymal components. The ethics committee of our institution approved the study protocol, and signed

Table 1 Clinicopathological factors and *RASSF1A* methylation status in 74 patients with hepatoblastoma

Clinicopathological factors		No. of patients	*RASSF1A*		p value[1]
			Methylated	Unmethylated	
Sex	Male	45	14	31	0.360
	Female	29	11	18	
Age at diagnosis	<365 days	22	0	22	0.000064
	≥365 days	52	25	27	
PRETEXT	I	5	1	4	0.319
	II	27	7	20	
	III	29	10	19	
	IV	13	7	6	
Metastasis	No	63	15	48	0.000039
	Yes	11	10	1	
Histological type	Fetal	28	9	19	0.508
	Mixed fetal and embryonal	40	14	26	
	Unknown	6			
Outcome	Alive	63	15	48	0.000039
	Dead	11	10	1	

[1] Fisher's exact test

informed consent was obtained in all cases by local physicians of the participating institutions.

Evaluation of *RASSF1A* methylation level

We examined the methylation status of the *RASSF1A* promoter region by bisulfite pyrosequencing, which can calculate the level of methylation at each CpG site in samples after bisulfite treatment. Genomic DNA (500 ng) was modified with sodium bisulfite using an EpiTect bisulfite kit (Qiagen, Netherlands). Bisulfite pyrosequencing was carried out as described previously [7]. After PCR, the biotinylated PCR product was purified, made single-stranded, and used as a template in the pyrosequencing reaction. Briefly, the PCR products were bound to streptavidin sepharose beads HP (Amersham Biosciences, USA), after which beads containing the immobilized PCR product were purified, washed, and denatured using a 0.2 mol/L NaOH solution. After the addition of 0.3 μmol/L sequencing primer to the purified PCR product, pyrosequencing was carried out using a PSQ96MA system (Biotaga) and Pyro Q-CpG software (Biotage). The mean value of the methylation levels at two CpG sites in the *RASSF1A* promoter region was calculated. Primer sequences used in this study were as follows: forward, GAAGGAGGGAAGGAAGGGTAAG; reverse, GCCTCC CCCAAAATCCAA; sequencing primer, TTGTATTTAG GTTTTTATTG.

Statistical analysis

Correlations between the *RASSF1A* methylation status and clinicopathological factors were analyzed using the Fisher's exact test. Survival curves were constructed according to the methods of Kaplan and Meier, and comparisons of survival curves were performed with a log-rank test. One-way ANOVA followed by Student's *t* test with Bonferroni correction was used to compare methylation values of histologically different types of cell. A P value <0.05 was considered statistically significant.

Results

RASSF1A methylation status in 74 hepatoblastomas

The average of the *RASSF1A* methylation values in 74 hepatoblastoma tumors was 25.8 % (2.0–74.8 %). We performed the ROC analysis to determine the cutoff value of the *RASSF1A* methylation and adopted a cutoff value of 36.2 % in this study (Fig. 1). On the basis of this cutoff value, 25 (33.8 %) tumors were classified as having methylated *RASSF1A*, and the sensitivity and specificity for

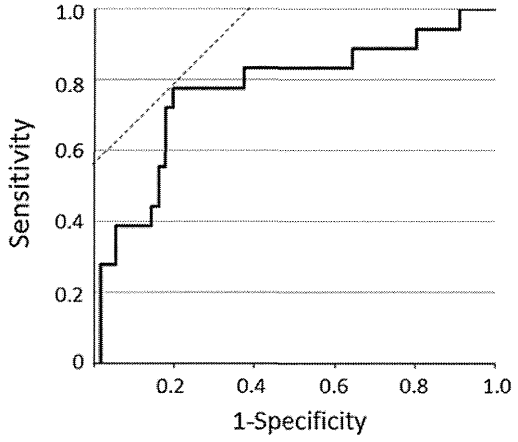

Fig. 1 ROC analysis to determine the cutoff value of the *RASSF1A* methylation

the patients having an event postoperatively were 77.8 and 80.4 %, respectively. There was only one patient who died in those with tumors with *RASSF1A* unmethylated (Fig. 2).

Associations between clinicopathological factors and *RASSF1A* methylation status

We evaluated the associations between the clinicopathological factors and *RASSF1A* methylation status in 74 patients. As Table 1 shows, there were no patients aged under 1 year who had a tumor with *RASSF1A* methylated; however, about half of the patients aged over 1 year were found to have a tumor with *RASSF1A* methylated. 10 of 25 patients (40 %) with a tumor with *RASSF1A* methylated suffered from metastatic tumors, although there was only one patient with metastasis in those with a tumor with *RASSF1A* unmethylated. This demonstrated that age at diagnosis and metastatic tumors were significantly associated with *RASSF1A* methylation. In Kaplan–Meier analyses, the patients with a tumor with methylated *RASSF1A* were significantly associated with a poor outcome: the 5-year overall survival and event-free survival rates were 63.6 and 35.5 %, respectively (Fig. 3).

The *RASSF1A* methylation was detected in 1 of 5 PRETEXT I tumors and 7 of 27 PRETEXT II tumors (Table 1). Despite complete resection, five PRETEXT II tumors showed recurrence or distant metastasis postoperatively, and three patients died. Among these cases, four tumors were found to have *RASSF1A* methylated.

Associations between histological types and *RASSF1A* methylation status

Next, we evaluated whether *RASSF1A* methylation is associated with the histopathological subtypes. Four of the

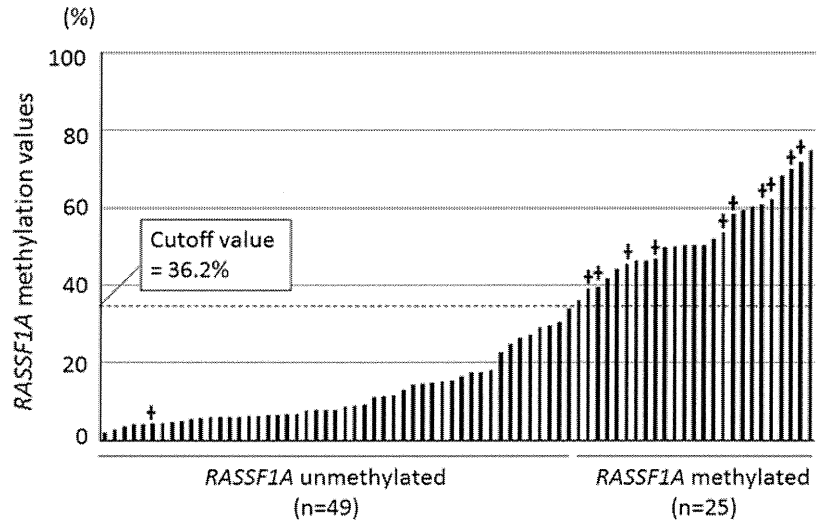

Fig. 2 *RASSF1A* methylation values in 74 patients with hepatoblastoma. *Plus* indicates the patient who died of the disease

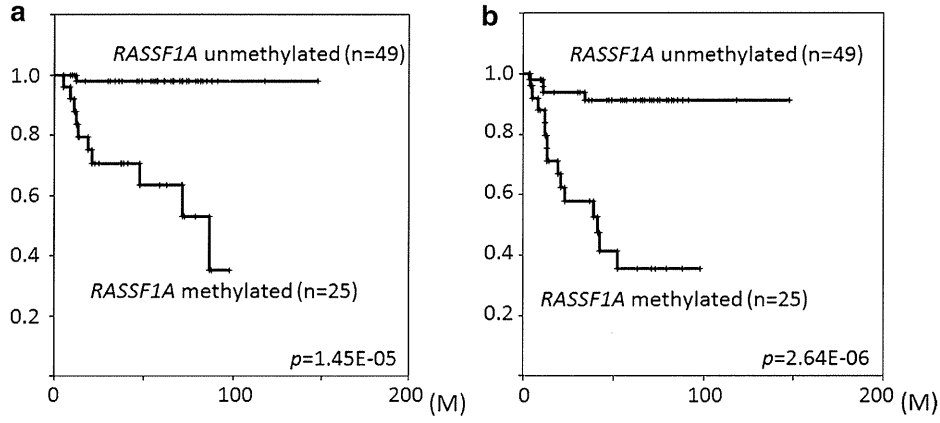

Fig. 3 **a** Overall survival curves and **b** event-free survival curves for hepatoblastoma patients classified by the methylation status of *RASSF1A*

nine tumors in FFPE specimens were classified pathologically into the mixed fetal and embryonal subtype, and DNA was extracted from the tumor cells of each subtype and the normal liver. The other five tumors were the pure fetal subtype, so DNA was extracted from fetal hepatoblastoma and normal liver cells.

The mean methylation values of *RASSF1A* in nine normal liver, nine fetal type, and four embryonal type samples were 8.6, 19.7, and 42.2 %, respectively (Fig. 4). This showed that fetal and embryonal types were significantly associated with *RASSF1A* methylation. Moreover, *RASSF1A* methylation values in samples of the normal liver, fetal type, and embryonal type were elevated in ascending order, when compared to each type of cell taken from the same patient.

Discussion

Complete surgical resection and chemotherapy including cisplatin remains the mainstay of hepatoblastoma treatment. In contrast to standard-risk patients, of who over 90 % achieve long-term survival, the treatment of patients with unrespectable and metastatic disease remains a challenge. Furthermore, there seems to exist a group of patients with high-risk tumors in PRETEXT II, which have a poorer prognosis despite the high-level resectability [5]. First, this study demonstrated that *RASSF1A* methylation was significantly associated with metastatic tumors and a poor prognosis, and that *RASSF1A* methylation may be useful to identify high-risk tumors in PRETEXT II. Secondly, *RASSF1A* methylation was also shown to be histologically correlated with different types of tumor. These findings suggest that *RASSF1A* may be a promising molecular marker to stratify the patients into appropriate risk groups in order to develop better therapeutic approaches.

The present factors predicting the outcome in hepatoblastoma patients include the age at diagnosis, histology, local growth pattern of the tumor, presence of metastasis, and the level of alpha–feto protein [1]. Chromosomal gains of 2q, 8q, and 20, high-level expression of *TERT* or *PLK1*, *CTNNB1* mutation, and *RASSF1A* methylation were shown

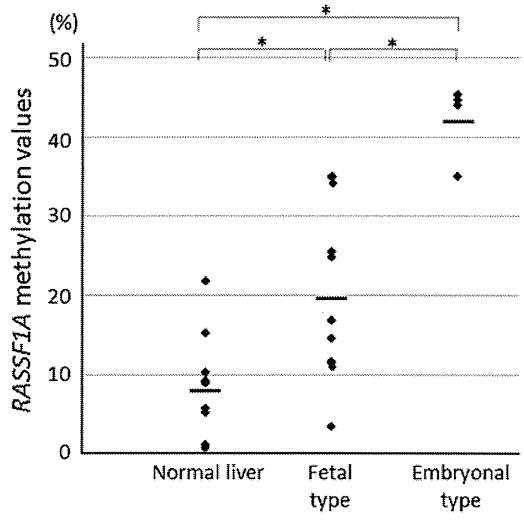

Fig. 4 *RASSF1A* methylation value for each sample is plotted by histological type. *Horizontal bars* indicate the mean value of each type. The p values were calculated by one-way ANOVA followed by Student's t test with Bonferroni correction ($*p < 0.017$)

to be molecular–genetic markers predicting a poor outcome [4, 8, 9]. We have been focusing on *RASSF1A* methylation in hepatoblastomas, since it has been proven to be an independent prognostic factor by multivariate analysis [4]. RASSF1A inhibits tumor formation by apoptosis, and regulates microtubule dynamics and mitotic arrest via multiple effectors. By dysregulation of the Ras-signaling pathway, *RASSF1A* methylation is correlated with poor differentiation and vascular invasion of cancer cells, and an unfavorable outcome [10]. In child cancers, *RASSF1A* methylation was shown to be associated with a poor outcome in neuroblastoma and Wilms tumor [11, 12]. In this study, we newly adopted bisulfite pyrosequencing as a tool for methylation analysis because it is a highly effective and practical method and offers higher throughput compared to quantitative methylation-specific PCR used in the previous study [4]. We believe that bisulfite pyrosequencing can be a reliable tool when used in a clinical setting.

Cairo et al. [13] identified a 16-gene signature discriminating tumors with a fairly well-differentiated histology and a favorable prognosis against advanced and poorly differentiated tumors with a dismal outcome. In this study, *RASSF1A* methylation was also shown to be correlated with different types of histological phenotype by examining FFPE samples dissected separately. As shown in Table 1, there was no apparent difference in *RASSF1A* methylation values between the fetal subtype and the mixed fetal and embryonal subtype, probably because contamination with different types of tumor cell, normal tissues, and mesenchymal components could not be avoided using fresh-frozen specimens. With these gene signatures based on different phenotypes, the molecular classification of hepatoblastoma tumors may become possible after thorough clinical testing. Although the number of cases in this study is too small to draw definite conclusions, we expect that these molecular markers can be used as prognostic markers predicting the treatment outcome when larger clinical trials are carried out.

In conclusion, *RASSF1A* methylation was significantly associated with metastatic tumors and a poor prognosis in hepatoblastoma patients, and it may be especially useful to identify high-risk tumors in PRETEXT II. The *RASSF1A* methylation was also shown to be correlated with different histological phenotypes. We hope that this work will contribute to establishing a useful molecular marker to predict the outcome of hepatoblastoma patients, stratify the patients efficiently, and develop better therapeutic strategies.

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Conflict of interest The authors who have taken part in this study declare that they do not have anything to disclose regarding funding or any conflict of interest with respect to this manuscript. The first and the corresponding authors are JSPS members, and this abstract was selected for presentation at the 50th Annual Meeting of the Japanese Society of Pediatric Surgeons.

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ORIGINAL ARTICLE

Percutaneous transhepatic gallbladder drainage followed by elective laparoscopic cholecystectomy in patients with moderate acute cholecystitis under antithrombotic therapy

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Abstract
Background Standard treatment for acute cholecystitis (AC) in patients receiving antithrombotic drugs has not been established. We evaluated the safety of percutaneous transhepatic gallbladder drainage (PTGBD) followed by elective laparoscopic cholecystectomy (LC) in patients with moderate AC who were receiving antithrombotics.
Methods Seventy-five patients received PTGBD from January 2006 to March 2013 followed by elective LC for moderate AC. Patients were divided into Group A, which consisted of patients receiving antithrombotic therapy (n = 23), and Group B, which included the remaining patients (n = 52). We analyzed clinical outcomes and perioperative complications between groups.
Results No hemorrhagic events occurred during PTGBD insertion regardless of antithrombotic treatment. The open conversion rate was not significantly different between the two groups. Postoperative complications were found in 10 patients (13.3%). The rate of postoperative complications in Group A was slightly higher than that in Group B, but the difference was not significant (21.7% vs. 9.6%; P = 0.15). Complications associated with PTGBD occurred in six patients (8%). There were no significant differences in the incidence of these complications, operation time, intraoperative blood loss, or length of postoperative hospital stay.
Conclusions Percutaneous transhepatic gallbladder drainage followed by elective LC may be an effective therapeutic strategy for moderate AC in patients receiving antithrombotic therapy.

Keywords Acute cholecystitis · Antithrombotic therapy · Laparoscopic cholecystectomy · Percutaneous transhepatic gallbladder drainage

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Introduction

Acute cholecystitis (AC) is one of the most commonly encountered diseases that is caused by obstruction of the cystic duct with or without gallstones. Early laparoscopic cholecystectomy (LC) has been widely accepted as a standard treatment for patients with AC. Many randomized studies and meta-analyses have indicated the clinical advantage of LC compared to open cholecystectomy (OC) [1, 2] and the value of early LC compared to delayed LC [3, 4]. The Japanese and Tokyo guidelines for acute cholangitis and cholecystitis were published sequentially in 2005 and 2007 [5, 6]. Since that time, early LC has been increasingly used for treating patients with AC [7].

However, there are situations in which surgeons are reluctant to perform early or emergency LC. For example, in elderly or critically ill patients, perioperative mortality rates are high (up to 19%) for emergency cholecystectomy in comparison to cholecystectomy in the elective setting [8]. Even in patients who are not elderly or critically ill, it is sometimes difficult to complete LC because of severe pericholecystic inflammation due to moderate AC

resulting from various conditions, including biliary peritonitis, pericholecystic abscess, hepatic abscess, gangrenous cholecystitis, and emphysematous cholecystitis. In addition, patients with coagulopathy have increased risks of perioperative thrombotic or hemorrhagic morbidities and mortalities in the emergency or early operative setting. Patients receive oral antiplatelet or anticoagulant therapies to prevent primary or secondary thrombotic cardiovascular or cerebrovascular events, which have increased in incidence due to aging of the population. However, the definitive therapeutic strategy for these patients with AC has not been established.

Percutaneous transhepatic gallbladder drainage (PTGBD) is a less invasive imaging-guided alternative designed to decompress the acutely inflamed gallbladder in patients who are unresponsive to medical therapy or are at high risk for cholecystectomy [9]. Some studies suggest that PTGBD allows subsequent elective cholecystectomy with minimal rates of conversion and perioperative morbidity and mortality in complicated AC [10–15]. This study was designed to evaluate the efficacy and safety of PTGBD followed by elective LC in patients with moderate AC receiving concurrent antithrombotic therapy.

Patients and methods

Patients

We retrospectively reviewed individual medical records from the Hokushinkai Megumino Hospital from January 2006 to March 2013. In this period, 206 patients were diagnosed with AC. The diagnosis of AC was based on clinical signs and findings from computed tomography (CT) scans and ultrasonograms. Severity grading for AC was based on the Japanese guidelines 2005 [5] and the Tokyo guidelines 2007 [16]. Early cholecystectomy including LC and OC is recommended in the Japanese and Tokyo guidelines, but is not adopted in our institute because of insufficient manpower and the lack of a system to do early or emergency operation. Thus, elective cholecystectomy after antibiotics therapy was performed on patients with mild (grade I) AC, and elective cholecystectomy after PTGBD was performed on patients with moderate (grade II) AC or who had not responded to medical therapy. The treatment flow chart according to the therapeutic strategy in our institute was shown in Figure 1. Here, we restricted our study to the patients who underwent elective cholecystectomy after PTGBD. Among them, we analyzed outcomes in 75 patients who received LC, and excluded from analysis 10 patients who received OC, one patient who underwent emergency OC due to failure of PTGBD insertion, and one patient who did not undergo cholecystectomy after PTGBD due to advanced age and poor condition. The distribution of patients according to severity criteria is shown in Table 1.

Antiplatelet or anticoagulant therapy

Twenty-three patients received oral antiplatelet and/or anticoagulant treatment for moderate AC. Thirteen patients received antiplatelet agents, including aspirin and thienopyridines. Seven patients received anticoagulant drugs, and three patients received a combination of antiplatelet and anticoagulant agents. Underlying diseases included ischemic heart disease in 12 patients, atrial fibrillation in seven patients, valvular heart disease in one patient, arteriosclerosis obliterans in two patients, and previous cerebral infarctions in five patients. Patients who were treated with antithrombotic therapy were placed in Group A ($n = 23$), and the remaining 52 patients were placed in Group B. All patients were admitted to our hospital, and oral antiplatelet and/or anticoagulant drugs were immediately discontinued following confirmation of the diagnosis of moderate AC. Nine patients at high risk for cardiovascular or cerebrovascular events needed heparin replacement therapy following discontinuation of oral drugs.

Percutaneous transhepatic gallbladder drainage

Percutaneous transhepatic gallbladder drainage was performed immediately or within a few days after confirming the diagnosis of moderate AC regardless of whether patients received antithrombotic drugs or not. Vitamin K was administered intravenously before PTGBD for one patient who received anticoagulant therapy due to an international normalized ratio of prothrombin time above 2.0. PTGBD was performed according to the Tokyo guidelines [17]. Briefly, an external cylinder with a mandolin was inserted into the gallbladder with ultrasonic guidance. The mandolin was removed, and the external cylinder remained. The backflow of bile was confirmed, and a guide wire was inserted into the gallbladder. The external cylinder was removed. After dilating the track, a 7-Fr drainage tube was passed over the guide wire into the gallbladder. The guide wire was withdrawn and cholangiograms were performed to confirm that the drainage tube was in the correct position within the gallbladder.

Timing for operation and operative technique

Laparoscopic cholecystectomy was electively performed at the appropriate time following PTGBD after the condition of the patient or the pericholecystic inflammation improved. LC was performed at least 7 days after PTGBD in group A, which was the time it took for the antithrombotic effects to

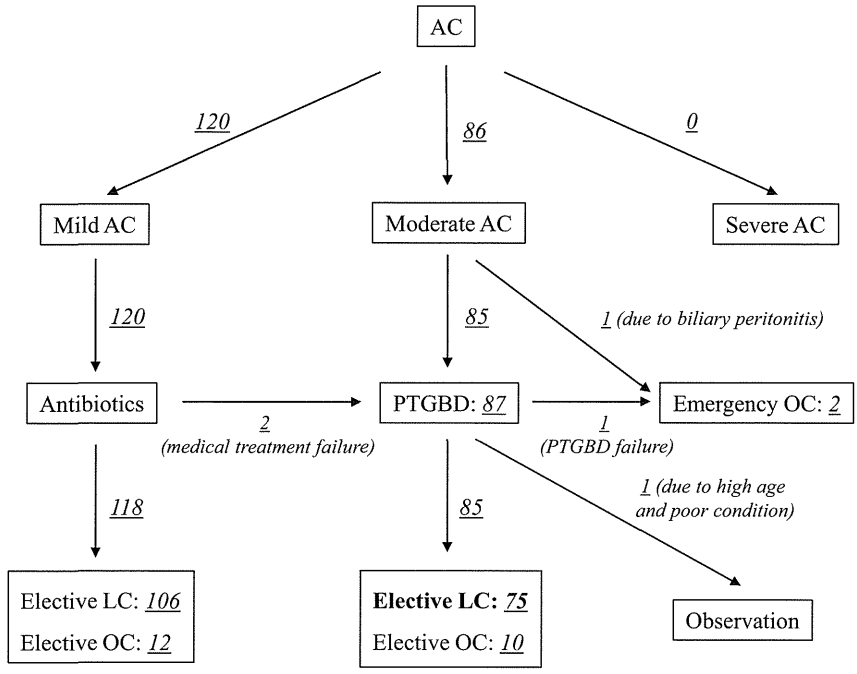

Fig. 1 The flow chart of treatment for acute cholecystitis (AC) according to the strategy in our institute. In our institute, early cholecystectomy including laparoscopic cholecystectomy (LC) and open cholecystectomy (OC) is not adopted. Thus, elective cholecystectomy after antibiotics therapy was performed on patients with mild AC, and elective cholecystectomy after percutaneous transhepatic gallbladder drainage (PTGBD) was performed on patients with moderate AC or who had not responded to medical therapy. Among 120 patients diagnosed with mild AC, two patients were refractory to medical treatment and finally inserted PTGBD. The other patients underwent elective LC in 106 and elective OC in 12. On the other hand, among 86 patients diagnosed with moderate AC, 85 underwent PTGBD, and one underwent emergency OC due to biliary peritonitis. One who failed to PTGBD insertion underwent emergency OC. One who was of advanced age and poor condition did not undergo the operation. The total 85 patients underwent elective cholecystectomy including LC in 75 and OC in 10. In this period, there were no patients diagnosed with severe AC

Table 1 Patient distributions according to severity assessment criteria for acute cholecystitis (AC)

		Group-A (n = 23)	Group-B (n = 52)	P-value
WBC (/μl)	<18,000	18	37	
	≥18,000	5	15	0.584
Palpable tender mass in the right upper abdominal quadrant	No	20	48	
	Yes	3	4	0.669
Duration of complaints	<72 h	18	36	
	≥72 h	5	18	0.579
Pericholecystic abscess	No	18	46	
	Yes	5	6	0.296
Gangrenous cholecystitis	No	20	49	
	Yes	3	5	0.689
Emphysematous cholecystitis	No	20	50	
	Yes	3	2	0.165
Refractory to medical therapy		0	2	0.999
Criteria of "moderate AC" based on the Japanese guideline but not the Tokyo guideline 2007		12	21	0.45

disappear. LC was performed with standard four-trocar technique in the presence of pneumoperitoneum. The PTGBD catheter was removed at the beginning of the operation. After release of inflammatory adhesions around the gallbladder, the triangle of Calot was dissected free of all tissue except for the cystic duct and artery, and the base of the liver bed was exposed. The cystic duct and artery were clipped and transected sequentially. The gallbladder was separated from the liver bed, placed into a disposable plastic bag, and removed from the abdominal cavity. A Penrose drain was inserted for all patients and removed within 24 h if no complications were found.

Statistical analysis

The patient demographics, perioperative characteristics, and rate of perioperative complications were compared between patients who received antiplatelet and/or anticoagulant therapy versus those who did not using the Mann–Whitney test or Fisher's exact test for independence. The data are shown as the median and range. Statistical analysis was performed with StatMate IV for windows (ATMS, Tokyo, Japan), and $P < 0.05$ was considered statistically significant.

Results

The success and response rate of PTGBD

Percutaneous transhepatic gallbladder drainage was performed for 87 patients and was successful in all patients except for one who had severe gangrenous cholecystitis. One patient who failed PTGBD received emergency OC due to poor response to other conservative therapies. All patients in whom PTGBD was successful improved within a few days and were subsequently able to receive elective cholecystectomy except for one who did not undergo operation due to advanced age and poor condition. Accordingly, the success rate and response rate were both 98.9%. Morbidity from PTGBD was 3.5% due to insertion failure in one patient, pleural effusion in another patient, and bile leakage in a third patient. However, the 27 patients who received antithrombotics did not suffer complications from PTGBD, including hemorrhagic events.

Patient demographics and clinical outcomes

In this study, a total of 75 patients were included, and the others were excluded due to OC. The patient demographics and clinical outcomes of PTGBD followed by elective LC are listed in Table 2. The median age was 71 years in Group A and 65 years in Group B ($P < 0.05$). There were no statistical differences in gender, weight, or body mass index (BMI) between Groups A and B. Both groups experienced similar operative times, blood loss, and postoperative clinical outcomes, including length of hospital stay and laboratory test values, such as white blood cell (WBC) count and C-reactive protein (CRP) on postoperative day 3 (Table 2).

Perioperative complications with PTGBD followed by elective LC

Perioperative complications were found in 15 patients (20%; Table 3). No significant differences were found between Groups A and B. Preoperative complications were found in two patients (2.7%), including one patient who developed a pleural effusion in the right thorax and one with bile leakage. Both patients improved with conservative treatment without drainage. Conversion from LC to OC was required in three patients (4%) because of uncontrollable intraoperative bleeding due to severe pericholecystic inflammation and adhesions. However, there were no significant differences between Groups A and B (0% vs. 5.8%; $P = 0.55$). According to the Clavien-Dindo classification [18], postoperative complications occurred in 10 patients (13.3%). Grade II complications occurred in seven patients (10.6%), and grade III occurred in three patients (4%, one with postoperative bleeding and two with bile leakages from the PTGBD route). The patient who developed postoperative bleeding had oozing from the liver bed and required re-operation by laparoscopic surgery. Two patients with bile leakages from the PTGBD route improved with endoscopic nasal biliary drainage and intra-abdominal drainage for several days. The patient who had postoperative subcapsular liver hemorrhage around the route of PTGBD recovered with conservative management after several days. Five patients had persistent postoperative inflammation, defined by high CRP values (more than 10 mg/dl) on postoperative day 3 or continuous fever greater than 37.5°C for more than 3 days. However, we did not find obvious intra-abdominal abscesses by CT scan or ultrasonography, and all five patients recovered with antibiotic therapy. The patient with postoperative pleural effusion improved with conservative therapy. Thus, we did not detect significant differences in outcomes between Group A and B patients, although the incidence of postoperative complications in Group A patients was slightly higher than in Group B patients (21.7% vs. 9.6%; $P = 0.15$). No surgery-related mortalities or serious cardiovascular or cerebrovascular events were observed within 30 days of operation. Complications related to PTGBD were found in six patients (8%, indicated in *Italic font* in Table 3). There were no significant differences in the incidence of these complications between the two patient groups (Table 3).

Table 2 Patient demographics and perioperative characteristics

	Group-A (n = 23)		Group-B (n = 52)		P-value
	Median	Range	Median	Range	
Demographics					
Gender					
Male	16		24		
Female	7		28		0.081
Age (years)	71	(57–95)	65	(22–88)	*0.033*
Height (cm)	164	(140–170)	161	(140–176)	0.438
Weight (kg)	62.7	(52.3–85.0)	62.3	(37.6–93.0)	0.405
BMI (kg/m^2)	24.4	(19.3–35.0)	23.9	(18.6–33.8)	0.346
Preoperative factors					
WBC before PTGBD (/μl)	13310	(6060–24450)	14995	(5770–26470)	0.141
peak CRP before operation (mg/dl)	20.5	(4.1–28.9)	21.795	(0.5–32.7)	0.219
Time interval from onset to PTGBD (days)	2	(0–9)	3	(0–14)	0.158
Time interval from PTGBD to LC (days)	11	(8–23)	12	(4–106)	0.158
Laboratory test after PTGBD					
WBC (/μl)	5680	(3640–10450)	6150	(3500–9890)	0.526
CRP (mg/ml)	1.32	(0.3–10.5)	1.085	(0.1–8.4)	0.280
Intraoperative factors					
Operation time (min)	112	(45–265)	109	(65–180)	0.809
Blood loss (ml)	20	(0–200)	0	(0–840)	0.162
Postoperative factors					
Postoperative hospital stay (days)	4	(4–16)	4	(3–11)	0.425
Laboratory test at 3 postoperative day					
WBC (/μl)	7940	(4420–11750)	6650	(4200–12480)	0.195
CRP (mg/ml)	5.27	(1.3–25.6)	4.505	(0.2–24.5)	0.059

BMI body mass index, *CRP* C-reactive protein, *PTGBD* percutaneous transhepatic gallbladder drainage, *WBC* white blood cell count

Table 3 Perioperative complications after percutaneous transhepatic gallbladder drainage (PTGBD) followed by laparoscopic cholecystectomy (LC)

	Group-A (n = 23)	Group-B (n = 52)	P-value
Total number of complications	5	10	0.532
Preoperative complications	0 (0%)	2 (3.8%)	0.909
Pleural effusion[a]		1	
Intraabdominal bile leakage[a]		1	
Intraoperative complications (conversion to open)	0	3 (5.8%)	0.548
Postoperative complications	5 (21.7%)	5 (9.6%)	0.154
Persistent inflammation after LC	1	4	
Postoperative bleeding (Grade III)	1		
Bile leakage from PTGBD[a] *(Grade III)*	1	1	
Subcapsular hemorrhage of the liver[a]	1		
Pleural effusion[a]	1		
Complication associated with PTGBD	3 (13.0%)	3 (5.8%)	0.363

[a] *Italic* font indicates complications associated with PTGBD

Risk factors associated with complications

Finally, we analyzed the risk factors associated with complications by univariate analysis. Treatment with either antiplatelet or anticoagulant drugs did not increase the incidence of complications. Further, perioperative heparin replacement therapy was not an independent factor. Age older than 65 was an independent risk factor predicting

Table 4 Risk factors associated with perioperative complications

		Complication (n = 15)	Without complication (n = 60)	P-value
Use of anti-platelet or -coagulant agents	Yes	6	17	
	No	9	43	0.532
Use of anti-platelet agents	Yes	4	12	
	No	11	48	0.725
Use of anti-coagulant agents	Yes	1	8	
	No	14	52	0.677
Heparin replacement therapy	Yes	2	7	
	No	13	53	0.859
Gender (male/female)	Male	6	34	
	Female	9	26	0.265
Age (years)	<65	3	30	
	≥65	12	30	*0.045*
BMI (kg/m^2)	<25	9	38	
	≥25	6	22	0.812
Operation time (min)	<120	9	40	
	≥120	6	20	0.763
Blood loss (ml)	<50	9	48	
	≥50	6	12	0.173
Time interval from onset to PTGBD (days)	≤3	10	44	
	>3	5	16	0.749
Time interval from PTGBD to LC (days)	<14	9	42	
	≥14	6	18	0.540
WBC before PTGBD (/μl)	<18,000	13	42	
	≥18,000	2	18	0.328
Peak CRP before LC (mg/ml)	<20	4	26	
	≥20	11	34	0.377

BMI body mass index, *CRP* C-reactive protein, *LC* laparoscopic cholecystectomy, *PTGBD* percutaneous transhepatic gallbladder drainage, *WBC* white blood cell count

perioperative complications from PTGBD followed by elective LC for patients with moderate AC (Table 4).

Discussion

PTGBD is a relatively safe and effective procedure for AC and achieves clinical improvement within 48–72 h after insertion with minimal procedure-related mortality. The review by Itoi et al. showed that the technical success rate and response rate of PTGBD were nearly 100% and 78–95%, respectively. Adverse events related to PTGBD occurred in 0.3–12% of patients [19]. In our series, the success and response rate were nearly 100%. The incidence of complications during PTGBD was 3.5%, and no hemorrhagic events occurred. In addition, Dewhurst et al. have reported that performing percutaneous cholecystostomy in patients with coagulopathy or in those receiving anticoagulant medications did not alter the incidence of hemorrhagic complications in comparison with those who have normal coagulation (1.5% vs. 1.8%) [20]. These data suggest that PTGBD can be performed safely irrespective of the use of antithrombotic drugs.

In patients who received antithrombotic therapy, the conversion rate was 0%, and the rate of postoperative complications was 21.7%. These rates were not different from those observed in patients who did not receive antithrombotic therapy (Table 3). In contrast, hemorrhagic postoperative complications occurred in only two patients receiving antithrombotic therapy. The patient who had postoperative oozing from the liver bed did not suffer any complications during PTGBD insertion, and the duration time from the cessation of aspirin to LC was greater than 14 days. Therefore, the complication was attributed to inflammation from moderate AC and not to PTGBD or aspirin. Another case of subcapsular hemorrhage around the PTGBD route did not occur immediately after insertion of the drainage tube but occurred after the operation. The interval time from the discontinuation of antiplatelets to LC was 10 days. Thus, excessive or forceful intraoperative traction most likely

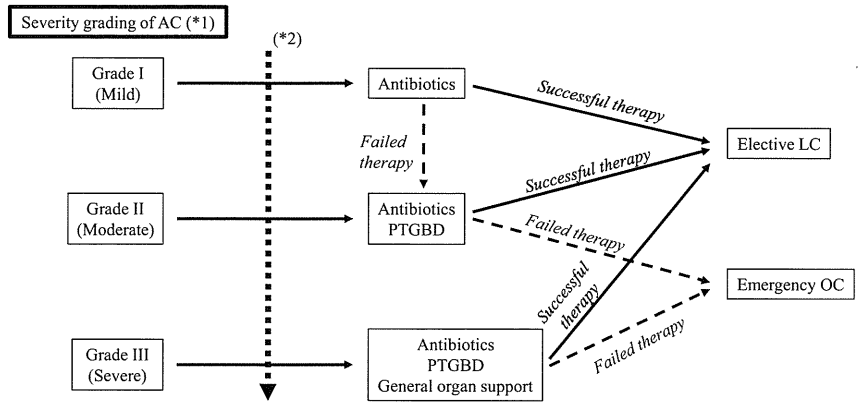

Fig. 2 The treatment strategy for acute cholecystitis (AC) in patients under antithrombotic therapy in our institute. (*1): Severity grading of AC is based on Tokyo guideline 2013. (*2): Antithrombotic drugs are immediately discontinued and heparin replacement is considered if needed. *PTGBD* percutaneous transhepatic gallbladder drainage

induced a slight tear or laceration of the liver parenchyma around the PTGBD route. This is probably related to the PTGBD procedure but not to the influence of antithrombotic therapy. These results suggest that antithrombotic drugs do not increase the risk of perioperative morbidities in PTGBD followed by elective LC for moderate AC.

It is controversial whether PTGBD followed by elective LC can be a standard therapy for moderate AC in non-clinically ill patients. In the 2013 Tokyo guidelines, the indication of early gallbladder drainage and subsequent delayed cholecystectomy, including laparoscopic or open, is restricted to patients with moderate AC who have severe local inflammation [21]. However, the clinical benefits of PTGBD followed by elective LC for complicated AC have recently been shown. The rate of conversion into OC was 3–8%. The incidence rate of postoperative complications was 3.2–16% [10–15], although there have been no randomized controlled studies directly comparing these results with early LC. In our series, the conversion rate and the occurrence rate of postoperative complications was 4% and 13.3%, respectively. These results compared favorably with the above reports. In contrast, perioperative complications associated with PTGBD were found in 8% of patients, including 2.7% with Clavien-Dindo Grade III complications. Our results indicate that it is important to adequately comprehend and pay attention to the particular complications induced by PTGBD if PTGBD followed by LC is performed.

It is assumed that early or emergency LC without PTGBD increases the risk for hemorrhagic events in patients with moderate AC who are receiving antithrombotic therapy due to residual effects from the antithrombotics. This is based in part on the observations that 8.5–27.2% of LC to OC conversions were due to intraoperative bleeding [22–24], and AC significantly increased risk for open conversion and postoperative complications [23–26]. In contrast, by preceding PTGBD, we can wait for the effects of antithrombotics to wear off. In addition, we can appropriately assess the perioperative risk for cardiovascular or cerebrovascular disease during the waiting time. We consider these the greatest benefits of PTGBD followed by elective LC. Thus, this therapeutic strategy seems to be a feasible approach for moderate AC in patients who are receiving antithrombotic therapy. Based on our study, we have developed a new treatment strategy for AC patients with antithrombotic therapy in our institute (Fig. 2). However, our study was small and retrospective, and we did not directly compare our results with those in patients receiving early LC and continued antithrombotic treatment. Further investigation and data accumulation are expected.

Conclusion

The risks of postoperative complications, including severe hemorrhagic complications, were not increased by PTGBD followed by elective LC for moderate AC in patients who received antithrombotic therapy. We therefore conclude that PTGBD followed by elective LC for moderate AC is an acceptable treatment in patients who have received antithrombotic therapy. However, we must pay attention to all PTGBD-related complications, including minor complications.

Conflict of interest None declared.

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RESEARCH Open Access

Surgical management of hepatocellular carcinoma with tumor thrombi in the inferior vena cava or right atrium

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Abstract

Background: The prognosis for advanced hepatocellular carcinoma (HCC) with tumor thrombi in the inferior vena cava (IVC) or right atrium (RA) is poor, and there is no established effective treatment for this condition. Thus study aimed to evaluate the efficacy of surgical resection and prognosis after surgery for such cases.

Methods: Between January 1990 and December 2012, 891 patients underwent hepatectomy for HCC at our institution. Of these, 13 patients (1.5%) diagnosed with advanced HCC with tumor thrombi in the IVC or RA underwent hepatectomy and thrombectomy. Data detailing the surgical outcome were evaluated and recurrence-free and overall survival rates were calculated using the Kaplan-Meier method.

Results: Seven patients had an IVC thrombus and six had an RA thrombus. Extra-hepatic metastasis was diagnosed in 8 of 13 patients. Surgical procedures included three extended right lobectomies, three extended left lobectomies, five right lobectomies, and two sectionectomies. Right adrenal gland metastases were excised simultaneously in two patients. All IVC thrombi were removed under hepatic vascular exclusion and all RA thrombi were removed under cardiopulmonary bypass (CPB). Four patients (30.8%) experienced controllable postoperative complications, and there was no surgical mortality. The mean postoperative hospital stay for patients with IVC and RA thrombi was 23.6 ± 12.5 days and 21.2 ± 4.6 days, respectively. Curative resection was performed in 5 of 13 cases. The 1- and 3-year overall survival rates were 50.4%, and 21.0%, respectively, and the median survival duration was 15.3 months. The 1- and 3-year overall survival rates for patients who underwent curative surgical resection were 80.0% and 30.0%, respectively, with a median survival duration of 30.8 months. All patients who underwent curative resection developed postoperative recurrences, with a median recurrence-free survival duration of 3.8 months. The 1-year survival rate for patients who underwent noncurative surgery and had residual tumors was 29.2%, with a median survival duration of 10.5 months.

Conclusions: Aggressive surgical resection for HCC with tumor thrombi in the IVC or RA can be performed safely and may improve the prognoses of these patients. However, early recurrence and treatment for recurrent or metastatic tumors remain unresolved issues.

Keywords: Hepatocellular carcinoma, Inferior vena cava, Right atrium, Tumor thrombus, Surgery

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Background

Hepatocellular carcinoma (HCC) is a highly malignant tumor with a propensity for invading intrahepatic blood vessels such as the portal vein (PV) or hepatic vein in advanced stages [1]. Further extension of tumor thrombi from any of the three main hepatic veins or the right inferior hepatic vein can give rise to thrombi in the inferior vena cava (IVC) or right atrium (RA) [1-3]. Commonly, the prognosis of HCC patients presenting with IVC or RA thrombosis is extremely poor [4-6], and there is no established management for such cases [4,5,7-17]. Surgical removal of IVC and RA thrombi combined with hepatectomy is the only radical treatment to decrease the risk of systemic metastasis and sudden death due to pulmonary embolism or occlusion of the tricuspid valve with a tumor thrombus [18-20]. However, aggressive surgical resection is not common because the surgical approach to IVC and RA thrombi is considered complicated and hazardous and is applicable only in limited cases with good hepatic reserve [4,6,9,16,17]. Therefore, the efficacy of surgical treatment for HCC with IVC or RA thrombi remains unclear. In this study, we retrospectively investigated the surgical outcomes and prognoses of patients who underwent surgery for HCC with IVC or RA tumor thrombi in a single institution to clarify the safety and efficacy of surgical resection.

Methods

Patients and diagnoses

Between January 1990 and December 2012, 891 patients underwent hepatectomy for HCC at the Department of Gastroenterological Surgery, Hokkaido University, Japan. The diagnosis of HCC was determined by enhanced computed tomography (CT) and magnetic resonance imaging (MRI). IVC or RA thrombi were evaluated by CT (Figure 1). Among those studied, 13 patients (1.5%) diagnosed with advanced HCC and tumor thrombi in the IVC or RA underwent hepatectomy. This study was approved by the Institutional Review Board of the Hokkaido University School of Advanced Medicine.

The mean age at diagnosis was 63.4 years. The most common cause of HCC was hepatitis B virus (HBV) infection (53.8%), followed by hepatitis C virus (HCV) infection (15.4%). A total of 12 (92.3%) patients were male, and according to the Child-Pugh classification, all cases had Child-Pugh class-A disease. Six (46.2%) patients had a single tumor and seven (53.8%) had multiple tumors. The mean main tumor size was 11.8 cm, with nine tumors (69.2%) located in the right lobe and four (30.8%) in the left lobe. Extra-hepatic metastases were detected in eight of thirteen (61.5%) patients (five with lung, two with right adrenal gland and one with mediastinal lymph node metastases). Seven (53.8%) patients had an IVC thrombus and five (46.2%) had an RA thrombus (Table 1).

The tumor thrombus arose from the right hepatic vein in four patients (30.8%), middle hepatic vein in three (23.1%), left hepatic vein in one (7.7%), inferior right hepatic vein in two (15.4%), right hepatic vein combined with the middle hepatic vein in one (7.7%), and right hepatic vein combined with the inferior right hepatic vein in one (7.7%). In one patient with right adrenal gland metastasis, the tumor thrombus arose from the right adrenal vein (7.7%). Two patients had a mural thrombus and eleven had a massive thrombus. The massive thrombi in 10 patients did not completely occlude the IVC because circinate or arc-like luminal flow in the IVC around the tumor thrombi was present and the outflow canals of the intact hepatic veins were maintained. The tumor thrombus of one patient completely occluded the IVC inferior to the influx of hepatic veins accompanied by an aggregating blood thrombus. The outflow canals of the intact hepatic veins were severely narrowed but not completely occluded. One patient with a thrombus completely occluding the IVC and two patients with a massive IVC thrombus suffered preoperative renal insufficiency, and two had evident leg edema (Table 2).

Surgical procedures

A lobectomy was performed for patients with an indocyanine green retention rate at 15 minutes after injection (ICG R_{15}) of <15% and total bilirubin levels of <1.5 mg/dl without ascites. Patients with an ICG R_{15} of 15 to 20% and total bilirubin levels of 1.5 to 2.0 mg/dl were eligible for sectionectomy according to our criteria [21]. The type of surgical procedure was selected based on Couinaud's classification [22] and included three extended right hepatectomies, three extended left hepatectomies, five right hepatectomies, and two sectionectomies accompanied by thrombectomies. The right adrenal gland was resected simultaneously in two patients with right adrenal gland metastases (Table 3).

Hepatic resections were performed with an ultrasonic dissector using the Pringle maneuver in all cases. All IVC thrombi were removed under hepatic vascular exclusion (HVE). Before thrombectomy, hepatic transection was performed and the IVC was clamped below and above the liver. The IVC thrombus was excised en-bloc from the incised IVC, with satisfactory visualization of the intraluminal space under HVE. The IVC incision was closed by a simple continuous suture without a patch. All RA thrombi were removed under cardiopulmonary bypass (CPB). Following a laparotomy, a median sternotomy was performed to prepare for prompt CPB in anticipation of an undesirable pulmonary tumor embolism from a dislodged thrombus. Hepatic transection was then performed. The liver was handled gently, particularly if the thrombus had a long, thin neck, to

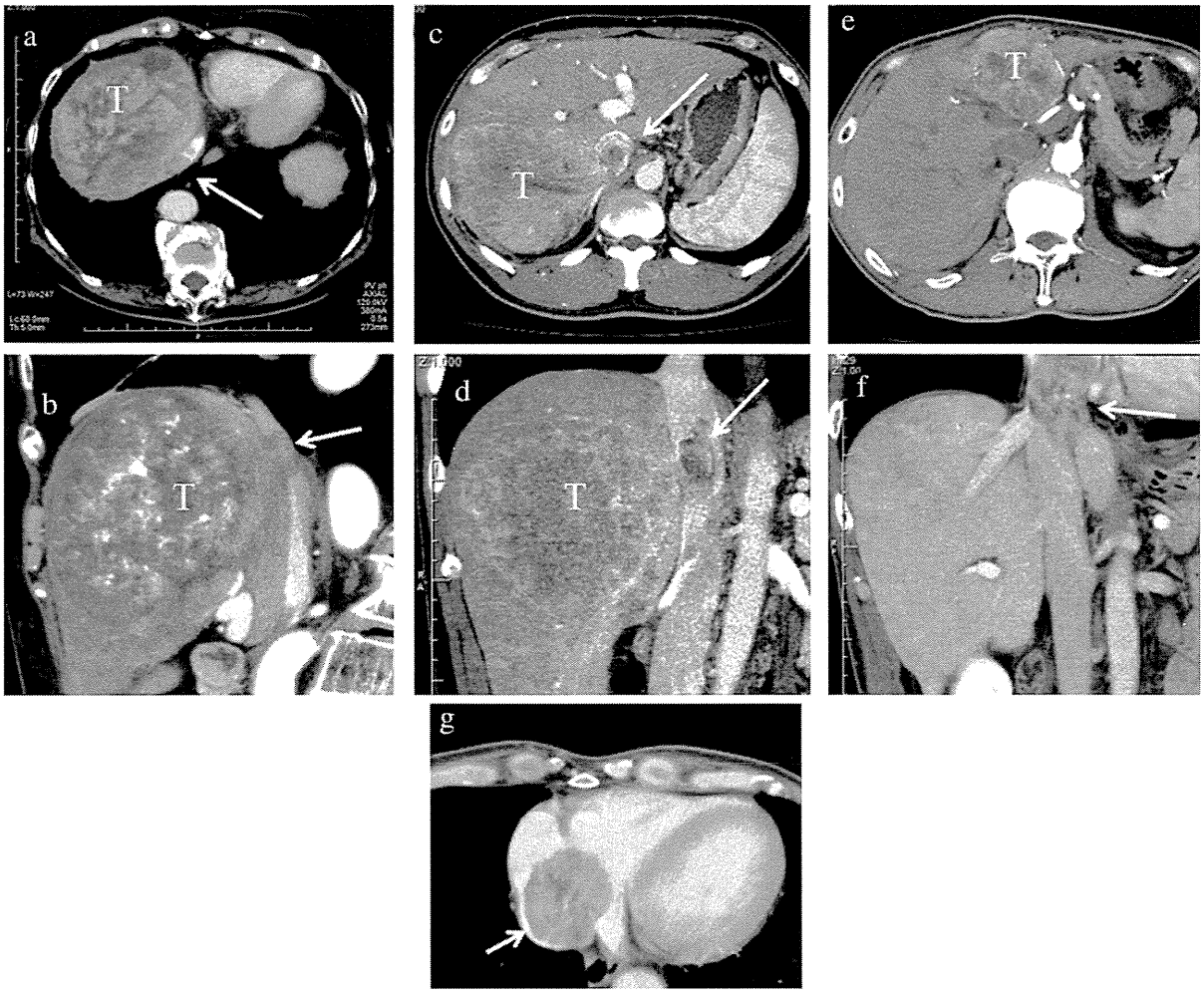

Figure 1 Representative computed tomography findings of hepatocellular carcinoma (HCC) with inferior vena cava (IVC) and right atrium (RA) thrombi. (**a** and **b**) A large HCC lesion in the right lobe (T), with a tumor thrombus arising from the right hepatic vein into the IVC (arrow). (**c** and **d**) HCC in the right lobe (T), with a tumor thrombus arising from the inferior right hepatic vein into the IVC (arrow). (**e**, **f**, and **g**) HCC in the left lobe (T), with a tumor thrombus arising from the left hepatic vein into the RA (arrow).

prevent dissemination of tumor thrombi. Then, the superior vena cava (SVC) and IVC below the liver were clamped and blood flow was bypassed to the ascending aorta via an oxygenator. The RA was incised and the thrombus was excised en-bloc under direct vision. In most cases, the RA was reconstructed by simple sutures, but in two cases, an invaded RA wall was partially excised and reconstructed using an artificial graft or pericardial patch. In addition to CPB, one patient with complete IVC occlusion accompanied by severe obstruction of intact hepatic outflow and one patient with tumor thrombi that arose from two major hepatic veins showed gross hepatic congestion due to outflow block at surgery. These cases needed extracorporeal bypass from the portal vein (PV) and IVC to SVC (Table 3). In all cases, thrombi were intraoperatively monitored by transesophageal echocardiography. In this study, we defined curative resection as macroscopic complete excision of the tumors, including metastatic lesions.

Follow up

The median duration of follow up was 11.2 (range, 1.8 to 51.8) months. Hospital death was defined as death occurring within 30 days of the first hospitalization. After surgery, CT or MRI was performed at 1- to 3-month intervals to determine recurrence. Data on surgical outcomes, postoperative management, recurrence, treatment of recurrence, and survival was analyzed for all cases.

Statistical analysis

Survival rates were analyzed by the Kaplan-Meier method and statistical significance was determined by

Table 1 Characteristics of patients and tumors

Characteristic	Value
Total number of patients	13
Age, years	
Mean ± SD (range)	63.4 ± 11.8 (37 to 86)
Sex	
Male/female	12/1
Hepatitis B virus	
Positive/negative	7/6
Hepatitis C virus	
Positive/negative	2/11
Child-Pugh classification	
A/B/C	13/0/0
Main tumor location	
Anterior/posterior/median/lateral section	5/4/3/1
Tumor size, cm	
Mean ± SD (range)	11.8 ± 4.3 (3.5–19)
Number of tumors	
Single/multiple	6/7
Extension of thrombus	
Inferior vena cava/right atrium	7/6
Preoperative extrahepatic metastases	
None/lung/adrenal gland/lymph nodes	5/5/2/1
Status of metastases after surgery	
Resected/regressed or stationary/progressed	3/1/4
Postoperative metastatic recurrence	
Liver/lung/lymph node/adrenal gland/inferior vena cava/brain	8/7/4/2/2/2

the log-rank test using JMP Pro 10.0.0 software (SAS, Cary, NC, USA). Significance was defined as $P < 0.05$.

Results

Surgical outcomes and postoperative complications

With regard to patients with an IVC thrombus, the mean surgical duration was 349 ± 30 minutes, the median blood loss was 950 ± 100 ml, and the mean HVE duration was 8.8 ± 3.1 minutes. Two of seven (28.6%) patients needed blood transfusions. No patient required an ICU stay, and the mean postoperative hospital stay was 23.6 ± 12.5 days. After surgery, one patient experienced biloma and one experienced controllable ascites. With regard to patients with an RA thrombus, the mean surgical duration was 608 ± 169 minutes, the median blood loss was 6540 ± 5404 ml, and the mean CPB duration was 32.2 ± 18.3 minutes. Five of six (83.3%) patients needed blood transfusions. The mean postoperative ICU stay was 1.7 ± 0.8 days and the mean postoperative hospital stay was 21.2 ± 4.6 days. After surgery, one patient experienced acute renal failure and one experienced atrial fibrillation, but these patients recovered with medical therapy. There was no postoperative mortality. All IVC and RA thrombi were excised completely. Curative resection was performed in five of thirteen (38.5%) cases (Table 4).

Postoperative management

Among the five patients (38.5%) who underwent curative resection, adjuvant systemic chemotherapy was administered to four. The chemotherapeutic agents used in combination included intravenous 5-fluorouracil (5-FU; 500 mg weekly) and peroral tegafur uracil (UFT; 300 mg daily) in three patients and peroral UFT (300 mg daily) in one. One patient was followed up without adjuvant chemotherapy.

Tumors remained after surgery in eight (61.5%) patients, including lung metastases in four, intrahepatic metastases in two, both intrahepatic and lung metastases in one, and mediastinal lymph node metastases in one. Residual lung metastases were treated with oral administration of UFT in two patients, 5-FU + UFT in one patient, and oral administration of tegafur gimeracil oteracil potassium (S-1) followed by surgical resection in one patient. Unresectable intrahepatic metastases were treated with UFT in two patients and transarterial chemoembolization (TACE) in one patient. A patient with residual mediastinal lymph node metastasis received radiation after surgery.

Recurrence and survival

All five patients who underwent curative resection experienced postoperative recurrences. Intrahepatic recurrences appeared in all five patients, lung metastases in four, intra-IVC metastases in one, and left adrenal gland metastases in one patient. The median recurrence-free survival duration of the patients who underwent curative resection was 3.8 months. Intrahepatic recurrences were treated with TACE in three patients, radiofrequency ablation (RFA) in two, and radiotherapy in one patient. Lung metastases were treated with systemic chemotherapy in three patients (5-FU + UFT in two, cisplatin (CDDP) + S-1 followed by oral administration of sorafenib in one patient), and surgical resection in one patient. Left adrenal gland metastases were surgically excised.

Among the eight patients who underwent noncurative resection, four of five with lung metastases exhibited progression of the metastases. In one patient, lung metastasis was resected but recurred after resection. Intrahepatic residual tumors in three patients progressed after surgery; however, mediastinal lymph node metastases treated by irradiation remained unchanged. Among these eight patients, seven experienced further dissemination of the tumor to new locations, including the lung in three, lymph

Table 2 Characteristics of tumor thrombi

Patients age/sex	Involved veins	Extent of thrombus	Advance of thrombus	Symptoms associated with the thrombus
68/M	RHV	Massive	RA	Renal insufficiency/lower limb edema
57/M	RAdV	Massive	IVC	Renal insufficiency
70/M	RHV	IVC occlusive	RA	Renal insufficiency/lower limb edema
86/F	RHV	Massive	IVC	(–)
68/M	IRHV	Mural	IVC	(–)
66/M	RHV	Massive	RA	(–)
37/M	IRHV	Massive	IVC	(–)
56/M	RHV/IRHV	Massive	IVC	(–)
51/M	LHV	Massive	RA	(–)
72/M	MHV	Mural	IVC	(–)
59/M	LHV/MHV	Massive	RA	(–)
69/M	MHV	Massive	IVC	(–)
65/M	MHV	Massive	RA	(–)

M male, *RHV* Right hepatic vein, *RAdV* right adrenal vein, *IRHV* inferior right hepatic vein, *LHV* left hepatic vein, *MHV*, middle hepatic vein, *IVC* inferior vena cava, *RA* right atrium. (–), no symptom.

nodes in three, brain in two, IVC in one, and adrenal gland in 1 (Tables 1 and 5).

The 1-, and 3-year overall survival rates for all 13 patients were 50.4% and 21.0%, respectively, and the overall median survival duration was 15.3 months. The cause of postoperative death in all patients was cancer, which remained at surgery or recurred after surgery (Table 5). The overall survival rate for patients with IVC thrombi was 57.1% at 1 year and 42.9% at 3 years, with median survival duration of 15.3 months. The 1-year overall survival rate for patients with RA thrombi was 40.0%, with median survival duration of 11.2 months. There was no significant difference between the IVC thrombi and RA thrombi groups (Figure 2a). The survival rates for patients who underwent curative surgical resection were 80.0% at 1 year and 30.0% at 3 years, with a median survival time of 30.8 months. Meanwhile, the 1-year survival rate for patients who underwent noncurative surgery and had residual tumors was 29.2%, with a median survival time of 10.5 months (Figure 2b). The longest survival time was 51.8 months for patients who underwent complete resection and 29.3 months (to date) for those who underwent incomplete resection, and they are still alive (Table 5).

Table 3 Surgical procedure

	Inferior vena cava thrombus (n = 7)	Right atrium thrombus (n = 6)
Surgical procedure		
Extended right hepatectomy	1	1
Extended right hepatectomy + right adrenectomy	0	1
Right hepatectomy	3	1
Right hepatectomy + right adrenectomy	1	0
Extended left hepatectomy	1	2
Sectionectomy	1	1
Inflow vascular control		
Hepatic vascular exclusion	7	0
Cardiopulmonary bypass	0	4
CPB + portal vein/inferior vena cava to superior vena cava bypass	0	2
Vascular wall reconstruction		
Simple closure	7	4
Patch reconstruction	0	2

Discussion

IVC and RA tumor thrombi arising from HCC are uncommon and are found in approximately 3 to 4% of HCC patients [2,23]. It is recognized that tumor invasion into intrahepatic vessels, such as the portal or hepatic veins, is an important prognostic factor for patients with HCC [24]. In particular, the prognosis of patients presenting with ICV or RA thrombi is extremely dismal [6]. Although surgical treatments as well as nonsurgical treatments such as TACE, radiotherapy, and chemotherapy are reported, optimal therapeutic management of IVC and RA thrombi has not been established because of the paucity of data [5,7,8,10-16]. Some reports demonstrate the potential benefit of surgical resection, but there are few reports that consolidate the efficacy of a surgical approach because IVC and RA thrombi are rare and because these reports are typically case reports or descriptions of a small number of patients [4,9,16,17,20,25,26]. Reports detailing the surgical treatment of RA thrombi

Table 4 Surgical outcomes

	Inferior vena cava thrombus	Right atrium thrombus
Surgical duration (minutes)		
Mean ± SD (range)	349 ± 30 (288 to 377)	608 ± 169 (449 to 911)
Blood loss (ml)		
Median ± SE (range)	950 ± 100 (750 to 1,520)	6540 ± 5404 (1,050 to 35,820)
Blood transfusion		
Yes/no	2/5	5/1
HVE time (minutes)		
Mean ± SD (range)	8.8 ± 3.1 (8 to 13)	-
CPB time (minutes)		
Mean ± SD (range)	-	32.2 ± 18.3 (4 to 54)
Curative resection		
Yes/no	3/4	2/4
ICU stay (days)		
Mean ± SD (range)	-	1.7 ± 0.8 (0–2)
Hospital stay (days)		
Mean ± SD (range)	23.6 ± 12.5 (14 to 48)	21.2 ± 4.6 (16 to 28)
Complications		
Yes/no	2 (ascites, 1; biloma, 1)/5	2 (ARF, 1; Af, 1)/4

HVE hepatic vascular exclusion, *CPB* cardiopulmonary bypass, *ARF* acute renal failure, *Af* atrial fibrillation.

are particularly rare, and to our knowledge this is the first report on the surgical treatment of IVC and RA thrombi, including six cases of RA thrombectomy, from a single institute.

It is generally assumed that liver resection combined with IVC or RA thrombectomy is a challenging and hazardous procedure that involves a high surgical risk. According to past reports, hepatectomy together with IVC or RA thrombectomy was associated with a high morbidity of 40% and a high mortality of 15% [4,15]. However, recent surgical innovations such as the inflow vascular control method together with refinement of the assessment of preoperative hepatic reserve have improved the safety of hepatectomy and thrombectomy procedures [26,27]. This progress has encouraged us to accept the challenge of aggressive surgical treatment for IVC and RA thrombi.

Effective control of intraoperative hemorrhage plays a crucial role in hepatectomy procedures combined with

Table 5 Characteristics and prognosis of patients

Patients age/sex	Tumor thrombus	Residual tumor		Metastatic recurrence		Outcome (cause of death)
		Hepatic	Distant	Hepatic	Distant	
68/M	RA	(–)	(–)	(+)	(+) (lung, Ad)	30.8 months; dead (cancer)
57/M	IVC	(–)	(–)	(+)	(–)	10.1 months; dead (cancer)
70/M	RA	(+)	(–)		(+) (lung)	9.1 months; dead (cancer)
86/F	IVC	(–)	(–)	(+)	(+) (lung, IVC)	15.3 months; dead (cancer)
68/M	IVC	(–)	(–)	(+)	(+) (lung, LN)	51.8 months; alive
66/M	RA	(–)	(+) (lung)	(+)	(+) (LN)	11.2 months; dead (cancer)
37/M	IVC	(–)	(+) (lung)	(–)	(+) (Ad, LN, Brain)	10.5 months; dead (cancer)
56/M	IVC	(–)	(+) (lung)	(–)	(–)	29.3 months; alive
51/M	RA	(–)	(+) (lung)	(+)	(+) (Brain)	8.5 months; dead (cancer)
72/M	IVC	(+)	(+) (lung)		(+) (LN)	1.9 months; dead (cancer)
59/M	RA	(–)	(–)	(+)	(+) (lung)	16.5 months; alive
69/M	IVC	(–)	(+) (LN)	(+)	(+) (lung, IVC)	16.0 months; alive
65/M	RA	(+)	(–)		(+) (lung)	7.6 months; alive

Ad adrenal gland, *IVC* inferior vena cava, *LN* lymph node.