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Long-Term Outcome of Combined Interferon-α and 5-Fluorouracil Treatment for Advanced Hepatocellular Carcinoma with Major Portal Vein Thrombosis

Hiroaki Nagano[a] Hiroshi Wada[a] Shogo Kobayashi[a] Shigeru Marubashi[a] Hidetoshi Eguchi[a] Masahiro Tanemura[a] Yoshito Tomimaru[a] Keigo Osuga[b] Koji Umeshita[c] Yuichiro Doki[a] Masaki Mori[a]

Departments of [a]Surgery, [b]Radiology and [c]Health Science, Graduate School of Medicine, Osaka University, Suita, Japan

Key Words

Hepatocellular carcinoma · Interferon · Portal vein tumor thrombosis · Arterial infusion chemotherapy

Abstract

Background/Aim: We previously reported the beneficial effects of a combination therapy of interferon (IFN)-α/5-fluorouracil (FU) for advanced hepatocellular carcinoma (HCC) with tumor thrombi in the major portal branches. This report describes the results of longer follow-up and includes more than twice the number of patients relative to the previous report; it also evaluates the clinical predictor on the response to the combination therapy and long-term survival. ***Methods:*** The study subjects were 102 patients with advanced HCC and tumor thrombi in the major branches of the portal vein (Vp3 or 4). They were treated with at least 2 courses of IFN-α/5-FU. ***Results:*** No major treatment-related complications were noted. In the 102 patients, 40 (39.2%) showed objective response [11 (10.8%) showed complete response, 29 (28.4%) partial response], 8 (7.9%) showed no response and 54 (52.9%) showed progressive disease. ***Conclusion:*** IFN-α/5-FU combination therapy is a promising modality for advanced HCC with tumor thrombi in the major portal branches.

Introduction

The prognosis of patients with advanced hepatocellular carcinoma (HCC) remains poor, particularly in patients with tumor thrombi in the major trunk of the portal vein (Vp4) [1–3]. The mortality rate is very high in patients with unresectable tumors and the quality of life is poor due to intractable ascites or esophageal bleeding. Even in patients with resectable HCC, the prognosis is extremely poor despite aggressive surgery [4, 5]. In such a situation, conventional therapies generally have no clinical effect on HCC associated with portal tumor thrombi due to poor efficacy and possible complications [6, 7]. Arterial infusion chemotherapy has also been attempted, but its effectiveness is still unsatisfactory for portal venous tumor thrombus (PVTT) [8, 9]. Therefore, a new strategy is required for patients with intractable HCC and tumor thrombi in the major branch of the portal vein.

Several recent studies have indicated the beneficial effects of interferon (IFN)-α-based combination chemotherapies for HCC [10–15], in spite of the lack of satisfactory results from IFN-α monotherapy [16]. We also reported the clinical efficiency of IFN-α and 5-fluorouracil (5-FU) combination therapy for advanced HCC with PVTT and intrahepatic metastasis [17–22].

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Hiroaki Nagano, MD, PhD
Department of Surgery
Graduate School of Medicine, Osaka University
2-2, Yamadaoka E-2, Suita 565-0871 (Japan)
Tel. +81 6 6879 3251, E-Mail hnagano@gesurg.med.osaka-u.ac.jp

Table 1. Patient characteristics

	Patients (n = 102)
Age, years	59.6 ± 9.4
Gender, male/female	94/12
Hepatitis virus	
HBV (+), HCV (–)	27
HBV (–), HCV (+)	54
HBV (+), HCV (+)	14
HBV (–), HCV (–)	9
Unknown	2
Granulocytes, /ml	4,420 ± 1,648
Platelets, $\times 10^4$/ml	13.3 ± 6.7
Serum albumin, g/dl	3.24 ± 0.44
Serum bilirubin, mg/dl	0.98 ± 0.36
Prothrombin time, s	17.9 ± 2.0
Child–Pugh classification	
A	38
B	63
C	4
Unknown	1
AFP, ng/ml	
<5	4
≥5	101
Unknown	1
PIVKA-II, mAU/ml	
<40	3
≥40	102
Unknown	1

The present study is the long-term outcome of the clinical effects of the combination therapy of subcutaneous IFN-α and arterial infusion of 5-FU in 102 patients with HCC associated with Vp4 and multiple intrahepatic metastases (IM3) [1], as an extension to our previous work [18, 19].

Patients and Methods

Patients and Selection Criteria

This was a single-arm open-label study, based on our pervious reports [18, 19]. Between December 1997 and December 2008, 102 patients with advanced HCC were enrolled. All patients were confirmed radiologically to have tumor thrombi in the main trunk of the portal vein (Vp4) and IM3. The diagnosis was based on liver function tests, serum α-fetoprotein (AFP), serum protein induced by vitamin K absence or antagonist-II (PIVKA-II) and imaging techniques including computed tomography (CT) scan, magnetic resonance imaging (MRI), hepatic angiography and arterial portography.

The following were the eligibility criteria for selection for intra-arterial combination therapy: (1) age of more than 20 years and less than 75 years; (2) tumor thrombi invading at least one of the main branches of the portal vein; (3) presence of multiple intrahepatic metastases in more than three segments (IM3); (4) absence of extrahepatic metastases; (5) a granulocyte count of more than 2,500/μl and less than 12,000/μl; (6) a red blood cell count of more than 8.0 g/dl; (7) a platelet count exceeding 8×10^4/μl; (8) GOT and GPT of less than 100 IU/l; (9) total bilirubin less than 1.4 g/dl; (10) serum BUN less than 30 mg/dl; (11) serum creatinine less than 1.5 mg/dl; (12) successful implantation of intra-arterial catheter and drug delivery system; (13) a performance status of level 0–2 (Eastern Cooperative Oncology Group, ECOG) [23]. These eligibility criteria were based on our previous report [18, 19]. All patients signed informed consent documents approved by the institutional review board attesting to the fact that they were aware of the investigational nature of the study and were willing to try the combination therapy.

The baseline characteristics of the enrolled 102 patients who received IFN/5-FU combined treatment are shown in table 1 (age, gender, hepatitis virus, granulocytes, platelet, albumin, bilirubin, prothrombin time, Child-Pugh classification, AFP and PIVKA-II).

Treatment Protocol of IFN/5-FU Combination Therapy

In each of the 102 patients, an intra-arterial catheter was inserted through the subclavian or femoral artery, with a subcutaneously implanted drug delivery system [24]. Each patient was treated with subcutaneous IFN-α (OIF; Otsuka Pharmaceutical Co., Tokushima, Japan) and intra-arterial infusion of 5-FU (Kyowa Hakko Co., Tokyo, Japan). One cycle of the treatment consisted of 4 weeks. IFN-α (5×10^6 U, 5 MU) was administered subcutaneously on days 1, 3 and 5 of each week, resulting in a total dose of 60 MU in a cycle. Continuous infusion chemotherapy (5-FU, 300 mg/m^2/day) through the proper hepatic artery was performed on the 1st and 2nd weeks via a catheter connected to a subcutaneously implanted drug delivery system. Two- or three-week rest period (cessation of drug therapy) separated the treatment cycles. All anticancer therapies were discontinued when adverse effects reached level 2 of the ECOG classification [23] (with the exception of platelet and leukocyte counts of less than 40,000 and 2,000/mm^3, respectively, since these parameters were often low prior to treatment due to the associated liver cirrhosis) [18, 19].

Evaluation of Response to IFN/5-FU Combination Therapy

A pretreatment evaluation was conducted at the commencement of IFN-α/5-FU protocol and posttreatment evaluation after completion of the 2-cycle treatment, almost 3 months later. The evaluation was performed using CT or MRI, and changes in serum tumor markers, such as AFP and PIVKA-II. All cases were compared at these two time points for the evaluation of antitumor effect. The objective response was classified according to the ECOG criteria [23]. Complete response (CR) was defined as normalization of tumor markers and disappearance of all tumors and portal vein thrombosis on CT and/or MRI. Partial response (PR) represented a decrease in tumor markers and 50–99% regression on the two-dimensional measurement. No change (NC) represented less than 50% regression or less than 25% progression. Progressive disease (PD) represented more than 25% progression. In addition, we also evaluated progression-free and overall survival rates. The follow-up period was 12–120 months.

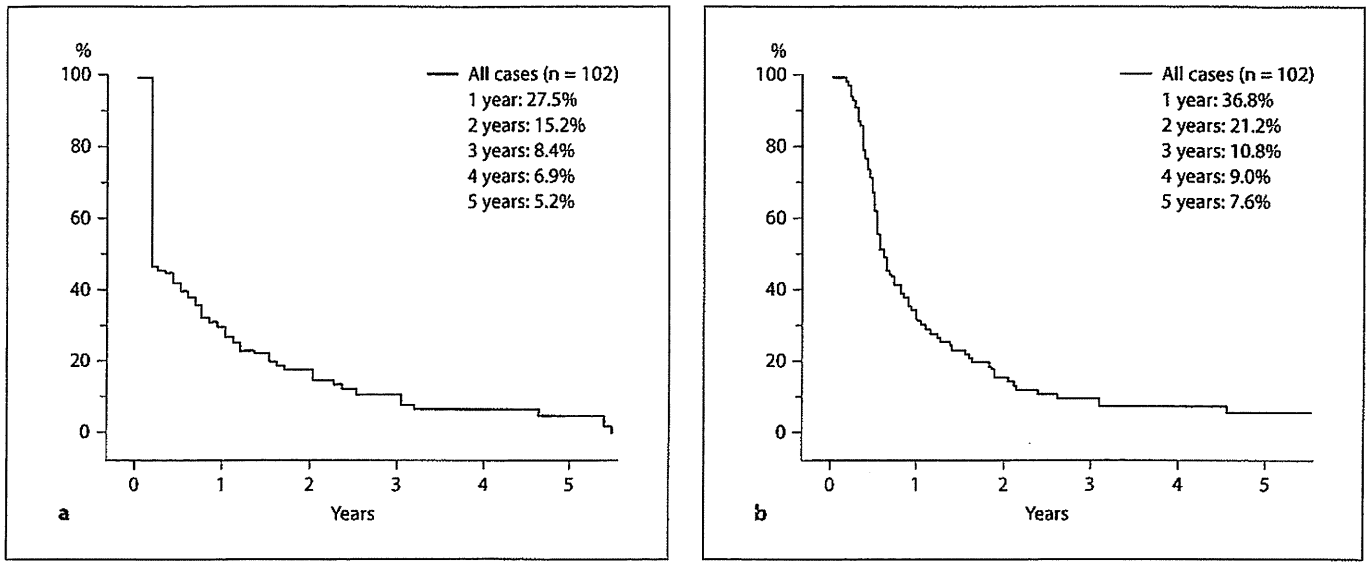

Fig. 1. Kaplan-Meyer analysis for efficiency of IFN/5-FU combination therapy. **a** Progression-free survival curve in all cases. The median progression-free survival period was 2.0 months, and the 1-, 3- and 5-year progression-free survival rates were 27.5, 8.4 and 5.2%, respectively. **b** Overall survival curve in all cases. The median overall survival period was 9 months, and the 1-, 3- and 5-year survival rates were 36.8, 10.8 and 7.6%, respectively.

Statistical Analysis

The Breslow-Gehan-Wilcoxon univariate test was used to examine the possible relationship between the effect of therapy (CR, PR vs. NC, PD), Child-Pugh score, serum AFP, serum PIVKA-II, Okuda score and CLIP score [3]. Survival curves were constructed using the Kaplan-Meier method. Differences in distribution between groups were compared by the χ^2 test and differences in mean values by Student's t test. All data were expressed as means ± SD. A p value less than 0.05 denoted the presence of a statistically significant difference.

Results

Clinical Response to Combination Therapy

All patients completed at least two cycles of the IFN/5-FU combination therapy. For patients who showed clinical response, we continued this combination therapy, while in those who showed no effect, we stopped the treatment after the completion of the second cycle, because of the extensive progression of HCC.

With regard to the clinical effect, 40 (39.2%) showed objective response, 11 (10.8%) showed CR, 29 (28.4%) showed PR, 8 (7.9%) showed NC and 54 (52.9%) showed PD. With respect to time to progression, the median progression-free survival period was 2.0 months, and the 1-, 3- and 5-year progression-free survival rates were 27.5, 8.4 and 5.2%, respectively. Furthermore, the median overall survival period was 9 months, and the 1-, 3- and 5-year survival rates were 36.8, 10.8 and 7.6%, respectively. The median progression-free survival period of CR/PR cases (n = 40) was 18.5 months and that of NC/PD cases (n = 62) was 2.0 months. The 1-, 3- and 5-year progression-free survival rates of CR/PR cases were 70.0, 21.3 and 13.3%, respectively, and those of NC/PD cases were 0, 0 and 0%, respectively.

The median survival time of CR/PR cases (n = 40) was 25 months and that of NC/PD cases (n = 62) was 6 months. The median follow-up time of survived patients was 30 months. The 1-, 3- and 5-year survival rates of CR/PR cases were 82.7, 28.6 and 18.9%, respectively, and those of NC/PD cases were 4.8, 0 and 0%, respectively. The progression-free survival and overall survival curves are shown in figures 1 and 2, respectively. There were significant differences in the progression-free survival and the overall survival between responders (CR/PR) and nonresponders (NC/PD) ($p < 0.0001$).

Adverse Effects

None of the patients developed side effects related to catheter insertion or subcutaneous implantation of the drug delivery system. However, 8.8% of patients developed grade 3 leukopenia, thrombocytopenia or anae-

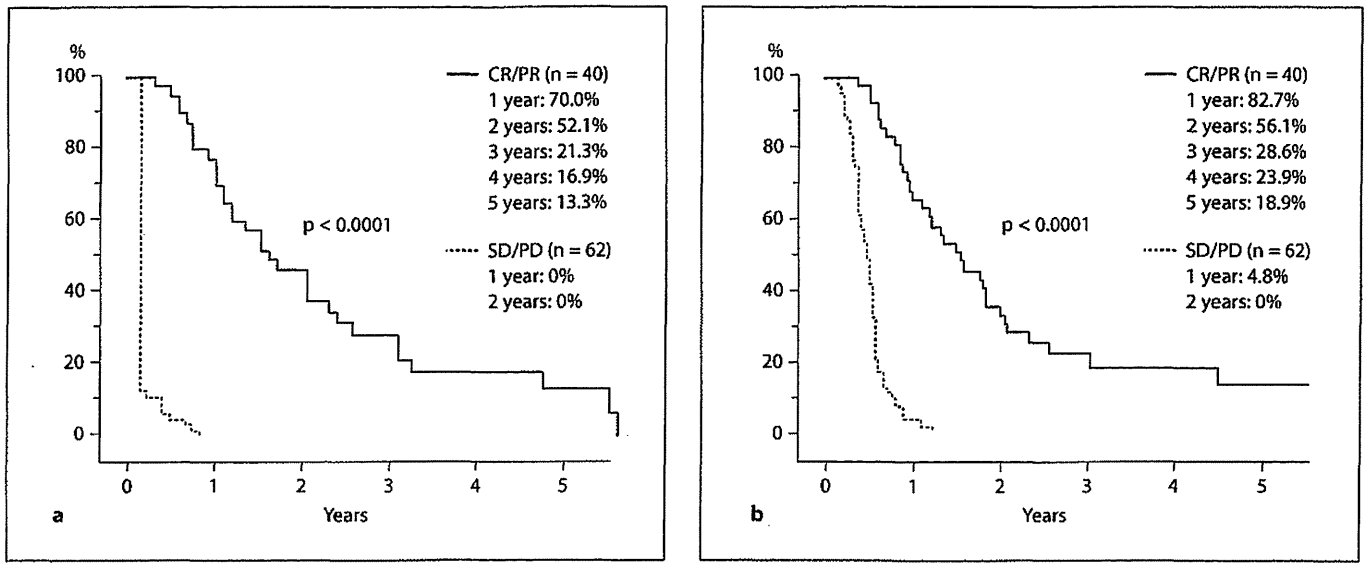

Fig. 2. Kaplan-Meyer analysis for efficiency of IFN/5-FU combination therapy. **a** Progression-free survival curves in CR/PR and NC/PD cases. The 1-, 3- and 5-year progression-free survival rates of CR/PR cases were 70.0, 21.3 and 13.3%, respectively, and those of NC/PD cases were 0, 0 and 0%, respectively. **b** Overall survival curves in CR/PR and NC/PD cases. The 1-, 3- and 5-year survival rates of CR/PR cases were 82.7, 28.6 and 18.9%, respectively, and those of NC/PD cases were 4.8, 0 and 0%, respectively. There were significant differences in the progression-free survival and the overall survival between responders (CR/PR) and nonresponders (NC/PD) ($p < 0.0001$).

mia, but drip transfusion of granulocyte colony-stimulating factors was not used during this study. Nonhematological toxicities included grade 1 or 2 fever (100% of patients), chilling sense (92.3%), nausea (6.9%), diarrhea (3.6%), gastric ulcer (2.9%), flu-like syndrome (100%), skin reaction (4.9%), general fatigue (31.3%) and depression (2.9%). The side effects are summarized in table 2.

Clinical Correlations

Finally, we compared the responders (CR/PR) (n = 10) with nonresponders (NC/PD) (n = 20) in terms of serum AFP (within normal range; <5), serum PIVKA-II (normal range; <45), Child-Pugh score, OKUDA score and CLIP score by univariate analysis. Serum AFP, PIVKA-II, Child-Pugh score, OKUDA score and CLIP score did not correlate with the response to combination therapy, similar to our previous report [19] (data not shown).

Table 2. Adverse effects

	Patients (n = 102)			
	grade 1	grade 2	grade 3	grade 4
Hematological				
Leukopenia	14	23	6	0
Anemia	0	1	3	0
Thrombocytopenia	16	20	9	0
Nonhematological				
Fever	97	5	0	0
Chilling sense	94	0	0	0
Nausea	7	0	0	0
Diarrhea	4	0	0	0
Gastric ulcer	0	3	0	0
Flu-like syndrome	102	0	0	0
Skin reaction	5	0	0	0
General fatigue	32	0	0	0
Depression	3	0	0	0

Discussion

In this study, we showed the beneficial effects of IFN-α/5-FU combination therapy in patients with multiple lesions and tumor thrombi in the major branches of the portal vein (Vp3 or 4), as our third report on this combined treatment. The efficacy of such treatment was 39.2% in our patients with highly advanced HCC, which was almost similar to the others and our previous reports of patients with the same stage HCC [15, 19, 25]. The

prognosis of such patients is extremely poor and survival is generally limited to a few months after diagnosis, despite multimodal therapies even in cases suitable for surgical resection [26]. The combination treatment IFN-α and 5-FU markedly decreased tumor size and levels of tumor markers with an encouraging response rate and prolonged survival time in the responders. Furthermore, the clinical response completely reflected the survival benefits, as shown in figures 1 and 2. There are several other reports about the possibilities as a treatment for advanced HCC with PVTT, such as intra-arterial infusion chemotherapy with 5-FU and CDDP [27–29] or transarterial chemoembolization [30]. A certain level of antitumor effect has been shown in 5-FU and CDDP intra-arterial chemotherapy for the lower stage of HCC patients, but not just for PVTT; antitumor effect for the HCC partially including PVTT patients were not significant compared to IFN-α/5-FU combined treatment in terms of median survival time, response rate and overall survival. Transarterial chemoembolization was reported as an effective treatment for advanced HCC with PVTT in RCT, but the clinical outcome was not better than IFN and 5-FU combined treatment. From these findings, the clinical result of IFN and 5-FU combined treatment was promising for the disastrous advanced HCC with PVTT patients.

On the other hand, no response to the combination therapy was seen in 60.8% (62/102) of the patients in this study. To advance the effect of IFN-α/5-FU combination therapy and to increase the response rate, it is necessary to investigate the mechanism of IFN-α/5-FU combination therapy. Among the nonresponders, there were only a few NC (8/102) in this study, in spite of the mostly chemo-resistant disease. We reasoned this finding to the following; the HCC in this series was far advanced and HCC progression was extremely rapid and aggressive. Under such conditions, almost all nonresponders died within 12 months (59/62); 40 of 62 cases (64.5%) within 6 months. For nonresponders to this treatment, however, the survival period was too short to allow receiving another treatment modality. Therefore, accurate prediction of chemosensitivity is desirable not only for loss of a limited chance for another possible treatment but also to avoid potentially serious side effects. However, there are no suitable markers that could distinguish patients who are likely to respond to this combination chemotherapy from those who are not. In this point, Obi et al. [15] recommended to start the combination therapy with close monitoring of response, preferably that of tumor biomarkers, and treatment should be continued if there is a response after the first cycle of chemotherapy.

Several mechanisms for the anticancer effects of IFN-α, with or without 5-FU, have been proposed [31–34]. We showed previously that IFN-α and 5-FU synergistically inhibit tumor cell proliferation with cell cycle arrest [35] and induced apoptosis by regulating the apoptosis-related molecules [36] as well as an antiangiogenic effect [37]. We also reported that tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), its receptor pathway [38] and Fas and Fas-L pathway [39] partially contributed to the antitumor effects of IFN-α and 5-FU combination therapy. About apoptosis induction, the close involvement of P53 has been reported [40, 41]. Moreover, IFN-α suppresses the proliferation of all type I interferon receptor 2 (IFNAR2)-positive HCC cell lines in vitro through mechanisms related to apoptosis or inhibition of cell cycle [42]. The importance of IFNAR2 expression for the anticancer effect of IFN/5-FU was highlighted in a similar situation in our previous report [35, 36, 43]. In addition, we reported the significance of Ep-CAM [44] and IGFR-7 [45] as a noble biomarker to assess the antitumor effect of IFN/5-FU combined treatment. CD133 may be related to antitumor effect of IFN and 5-FU as a predictor in perspective of cancer stem cell [46].

The combination of IFN/5-FU is not effective against extrahepatic metastases. This is understandable because 5-FU, administered into the hepatic artery, will not reach extrahepatic tissues in high concentration. However, systemic administration of 5-FU or related agents may be effective against extrahepatic lesions in combination with IFN-α [47]. This possibility is highly interesting since the implantation of dwelling catheter is one of the demerits of the present combination therapy [15]. Recently, several molecularly targeting agents have been developed and applied for HCC treatment [48–51]. Especially sorafenib is the first agent leading to improved overall survival with advanced HCC, revealed in a phase III clinical trial [51]. These molecularly targeting agents are a very effective therapeutic modality, which has the different mechanism of antitumor effect from IFN/5-FU combination as an cytotoxic medicine. We reported actually that PTK/ZK, a kind of molecularly targeting medicine, enhanced the antitumor effect of IFN/5-FU in vitro [52]. After this, mutual interaction and sharing roles might be very important for the progression of the treatment for intractable advanced HCC.

In conclusion, we demonstrated the long-term outcome about the efficacy of IFN/5-FU combination therapy for advanced HCC patients with tumor thrombi in major branches of the portal vein.

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Prognostic Value of Endoscopic Biopsy Findings After Induction Chemoradiotherapy With and Without Surgery for Esophageal Cancer

Hiroshi Miyata, MD, Makoto Yamasaki, MD*, Shuji Takiguchi, MD*, Kiyokazu Nakajima, MD*, Yoshiyuki Fujiwara, MD*, Koji Konishi, MD†, Eiichi Morii, MD‡, Masaki Mori, MD*, and Yuichiro Doki, MD**

Objective: To investigate the value of endoscopic biopsy in predicting the clinicopathological response and survival in patients with esophageal cancers who received chemoradiotherapy (CRT) alone or CRT followed by surgery.

Background: Endoscopic biopsy examination after CRT for esophageal cancer has been used to confirm the presence of residual tumor before surgery, but there is little or no information on the clinical significance of the results of endoscopic biopsy in neoadjuvant or definitive CRT.

Methods: We studied 189 patients who underwent endoscopic biopsy after induction CRT (40 Gy) for esophageal cancer, consisting of 123 patients who received neoadjuvant CRT (40 Gy) followed by surgery and 66 patients who underwent definitive CRT (mostly more than 60 Gy). The correlations between the results of endoscopic biopsy and clinicopathological factors, including response to CRT and survival, were examined.

Results: For neoadjuvant CRT, endoscopic biopsy findings correlated significantly with pathological tumor regression and lymph node involvement, although the majority of cases with negative biopsy (64%) displayed residual tumor cells in the surgical specimen. The 5-year survival rate was significantly higher in patients with negative biopsy (48.3%) than in those with positive biopsy (21.8%, $P = 0.006$). For definitive CRT, patients with negative biopsy at the time of 40 Gy showed clinical complete response to CRT ($P = 0.002$) and had significantly better 3-year survival (57.0%) than those with positive biopsy (22.5%, $P = 0.0008$).

Conclusions: The results of endoscopic biopsy examination after induction CRT can predict the response to CRT and prognosis of patients who receive CRT with and without surgery.

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Esophagectomy is traditionally used as the standard treatment of locoregional esophageal cancer. However, the majority of patients who undergo curative resection subsequently develop locoregional or systemic recurrence, leading to unfavorable prognosis.[1–4] To improve prognosis, multimodal therapy, including chemotherapy and radiotherapy, in addition to surgery, has been used.[5–7] In fact, preoperative chemoradiotherapy (CRT) is widely used for treatment of patients with locally advanced esophageal cancers. Although controversy exists as to whether preoperative CRT offers survival benefits, previous studies reported that neoadjuvant CRT before surgery increased complete resection rates and improved prognosis in patients with good response to CRT,[5–14] with pathological complete response (pCR) being achieved in 15% to 32% of patients who received preoperative CRT.[15–20]

From the Departments of *Gastroenterological Surgery, †Radiation Oncology, and ‡Pathology, Graduate School of Medicine, Osaka University, Osaka, Japan.
Reprints: Hiroshi Miyata, MD, Department of Gastroenterological Surgery, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan. E-mail: hmiyata@gesurg.med.osaka-u.ac.jp.

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Chemoradiotherapy alone without surgical resection is used as another treatment option for locally advanced esophageal cancer. Previous studies that compared neoadjuvant CRT followed by surgery with CRT alone suggested that the prognosis of patients treated with CRT alone is comparable with that of patients who underwent neoadjuvant CRT followed by surgery, especially in those who show good response to preoperative CRT.[21,22] This raises the question of whether or not the patients who achieve complete response (CR) after preoperative CRT actually need subsequent surgical resection. Thus, early assessment of the response to induction therapy may make it possible to individualize therapy on the basis of the response to CRT.

Various imaging studies, including computed tomography (CT), endoscopy, and endoscopic ultrasound, have been used to evaluate the clinical response to preoperative (induction) CRT.[23–27] Furthermore, several recent studies have demonstrated that 18-fluorodeoxyglucose positron emission tomography (PET) imaging after induction therapy can predict outcome.[28–31] Several studies carried out previously examined whether endoscopic biopsy can accurately predict the presence of residual tumor after neoadjuvant CRT for esophageal cancers, but the value of post-CRT endoscopic biopsy in predicting the response to treatment and survival in patients who underwent CRT with or without surgery is not clear.[32–35]

In the present study, we determined whether endoscopic biopsy after induction CRT (40 Gy) can predict the histopathological response and prognosis of patients who were subsequently treated with surgery or continued the course of definitive CRT only. Moreover, we also analyzed whether endoscopic biopsy after induction CRT provides useful information for selection of further therapy.

MATERIALS AND METHODS

Patients and Treatment Protocols

Between January 1994 and December 2007, 584 patients with thoracic esophageal cancers underwent surgery at the Department of Gastroenterological Surgery, Graduate School of Medicine, Osaka University, Osaka, Japan. Among them, 128 patients underwent esophagectomy after neoadjuvant CRT for thoracic esophageal cancer. During the same period, 141 patients with thoracic esophageal cancers received definitive CRT alone without surgical resection. Of these 269 patients who received CRT as initial treatment of thoracic esophageal cancer, 123 patients who underwent endoscopic biopsy after neoadjuvant CRT and 66 patients who underwent endoscopic biopsy at the time of 40 Gy irradiation in the definitive CRT group were included in the present study. Basically, neoadjuvant CRT followed by surgery was selected for patients who had invasive thoracic esophageal cancers (T3-T4) without distant organ metastasis or those whose tumor was located in the upper third of the thoracic esophagus with infiltration of the cervical esophagus. On the contrary, definitive CRT was basically provided for patients who elected to be treated with CRT as curative treatment. During the time of this study, all patients who had started receiving CRT as neoadjuvant intent underwent esophagectomy even if clinical CR was achieved after neoadjuvant

CRT. All 189 patients were diagnosed with squamous cell carcinoma of the thoracic esophagus by pretreatment biopsy samples.

To evaluate the response to CRT, endoscopy was routinely performed after neoadjuvant CRT and at the time of 40 Gy irradiation during the course of definitive CRT. Basically, 3 or more biopsies were taken at endoscopic evaluation. Patients who did not undergo endoscopic examination because of CRT-related toxicity, those who did not have a biopsy by endoscopic examination, and those who did not have sufficient amounts of tissue biopsy samples for histopathological examination were excluded from the study. Endoscopic evaluation was necessary after 40 Gy in the neoadjuvant CRT group and after 60 Gy in the definitive CRT group. However, endoscopic evaluation after 40 Gy in the definitive CRT group was optional for this study. There were no significant clinical differences between patients with endoscopic biopsy and those without endoscopic biopsy for patients who underwent definitive CRT (Table 1).

The CRT treatment regimen included administration of a single daily fraction of 2 Gy concurrently with cisplatin and 5-fluorouracil. 5-fluorouracil was administered by continuous intravenous administration at a dose of 400 mg/m^2 in combination with cisplatin at 10 mg/m^2 administered by drip for 5 days per week. Patients who underwent neoadjuvant CRT followed by surgery received a total dose of 40 Gy radiation, in combination with chemotherapy. On the contrary, those on definitive CRT received a total dose of more than 54 Gy (range, 54–68 Gy). In the neoadjuvant CRT, patients underwent endoscopic biopsy within 1 week of the completion of neoadjuvant CRT and underwent surgical resection 4 to 6 weeks after the completion of neoadjuvant CRT. Among the 123 patients who underwent surgical resection, 34 patients underwent transthoracic esophagectomy with 2-field lymphadenectomy, 63 underwent transthoracic esophagectomy with 3-field lymphadenectomy, and 26 patients underwent esophagectomy using the trans-hiatal approach. In the definitive CRT, patients underwent endoscopic biopsy at the time of 40 Gy irradiation (within 1 week), and after an interval of 1 to 2 weeks, radiation was delivered up to 54 to 68Gy. Four to 6 weeks after the completion of definitive CRT, endoscopic biopsy was obtained again to evaluate the clinical response of CRT. Complete follow-up information until death or July 2009 was available for all patients.

In this study, all patients were staged before and after surgery according to the criteria of the International Union Against Cancer (UICC). Pretreatment clinical staging was based on oesophagogeography, endoscopy, and CT of the neck, chest, and upper abdomen by using continuous 5-mm-thick slices. Bronchoscopy was performed when tracheobronchial involvement was suspected. From March 2000, PET was also used in our facility for clinical staging where possible. Lymph nodes were diagnosed as metastasis-positive on CT scan if they were greater than 1.0 cm in maximum transverse diameter. Lymph nodes visible but smaller than 1.0 cm on the long axis on CT scan were regarded as metastasis-positive only if focal prominent 18-fluorodeoxyglucose uptake, relative to normal mediastinal activity, was also detected on the PET scan.

The protocol of this retrospective study was approved by the Human Ethics Review Committee of Osaka University Graduate School of Medicine and a signed consent form was obtained from each subject.

TABLE 1. Patients' Characteristics

	Neoadjuvant CRT	Definitive CRT		
		Biopsy (+)	Biopsy (−)	*P* value
n	123	66	75	
Mean age	61.0	66.1	65.6	0.623
Gender (male/female)	107/16	58/8	68/7	0.592
Tumor location				
Upper	61 (50)	19 (29)	18 (24)	0.189
Middle	44 (36)	33 (50)	48 (64)	
Lower	18 (14)	14 (21)	9 (12)	
Tumor depth				
cT1	0 (0)	20 (30)	22 (29)	0.417
cT2	19 (15)	8 (12)	6 (8)	
cT3	39 (32)	20 (30)	20 (27)	
cT4	65 (53)	18 (28)	27 (36)	
Nodal status				
cN0	36 (29)	32 (48)	38 (51)	0.796
cN1	87 (71)	34 (52)	37 (49)	
Mean radiation dose (Gy)	40.0	61.6	60.6	0.173
Clinical response				
CR	12 (10)	37 (56)	32 (43)	0.125
PR	86 (70)	16 (24)	23 (31)	
NC/PD	25 (20)	13 (20)	20 (26)	

CR indicates complete response; NC/PD, no change or progressive disease; PR, partial response.

Evaluation of Clinical Response

After completion of neoadjuvant or definitive CRT, all patients were restaged by CT scan, endoscopy, and, in recent cases, PET to evaluate the clinical response to CRT. The response was categorized on the basis of the World Health Organization response criteria for measurable disease and the criteria of the Japanese Society for Esophageal Diseases.[36] A CR was defined as complete regression of disease for at least 4 weeks on the basis of CT scan and/or PET scan and endoscopy. The patient was not considered as to have achieved CR when persistent ulceration and/or presence of cancer cells in biopsy samples were confirmed on endoscopy.[37] Partial response was defined by more than 50% reduction in the size of the primary tumor and lymph node metastasis, as confirmed by CT and endoscopy. *Progressive disease* was defined by an increase of more than 25% in the size of the primary tumor or the appearance of new lesions. Cases that did not meet the criteria of partial response or progressive disease were defined as no change.[36,37]

Histopathological Examination

The histopathological findings were classified according to the UICC TNM classification. The degree of histopathological tumor regression in the surgical specimens was classified into 5 categories.[36,38] The extent of viable residual carcinoma at the primary site was assessed semiquantitatively, on the basis of the estimated percentage of viable residual carcinoma in relation to the macroscopically identifiable tumor bed that was evaluated histopathologically. Therapy-induced changes included reactive changes such as necrosis, fibrosis, foamy histiocytes, mucosal edema, vascular changes in the tumor periphery, and giant cell reactions. Such characteristics were considered signs of neoplastic regression after neoadjuvant CRT. The percentage of viable residual tumor cells within the entire cancerous tissue was assessed as follows: grade 3, no viable residual tumor cells (pCR); grade 2, less than one-third of residual tumor cells; grade 1b, one-third to two-thirds of residual tumor cells; grade 1a, more than two-thirds of residual tumor cells; grade 0, no significant response to CRT.[36,38]

Statistical Analysis

The Student t test, Mann-Whitney U test, and the χ^2 test were used to compare the baseline characteristics of the treatment group and to compare the results of endoscopic biopsy and clinicopathological factors of the 2 groups. Overall survival was calculated from the date of neoadjuvant or definitive CRT to the occurrence of the event or to the last known date of follow-up. Actual survival was calculated by the Kaplan-Meier method and evaluated statistically by the log-rank test. A P value less than 0.05 was considered to reflect statistical significance. These analyses were carried out using the StatView J5.0 software package (Abacus Concepts, Berkeley, CA).

RESULTS

Correlation Between Endoscopic Biopsy Findings After Neoadjuvant CRT and Pathological and Survival Outcomes

Table 2 lists the characteristics of patients who received neoadjuvant CRT. Of 123 patients, the biopsy obtained after neoadjuvant CRT was negative for malignancy in 61 (50.0%) and positive in 62 (50.0%). There was a significant association between the histopathological findings of endoscopic biopsy and pathological tumor depth and tumor regression. Positive biopsy correlated significantly with minor histopathological tumor regression (grade 1), whereas negative biopsy correlated significantly with major histopathological tumor regression (grade 2 or 3). The sensitivity and specificity of endoscopic biopsy after neoadjuvant CRT in predicting pCR were 58.9% (56 of 95) and 78.6% (22 of 28), respectively. The positive and negative predictive values were 90.3% (56 of 62) and 36.1% (22 of 61), respectively. The findings on endoscopic biopsy predicted the pathological nodal status. Lymph node involvement was more frequent in patients with positive biopsy than in those with negative biopsy (62.9% vs 37.7%, $P = 0.005$).

Patients who received neoadjuvant CRT and later had negative biopsy showed significantly better survival than their counterparts with positive biopsy (5-year survival rate: 48.3% vs 21.8%, $P = 0.006$, Fig. 1). Of the 123 patients who received neoadjuvant CRT, recurrence was observed in 69 (56.1%) patients during the study period. Local recurrence was observed in 18 (14.6%) patients, lymphatic recurrence in 32 (26.0%) patients, and distant metastasis in 27 (22.0%) patients.

Correlation Between Endoscopic Biopsy Findings During and After Definitive CRT and Survival Rate

Of 66 patients who received definitive CRT, 32 (48.5%) had negative biopsy for malignancy whereas 34 (51.5%) had positive biopsy for malignancy at the dose of irradiation used in neoadjuvant setting (40 Gy) (Table 3). All but 2 of the 32 patients with negative biopsy at 40 Gy showed negative biopsy at the time of completion of definitive CRT, and 25 patients achieved clinical CR. On the contrary, 14 of the 34 patients with positive biopsy at 40 Gy improved to negative biopsy at the time of completion of definitive CRT, and 12 of these patients achieved clinical CR. Thus, the incidence of clinical CR after definitive CRT was significantly higher in patients with negative biopsy (78.1%) than in their counterparts with positive biopsy (35.3%, $P = 0.002$). In addition, the recurrence rate was significantly lower in clinical CR patients with negative biopsy after CRT (24%, 6 of 25) than in their counterparts with positive biopsy (67%, 8 of 12, $P = 0.0002$). Of the 37 patients who achieved clinical CR after definitive CRT, local recurrence was observed in 10 (27.0%) patients, lymphatic recurrence in 3 (8.1%) patients, and distant metastasis in 5 (13.5%) patients.

In the definitive CRT group, the 3-year survival rate of patients with negative biopsy at 40 Gy (57.0%) was significantly better than that of the patients with positive biopsy at 40 Gy (22.5%, $P = 0.0008$, Fig. 2). We also analyzed the survival data according to the results of endoscopic biopsy at the time of 40 Gy CRT and completion of definitive CRT (Fig. 3). Both the results of endoscopic biopsy at the time of CRT and at completion of definitive CRT influenced the survival rate.

TABLE 2. Clinicopathological Characteristics of Patients Who Underwent Neoadjuvant Chemoradiotherapy Followed by Surgery

	Results of Biopsy Obtained After Neoadjuvant CRT (40 Gy)		
	Negative	Positive	P value
n	61	62	
Mean age (years)	61.5	60.4	0.475
Gender (male/female)	52/9	55/7	0.568
Pretherapy tumor depth			
cT2	9 (15)	10 (16)	0.402
cT3	17 (28)	22 (36)	
cT4	35 (57)	30 (48)	
Pretherapy nodal status			
cN0	17 (28)	19 (31)	0.735
cN1	44 (72)	43 (69)	
Mean radiation dose (Gy)	40.0	40.0	0.541
Pathological tumor depth			
pT0	22 (36)	6 (10)	<0.001
pT1	5 (8)	6 (10)	
pT2	15 (24)	12 (19)	
pT3	14 (24)	22 (35)	
pT4	5 (8)	16 (26)	
Lymph node involvement			
pN0	38 (62)	23 (37)	0.005
pN1	23 (38)	39 (63)	
Clinical response			
CR	12 (20)	0 (0)	<0.001
PR	42 (69)	44 (71)	
NC/PD	7 (11)	18 (29)	
Pathological response			
Grade 3 (pCR)	22 (36)	6 (10)	<0.001
Grade 2	27 (44)	24 (39)	
Grade 1	12 (20)	32 (51)	

CR indicates complete response; NC/PD, no change or progressive disease; PR, partial response.

DISCUSSION

Both neoadjuvant CRT followed by surgery and definitive CRT have been recognized as curative treatment options for locally advanced esophageal cancers. Although a significant survival advantage has not been established for each therapeutic option, patients who show good response to CRT are considered to show a favorable prognosis. In fact, previous studies showed that the extent of

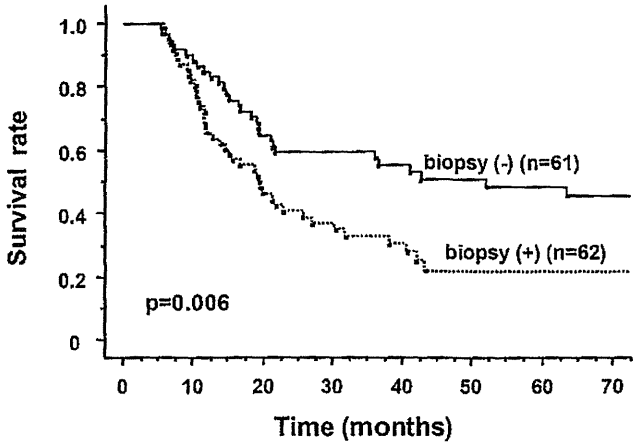

FIGURE 1. Overall survival rate in 123 patients with esophageal cancers who received neoadjuvant CRT followed by surgery, according to the results of endoscopic biopsy after induction CRT (40 Gy). The overall survival rate was significantly better in patients with negative biopsy than in those with positive biopsy.

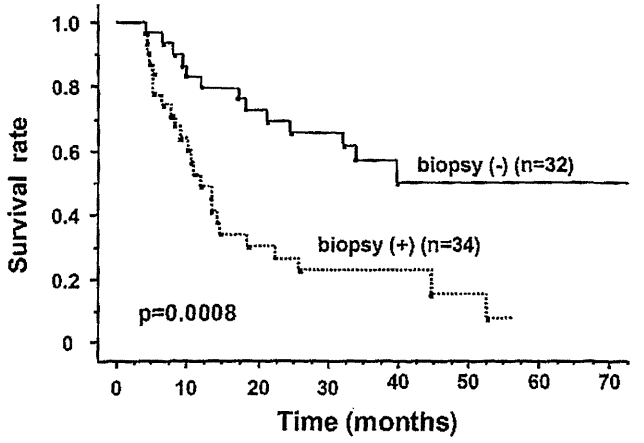

FIGURE 2. Overall survival rate in 66 patients with esophageal cancers who received definitive CRT, according to the result of endoscopic biopsy at the time of 40 Gy during the course of definitive CRT. The overall survival rate was significantly better in patients with negative biopsy than in those with positive biopsy.

TABLE 3. Characteristics of Patients Who Underwent Definitive Chemoradiotherapy

	Results of Biopsy Taken at Time of 40 Gy During Definitive CRT		
	Negative	Positive	*P* value
n	32	34	
Mean age (years)	65.2	66.8	0.483
Gender (male/female)	29/3	29/5	0.507
Pretherapy tumor depth			
cT1	13 (41)	7 (21)	0.088
cT2	3 (9)	5 (15)	
cT3	10 (31)	10 (29)	
cT4	6 (19)	12 (35)	
Pretherapy nodal status			
cN0	16 (50)	16 (47)	0.811
cN1	16 (50)	18 (53)	
Pretherapy stage			
I	12 (38)	7 (21)	0.575
II	3 (9)	7 (21)	
III	9 (28)	13 (37)	
IV	8 (25)	7 (21)	
Mean radiation dose (Gy)	60.6	62.6	0.091
Biopsy results after definitive CRT			
Negative	30 (94)	14 (41)	<0.001
Positive	2 (6)	20 (59)	
Clinical response after definitive CRT			
CR	25 (78)	12 (35)	0.002
PR	4 (13)	12 (35)	
NC/PD	3 (9)	10 (30)	

CR indicates complete response; NC/PD, no change or progressive disease; PR, partial response.

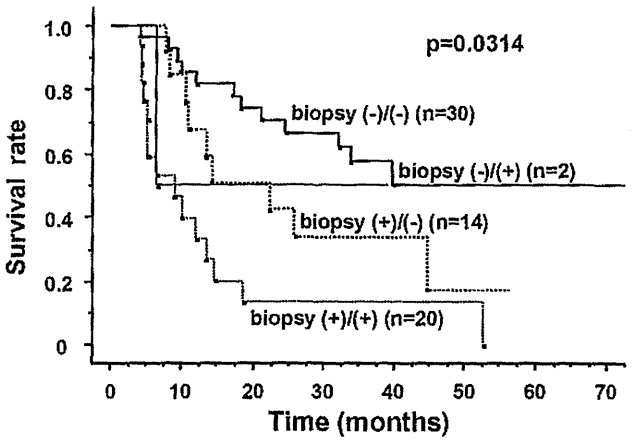

FIGURE 3. Overall survival rate in 66 patients with esophageal cancers who received definitive CRT, according to the results of endoscopic biopsy at the time of 40 Gy (represented by the first plus and minus sign after "biopsy") and at the time of completion of definitive CRT (represented by the second plus and minus sign after "biopsy").

histopathological tumor regression, especially pathological CR, correlated with improved prognosis.[17–19,39,40] Thus, the early detection of those patients who achieve good response to CRT is necessary so as to individualize therapy based on the response to induction therapy. In the present study, we investigated the prognostic significance of endoscopic biopsy findings at the dose of irradiation used in neoadjuvant setting (40 Gy), and we found that the findings of endoscopic biopsy at 40 Gy correlated with pathological tumor regression in neoadjuvant CRT and with clinical response in definitive CRT, and that such findings can predict the survival of patients who undergo CRT with and without surgery.

In neoadjuvant CRT, several studies investigated previously the use of endoscopic biopsy in predicting the pathological response to

neoadjuvant treatment.[27,32–35] However, these studies suggested that endoscopic biopsy findings were not useful in predicting pathological CR that would otherwise allow avoidance of surgical resection in patients with pathological CR. In the above studies, 70% to 80% of patients showed negative biopsy after neoadjuvant CRT, but pathological CR was observed in only 23% to 63% of patients with negative biopsy and the majority of those patients actually had residual cancer at resection. In our study, the endoscopic biopsy findings correlated significantly with histopathological tumor regression and survival after surgery. However, in agreement with previous studies, the biopsy findings were unreliable in predicting pathological CR, with pathological CR being observed in only 36% of patients with negative biopsy. The reason for this result is not clear at present but could be related to the fact that endoscopic biopsy can only detect cancer cells in the superficial mucosal layer. We reported previously that after CRT, few residual cancer cells were still present in deeper layers of the esophageal wall, such as the submucosal layer and muscularis propria.[41] These cancer cells can be missed even if endoscopic biopsy was taken accurately.

On the contrary, for definitive CRT, there is little or no information on the value of endoscopic biopsy during the course of radiotherapy. In the study of Kim et al,[35] which included examination of endoscopic biopsy samples from 94 patients with resectable esophageal cancer after neoadjuvant CRT (48 Gy), 27 of the 94 patients received a second course of CRT of up to 60 Gy as definitive CRT, but the value of endoscopic biopsy was not investigated in those patients who received definitive CRT. Our study demonstrated that the findings of endoscopic biopsy performed during the course of definitive CRT, at the time of 40 Gy irradiation, could predict the clinical response and survival after completion of definitive CRT. These results suggest that the findings of endoscopic biopsy conducted during the course of CRT are useful for selection of therapy after induction CRT, surgery, or completion of definitive CRT. In definitive CRT, the majority of patients whose biopsy results at the time of 40 Gy irradiation were negative achieved CR after additional irradiation up to the definitive dose, and the recurrence rate after CR was low in these patients. This indicates that a patient with negative biopsy at the time of 40 Gy has a good chance of complete cure after additional irradiation up to the definitive dose, regardless of neoadjuvant intent or definitive intent at the start of treatment. On the contrary, the rate of recurrence in patients with positive biopsy at the time of 40 Gy is considered high even if they achieved CR after further irradiation up to the definitive dose. This indicates that a patient with a positive biopsy at the time of 40 Gy has only a small chance of being cured by additional irradiation up to the definitive dose, and that perhaps surgery would be performed for such patients instead of further sessions of irradiation up to definitive dose. In fact, the present study showed that among patients with positive biopsies excluding patients with T1 tumors, there was a tendency of those who underwent neadjuvant CRT followed by surgery showing better survival than those with definitive CRT (31.8% vs 13.5%). However, in case of locally advanced tumors, if endoscopic biopsy at the time of 40 Gy is positive for malignancy, patients often do not achieve downstaging of those tumors and sometimes receive noncurative resection. Indication for surgery in such patients should be carefully considered by using diagnostic imaging. The usefulness of endoscopic biopsy in selecting treatment strategy after induction CRT needs further investigation.

In the present study, the incidences of positive biopsy after induction CRT in both the patients who received neoadjuvant CRT followed by surgery and those who received definitive CRT were relatively higher (nearly half of patients) than those reported in previous studies (range, 20%–30%).[27,32–35] One possible reason for the difference in the studies is that we performed endoscopic biopsy immediately, almost within 1 week after completion of 40 Gy irradiation, whereas endoscopic biopsy was often conducted some time after the completion of neoadjuvant CRT in the previous studies. Another possible reason is that the radiation dose at the time of endoscopic examination was relatively low in our study compared with those studies in which patients received more than 40 Gy irradiation (40–50.4 Gy) as neoadjuvant CRT, with the exception of the study by Schneider et al (36 Gy).[27]

In the present study, we performed endoscopic biopsy at the time of 40 Gy irradiation during the course of CRT, because the dose of irradiation used routinely in our hospital is 40 Gy for neoadjuvant CRT and mostly 60 Gy for definitive CRT. In previous studies of neoadjuvant CRT for locally advanced esophageal cancers, the total radiation dose varied with institutions and therapeutic regimens, ranging from 35 Gy to 50.4 Gy.[5–10,12–14] With regard to definitive CRT, a phase III randomized trial of combined-modality therapy for esophageal cancer, the RTOG 9405 (Intergroup 0123) study, which compared high-dose radiation therapy (64.8 Gy) with standard dose radiation (50.4 Gy), showed higher toxicity in the high-dose radiation group and no survival advantage.[42] Thus, the dose of 50.4 Gy has become the standard in definitive CRT in the United States. On the contrary, the current practice in Japan is to apply total radiation of more than 60 Gy in patients who undergo definitive CRT.[37,43] Although our results suggest that endoscopic biopsy at the time of 40 Gy irradiation may provide useful information for selection of therapy, the most appropriate time to obtain endoscopic biopsy to individualize therapy (surgery or completion of definitive CRT) may vary according to the dose of irradiation used in neoadjuvant and definitive CRT.

In conclusion, the present study demonstrated that endoscopic biopsy conducted at the time of 40 Gy irradiation predicts histopathological tumor regression and survival of patients who receive neoadjuvant CRT followed by surgery and it also predicts the clinical response and survival of patients who receive definitive CRT. Further studies are required to confirm the usefulness of endoscopic biopsy in the course of CRT for selection of treatment strategy based on response to induction CRT, surgery, or completion of CRT up to the definitive dose.

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Scientific Research

Comparison of 4-Weekly vs 3-Weekly Gemcitabine as Adjuvant Chemotherapy Following Curative Resection for Biliary Tract Cancer: A Prospective Randomized Controlled Trial

Shogo Kobayashi[1], Atsushi Miyamoto[2*], Junzo Shimizu[3], Masaki Kashiwazaki[4], Yutaka Takeda[5], Shigeyuki Ueshima[6], Yongkook Kim[7], Toru Kitagawa[1], Keizo Dono[3], Masaki Mori[1], Yuichiro Doki[1], Hiroaki Nagano[1]

[1]Department of Surgery, Graduate School of Medicine, Osaka University, Suita, Japan; [2]Department of Surgery, National Hospital Organization, Osaka National Hospital, Osaka, Japan; [3]Department of Surgery, Toyonaka Municipal Hospital, Toyonaka, Japan; [4]Department of Surgery, Hyogo Prefectural Nishinomiya Hospital, Nishinomiya, Japan; [5]Department of Surgery, Kansai Rosai Hospital, Amagasaki, Japan; [6]Department of Surgery, Osaka Police Hospital, Osaka, Japan; [7]Department of Surgery, Osaka Rosai Hospital, Sakai, Japan.
E-mail: *amiyamo@onh.go.jp

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ABSTRACT

Background: *Surgery for biliary tract cancer, including pancreatoduodenectomy and major hepatectomy, is too aggressive and does not allow postoperative gemcitabine to be administered by the usual dosage protocol. We hypothesized that the feasibility of* 3*-weekly protocol* (*days* 1 *and* 8, *every* 3 *weeks*) *of adjuvant gemcitabine therapy may be superior to the usual* 4*-weekly protocol* (*days* 1, 8, *and* 15 *every* 4 *weeks*). ***Method***: *We compared the outcomes of* 6 *cycles of the* 4*-weekly protocol and* 9 *cycles of the* 3*-weekly protocol in a prospective randomized setting. The primary endpoint was the completion rate, and the secondary endpoints were the adverse events and the recurrence-free survival rate.* ***Results***: *Totally,* 27 *patients were enrolled. The protocol could be completed without any omittances and/or dose modifications in two patients* (14%) *of the* 4*-weekly protocol, and three patients* (23%) *of the* 3*-weekly protocol* (*p* = 0.8099); *grade* 3/4 *neutropenia occurred in almost all the remaining* (70%) *patients. The relative dose intensity was* 72% *in the* 4*-weekly protocol and* 78% *in the* 3*-weekly protocol. There was no significant difference in the recurrence-free survival rate.* ***Conclusion***: *The* 3*-weekly protocol did not yield superior completion, adverse events or recurrence-free survival rates as compared to the* 4*-week protocol.* ***Trial Registration***: *UMIN-CTR, UMIN*000001020.

Keywords: *Biliary Tract Cancer, Adjuvant Therapy, Gemcitabine*

1. Introduction

The prognosis of biliary tract cancer (BTC) is still poor [1-6]. Although surgical treatment remains the only potentially curative treatment, the overall 5-year survival rate remains approximately 40% [6,7]. This uncommon cancer is not yet well-studied because of the complexity of its classification and surgical procedures, andthe high perioperative morbidity, including liver dysfunction and cholangitis. Therefore, no (neo-) adjuvant chemotherapy has been established for these patients [8]. On the other hand, a pooled analysis [9] and multicenter retrospective analysis [10] revealed the potential efficacy of gemcitabine for unresectable and recurrent BTC, and a prospective randomized study revealed the survival benefit of gemcitabine-based chemotherapy in these patients [11,12]. Moreover, this drug has been shown to have a good safety profile, with a low incidence of grade 3/4 toxicities [13].

Based on this background, gemcitabine was introduced for adjuvant therapy after curative resection of BTC. However, it is difficult in the clinical setting to continue the usual4-weekly protocol (1000 mg/m^2 on days 1, 8

and 15 every 4 weeks) after BTC surgery. And, there have been several reports of gemcitabine-based adjuvant chemo therapy following major hepatic to mine for BTC or pancrea to duodenectomy for pancreatic cancer, which have suggested that dose modification is often necessary or that the usual 4-weekly protocol could not be, or was not applied [14-16]. Because of the morbidity, liver dysfunction, and low performance status (PS) after BTC surgery (major hepatectomy/pancreatoduodenectomy), it would seem difficult to complete the usual 4-weekly protocol, and frequent pauses during the adjuvant during therapy would be necessary.

For the above reasons, we hypothesized that postoperative gemcitabine therapy by the3-weekly protocol (1000 mg/m^2 on days 1 and 8 every 3 weeks) might be more feasible and superior to the 4-weekly protocol, because it would allow more treatment pauses. In this study, we compared the completion rate between patients assigned to the 4-weekly and 3-weekly protocols, for the same planned total dosage (6 cycles for the 4-weekly protocol and 9 cycles for the 3-weekly protocol). Because no feasibility studies of adjuvant gemcitabine therapy have been reported yet for BTC, we also comparatively estimated the frequency and severity of adverse events and the treatment efficacy (recurrence-free survival) between the two protocols. This study was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR, UMIN 000001020) in JAPAN.

2. Patients and Methods

2.1. Study Design and Endpoints

We designed this open, multicenter, randomized controlled trial to explore the feasibility and efficacy of adjuvant gemcitabine therapy for BTC. The trial was initiated by the Osaka University Biliary Tract Cancer Treatment Group (OBCG, affiliated to the Multicenter Clinical Study Group of Osaka), Department of Surgery, Graduate School of Medicine, Osaka University. The protocol was approved by the institutional review board a teach hospital, and written informed consent was obtained from each of the patients.

The primary end point was the completion rate of adjuvant therapy. As control, we employed the 4-weekly protocol of gemcitabine treatment, which was studied in the CONKO-001 study after similar surgeries for pancreatic cancer [15].

The secondary end points included the frequency and severity of adverse events, for the purpose of collecting data on adverse events associated with adjuvant therapy, and the recurrence-free survival.

We determined that alpha and beta errors were 10% and 20%, respectively, to explore the feasibility and efficacy. After calculation of the sample seize, we determined that a total of 40 patients would be required. The study was started in August 2007 and completed in March 2010.

2.2. Patient Selection

Patients with histologically confirmed BTC (extrahepatic bile duct cancer, gall bladder cancer, or cancer of the papilla of Vater, UICC-stage II to IV [17]), who underwent macroscopic complete resection and no other therapy than surgery, were eligible for the study, and adjuvant therapy was to be started from 4 to 12 weeks after the surgery. Other eligibility criteria included age ≥20 years, Eastern Cooperative Oncology Group (ECOG) PS 0/1 [18], adequate hematological, liver and renal function (hemoglobin > 10 g/dl, leukocytes > 4000/μl, neutrophils > 2000/μl, platelets > 100,000/μl, serum transaminases < 4× the upper limit of normal (ULN), serum bilirubin < 2× ULN, andserum creatinine <ULN).

Patients were excluded if they had active interstitial pneumonia, severe edema, pregnancy, active infection, severe underlying disease (impaired cardiac function, active peptic ulcer, ileus, uncontrolled diabetes mellitus, etc.), severe allergy, severe mental disorders, or active another cancer.

2.3. Treatment and Dose Modification

Standard surgical procedures were used depending on BTC involvement. Eligible patients were randomly assigned by a computer-generated central randomization with stratification for institution and surgical procedure (pancrea to duodenectomy vs. others).

Patients assigned to the 4-weekly protocol received 6 cycles, with each cycle consisting of three weekly administrations of intravenous gemcitabine at 1000 mg/m^2, followed by a1-week drug-free pause. Patients assigned to the 3-weekly protocol received 9 cycles, each cycle-consisting of two weekly administrations of gemcitabine, followed by a1-week drug-free pause.

The first administration in each cycle was started with adequate hematological, liver, and renal functions (leukocytes > 3000/μl or neutrophils > 1500/μl, platelets > 100,000/μl, serum transaminases < 5 × ULN, serum bilirubin < 3 × ULN, and serum creatinine < ULN). When the first administration of any cycle could not be started within 28 days, the patient was withdrawn from the study.

For the second or third administrations in each cycle, the following were set in addition to first administration criteria; leukocytes > 2000/μl or neutrophils > 1000/μl, and platelet > 70,000/μl. When the above-mentioned criteriawere not fulfilled, dose modification was necessary for the next administration, as follows: 1000 mg/m^2

> 800 mg/m^2 > 600 mg/m^2 > withdrawal from the study. Omitted doses of gemcitabine were not replaced. During the study, neither anti-cancer therapies were allowed. Patients were withdrawn from the study for any of the following reason: disease recurrence, patient's desire to discontinue, or unacceptable treatment toxicity

2.4. Assessments

Prior to enrollment in the study, all patient sunder went routine examinations and laboratory studies. Tumor assessments were performed on the chest X-ray and abdominal computed tomo graphic or magnetic resonance images, prior to the adjuvant therapy and every 3 months.

During the study, vitalsigns, laboratory studies, PS, and toxicities/adverse events were evaluated prior to each administration. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE version 3.0).

The overall survival and recurrence-free survival rates were calculated by the Kaplan–Meiermethod, and the log-rank test was used for comparisons. Student's t-test or chi-square test was used to compare any differences. P-values <0.05 were considered to indicate statistical significance. All statistical analyses were performed using the StatView J-5.0 software (SAS, Cary, NC).

3. Results

3.1. Results

A total of 27 patients were recruited into the study from 13 centersinthe Kansai area, Japan. Recruitment was planned for ending in March 2010, and we analyzed the data at thistime to determine whether the study should be extended or not. There were no differences in the completion rate between the protocols, and we completed the study in 27 patients. The patients were randomized to the 4-weekly protocol (n = 14) and or the 3-weekly protocol (n = 13).The baseline characteristics of the eligible patients are shown in **Table 1**, and there were no statistically differences between the two protocols. All patients had adenocarcinoma, the majority had Stage IIB BTC, 15 (56%) underwent pancreatoduodenectomy, and 8 (30%) underwent major liver surgery. The median time from surgery to the start of chemotherapy was 62 days (24 - 86), with no significant difference between the protocols.

3.2. Treatment Delivery

The number of patients in whom the adjuvant therapy could be completed without any omittances and/or dose modifications was 2 (14%) in the 4-weekly protocol and 2 (15%) in the 3-weekly protocol (p = 0.8099, **Table 2**). The scheduled treatment cycles could not be completed in 3 patients of the 4-weekly protocol and in 4 patients of the 3-weekly protocol. The median number of administrations was16 for both protocols, and the median durations of administration were 168 days and 189 days, depending on the protocol bias. The median total dosages of adjuvant gemcitabine were 13,000 mg/m^2 in the 4-weekly protocol, and 14,000 mg/m^2 in the 3-weekly protocol. The median relative dose intensities were 72.2% and 77.8%, respectively. The potential need for omittance on account of grade 3 hematological or other events occurred first at the 3rd administration in the 4-weekly protocol and at the 10th administration in the 3-weekly protocol.

Table 1. Patient characteristics.

	4-Weekly Protocol	3-Weekly Protocol	p-value
Age	64 (56 - 76)*	68 (57 - 77)	0.6334
Sex (male/ female)	8/6	6/7	0.5680
Performance status (0/1)	11/3	10/3	0.9180
Body weight (kg)	52 (41 - 65)	54 (34 - 70)	0.9478
Primary lesion			0.3624
Hilar cancer	2	5	
Inferior bile duct cancer	4	4	
Gall bladder cancer	4	1	
Cancer of the Papilla of Vater	4	3	
Surgery			0.4649
Pancreatoduodenectomy	8	7	
Liver bed resection	3	1	
Hemihepatectomy or more	3	5	
UICC-Stage			0.3576
IIA	5	6	
IIB	7	7	
III	2	0	

*The number indicates the median (minimum-maximum).

In the subcategory analysis, the completion rates were 26.7% (4/15) and 9.0% (1/11) in pancreatoduodenectomy and other-surgery, respectively (p = 0.5691). The potential need for omittance occurred first at the 8th administration in pancreatoduodenectomy and at the 2nd administration in other-surgery. The dose intensities were 78% and 73%, respectively. In 4-weekly and 3-weekly protocol after pancreatoduodenectomy, the completion rates were 12.5% (1/8) and 42.9% (3/7), respectively (p = 0.5263). The potential need for omittance occurred first at the 3rd administration in the 4-weekly protocol, and at the 12th in the 3-weekly protocol (p = 0.0188). The dose intensities were 72% and 86%, respectively (p = 0.1152). After other-surgery, the completion rateswere 16.7% (1/6) and 0% (0/6), the potential need for omittance occurred first at the 3rd and 2nd administrations, and the dose intensities were 71% and 75%, respectively, in the 4-weekly and 3-weekly protocols, with no statistically significant differences between the two protocols.

3.3. Toxicity

Grade 4 toxicities were encountered only in the 4-weekly protocol (grade 4 neutropenia), while grade 3 neutropenia was noted at a high frequency (64% and 69%, respectively) in both protocols (Table 3). Grade 3/4 nonhematologic toxicity occurred only infrequently in either

Table 2. Completion rate, number of administrations, and total dose.

	4-Weekly Protocol	3-Weekly Protocol	p-value
n (Pancreatoduodenectomy / other surgery)	14 (8/6)	13 (7/6)	
Complete all cycles	2 (1/1) 14%	3 (3/0) 23%	0.8099
Adjuvant therapy completed with omittance and/ or dose modification	9 (5/4) 64%	6 (2/4) 46%	
Could not complete all cycles	2 (1/1) 14%	3 (1/1) 23%	
Recurrence during therapy	1 (1/0)	1 (0/1)	
Number of administrations	16	16	
Total dose (mg/m^2)	13000 (2600 - 18000)	14000 (2400 - 18000)	0.7017

Table 3. Number of patients with maximum grade of adverse events during the treatment.

		4-Weekly Protocol				3-Weekly Protocol			
		Gr1*	Gr2	Gr3	Gr4	Gr1	Gr2	Gr3	Gr4
Performance status		5				5	3	1	
Hematological	Leukocytes	1	8	2		1	8	2	
	Neutrophils		3	9	1		3	9	
	Hemoglobin	11	3			6	5	1	
	Platelets	8	3	1		3	2		
Laboratory	Aspartate aminotransferase	6	1	1		4	1		
	Alanine aminotransferase	4	1			4	1		
	Bilirubin		1			1			
	Creatinine								
Constitutional symptom	Fatigue	5	2			8	2		
	Fever	3	1			3	1		
Gastrointestinal	Nausea / Vomiting	3				5	1		
	Anorexia	2	2			2	3		
	Diarrhea	1				8			
	Stomatitis	2							
	Constipation	2							
Dermatology	Alopecia					1			
	Rash		1						
Edema							1		

*Grade 1.

protocol. A total of 27 serious adverse events (grade 3/4) were reported in the 21 patients (12 patients of the 4-weekly protocol and 9 patients of the 3-weekly protocol). In regard to the development of constitutional symptoms (PS and general fatigue) and gastrointestinal toxicity (nausea/vomiting), 52% and22% of patients developed grade 1/2 toxicities in the 4-weekly and 3-weekly protocolsandonly one patient experienced grade 3/4 toxicity (PS 3, just before withdrawal from the study).

3.4. Efficacy

The survival curves are shown in **Figure 1**. For a median follow-up of 17 months, the 1- and 2-year recurrence-free survival rates were 77% and 55% in the 4-weekly protocol, and 84% and 53% in the 3-weekly protocol, respectively (p = 0.8295). The 1- and 2-year overall survival rates were 100% and 75% in the 4-weekly protocol, and 100% and 71% in the 3-weekly protocol, respectively (p = 0.5876).

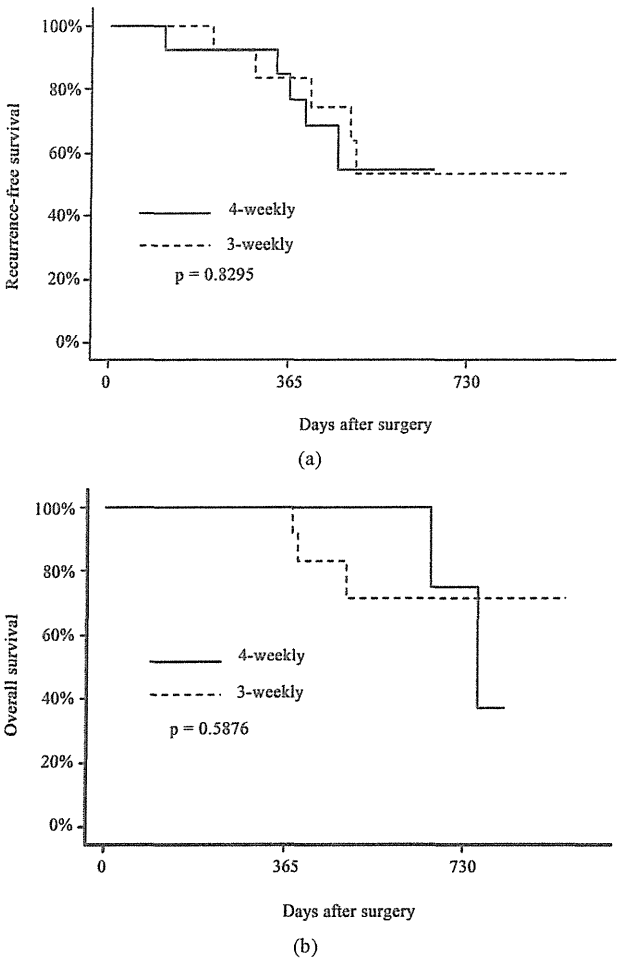

Figure 1. Recurrence-free survival (a) and overall survival (b) of biliary tract cancer patients after surgical resection. Solid line indicates the survival curve of the patients who received 6 cycles of adjuvant gemcitabine therapy by the 4-weekly protocol (1000 mg/m^2 on days 1, 8 and 15 every 4 weeks), and the dotted line indicates that of those who received 9 cycles of adjuvant therapy by the 3-weekly protocol (1000 mg/m^2 on days 1 and 8 every 3 weeks). There were no significant differences in either parameter between the two groups.

4. Discussion

There is as yet no feasibility-certified adjuvant therapy for BTC, especially when gemcitabine is used. In pancrea to duodenectomy for pancreatic cancer, the CONKO-001 and JSAP-02 trials revealed that dose modification was frequently necessary for completion [15,16]. In BTC, Murakami *et al.* presented gemcitabine-based adjuvant chemotherapy, however, all patients could not complete the protocol, even for biweekly 700 mg/m^2 gemcitabine-based chemotherapy [14]. Clinically, several omittances are necessary in the usual 4-weekly protocol of gemcitabine after BTC surgery, and it become like the 3-weekly protocol. Therefore, we hypothesized that the 3-weekly protocol may be more feasible and superior (higher completion rate). However, the 3-weekly protocol was not superior to the 4-weekly protocol in the completion rate, frequency of adverse events, or disease-free survival. In other words, the administration protocol did not influence the completion rate of adjuvant gemcitabine therapy for BTC.

In regard to the relative dose intensity, approximately 75% was achieved on average. In CONKO-001 and JSAP-02 (Note; including distal pancreatectomy), the relative dose intensity was approximately 90% [15,16]. In our data, the dose intensity was 78% in pancreatoduodenectomy, but only 73% in other-surgery (including liver resection). It would therefore seem that the dose intensity is related to the surgical stress. On the other hand, the dose intensity in pancrea to duodenectomy treated by the 3-weekly protocol was 86%, whereas that in the subgroup treated by the 4-weekly protocol was approximately 70%. Similar data were obtained for the first drug omittance. In pancrea to duodenectomy, the first potential omittance was necessitated much later in the 3-weekly protocol than in the 4-weekly protocol, the difference being statistically significant. In other-surgery, the dose intensity was only 70% in both protocols and the first omittances were necessitated at the 2nd - 3rd administrations; these data were inferior to the data in the 3-weekly subgroup of pancrea to duodenectomy. In addition, some patients who underwent hepatectomy received only few gemcitabine administrations before withdrawal. This led us to speculate that in pancrea to duodenectomy, the 3-weekly protocol might be better, but that in more

aggressive surgery, like major liver resection, neither the 4-weekly nor the 3-weekly protocol might be feasible for adjuvant therapy. This study might not have enough power to mention these speculations. It would be necessary to perform phase I study to yield a higher dose intensity, followed by phase II/III study in a larger study group.

In relation to treatment toxicity, we encountered a high frequency of grade 3/4 neutropenia, and also of grade 2 constitutional symptoms and gastrointestinal adverse events. Although there is little information about neutropenia in previous reports [14,15], the JSAP-02 reported a high frequency of grade 3/4 neutropenia [16], similar to our study. This adjuvant therapy seemed to yield a high frequency of not grade 3/4 leukopenia, but of grade 3/4 neutropenia. After surgery for BTC, the patients sometimes develop cholangitis, and with the occurrence of severe neutropenia, liver abscess and/or sepsis could occur. Patients must therefore be closely monitored for the development of neutropenia. In regard to constitutional symptoms and gastrointestinal adverse events, approximately 25% of the patients with such adverse events were unable to carry on with their work activities or needed drip infusions (grade 2 toxicity), suggesting that treatment of these patients in the outpatient setting might be difficult.

In regard to the efficacy, we compared our historical data [4-6] and the report from the Japanese Society of Biliary Surgery (JSBS) [7,19], and data on gemcitabine-based adjuvant therapy by Murakami et al. [14]. In regard to the recurrence-free survival, the data in our present study (77% - 84% at 1 year and 52% - 55% at 2 years) were similar to those reported by Murakami et al. (recurrence-free survival: 79% at 1 year and 60% at 3 years) [14]. In terms of overall survival, the rate in our study 75% - 71% at 2 years, as compared to historical data (without adjuvant therapy) of approximately 65% at 2 years and 60% - 63% at 3 years. The JSBS reported an overall survival rate of 40% - 65% at 3 years. Based on the above findings, we suggest that there remains the possibility of a survival benefit of adjuvant gemcitabine therapy after BTC surgery.

In conclusion, the 3-weekly gemcitabine treatment protocol was not superior to the 4-weekly protocol in terms of the completion rate, relative dose intensity, adverse events or recurrence-free survival, among patients receiving adjuvant therapy following BTC surgery; a high frequency of grade 3/4 neutropenia was found in both the protocols. Furthermore, the treatment could be completed without any interruptions and/or dose modifications in only approximately 10% of the patients. Our findings suggest the possibility of the dose intensity depending on the aggressiveness level of the surgical procedures, and further investigation is warranted. For a precise evaluation of the efficacy in a feasibility study for adjuvant therapy after aggressive BTC surgery, a prospective randomized study with a large number of patients would be necessary.

5. Disclosure

The authors have no conflict of interests to declare.

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