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Original Paper

Adjuvant Hepatic Arterial Infusion Chemotherapy after Curative Resection for Dukes C Colorectal Cancer: A Pilot Study

Mitsuyoshi Ota, Hiroshi Shimada, Hidenobu Masui, Kuniya Tanaka, Shigeki Yamaguchi Yasushi Ichikawa, Shinji Togo, Hideyuki Ike, Shigeo Oki

Second Department of Surgery, Yokohama City University, School of Medicine, Yokohama, Japan

Corresponding Author: Mitsuyoshi Ota, MD, Second Department of Surgery, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama, 236-0004, Japan

Tel: +81 45 787 2650, Fax: +81 45 782 9161, E-mail: otakun@ra2.so-net.ne.jp

KEY WORDS:
Colorectal cancer; Hepatic arterial infusion chemotherapy; 5-fluorouracil; Liver metastasis

ABBREVIATIONS:
Hepatic Arterial Infusion (HAI); Hepatic Arterial Infusion Chemotherapy (HAIC); 5-Fluorouracil (5-FU)

ABSTRACT

Background/Aims: The aim of this study was to evaluate the effect and the toxicity of prophylactic adjuvant hepatic arterial infusion chemotherapy (HAIC) on liver metastases and on overall survival of Dukes C colorectal cancer patients.

Methodology: Ninety patients in whom Dukes C colorectal cancer was diagnosed and were treated with curative resection between 1993 and 1997 underwent HAIC. The HAIC regimen consisted of a 24-hour continuous infusion of 1500mg of 5-fluorouracil, administered once a week for 8 weeks, utilizing a portable infusion drug delivery system to ambulatory patients. Patients to whom 7g or more of 5-fluorouracil could be given were included in the HAIC group, which resulted in 70 of the 90 patients being in this group. The HAIC group overall survival and liver recurrence rates were compared with those of 62 non-treated cases of Dukes C, which formed the non-HAIC control group.

Results: There were no serious toxic effects in this study. Significant differences were seen in the cumulative overall 5-year survival (HAIC group, 84.1%; non-HAIC group, 65.2%; $p=0.0369$). The cumulative 5-year liver metastasis-free rate was 92.7% in the HAIC group and 78.6% in the non-HAIC group ($p=0.0649$). In cases of distal lymph node metastasis, a risk factor for liver metastasis, the cumulative 5-year liver metastasis- free rate in the HAIC group (91.7%) was significantly higher than that in the non-HAIC group (58.6%; $p=0.0268$).

Conclusions: HAIC effectively prevents metachronous liver metastasis, especially in patients with pre-existing distal lymph node metastases, and improves the prognosis of advanced colorectal cancer.

INTRODUCTION

In Dukes C colorectal cancer, even when curative surgery is performed, the overall 5-year survival is only around 60% because of tumor recurrence. The liver is the most frequent site of recurrence, accounting for 40 to 50% of all colorectal cancer recurrences (1). The best treatment for liver metastases of colorectal cancer is major hepatic resection. The 5-year survival rate of cases with curative resection for liver metastasis is 25% (2), but in fact, only 10 to 20% of patients with liver metastases undergo surgical treatment. An effective adjuvant treatment should therefore be devised to prevent liver metastases.

Metachronous liver metastasis may arise from microscopic metastases that are undetected at initial surgery. Otherwise, it may originate from tumor cell emboli reaching the portal system via the mesenteric vein during the initial surgery. Consequently, previous studies have advocated portal injection of cytotoxic agents at the time of surgery and during the postoperative period to prevent metachronous liver metastasis (3,4). However, a large randomized trial of portal vein infusion of fluorouracil and heparin showed that they had no significant impact on survival (5).

In the present study, we investigated whether adjuvant hepatic arterial infusion chemotherapy could reduce the incidence of metachronous liver metastasis and prolong patient survival.

METHODOLOGY

At Yokohama City University Hospital, from 1993 to 1997, out of 191 patients with histologically confirmed Dukes C colorectal cancer who had had curative surgery, 90 patients received adjuvant hepatic arterial infusion therapy. Informed consent was obtained preoperatively from all patients eligible for the trial. Physical examination, computed tomography of the abdomen and pelvis, chest radiography or computed tomography were performed so as to demonstrate that there was no evidence of synchronous liver metastasis or other distant metastases.

During the operation, ultrasound sonography was also performed to confirm that no liver metastases were present. At the time of pump insertion, cholecystectomy was performed if it had not been done previously. The tip of an arterial catheter was placed at the point where the gastroduodenal artery branched off from the common hepatic artery. The right gastric

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artery and small branches supplying the duodenum were ligated.

Arterial catheterization using an interventional radiologic procedure was performed in patients where lymph node metastases were confirmed for the first time by postoperative pathologic study,

Hepatic arterial infusion chemotherapy (HAIC) was performed in the Outpatient Department. The adjuvant treatment regimen used was as follows; from 1993 to 1995, 5-fluorouracil (5-FU) (350mg/24 h) was administered by continuous infusion pump for 5 days at two-week intervals, and was repeated six times. This regimen resulted in frequent catheter occlusion and was changed in 1996 to a weekly dose of 5-FU (1500mg/24 hours) for eight weeks. After completing the regimen, all patients were given oral fluoropyrimidine for two years.

During the 5-FU infusions, patients were checked every week for side effects. Assessment of toxicity was carried out according to the WHO criteria after each cycle of treatment. Computed tomography of the abdomen and pelvis, and chest radiography were performed every three months, and laboratory examinations, including tests for tumor markers, were performed every month to prove or exclude tumor recurrence.

As a historical study, the clinical courses of the HAIC group patients were compared with those of the 62 patients in the non-HAIC control group, in which patients were also given oral fluoropyrimidine for two years.

We drew Kaplan-Meier curves to estimate the overall duration of survival and of disease-free survival. Data for the two groups were compared by means of the log-rank test using a significance level of 5%.

TABLE 1 Regimen and Toxicity

Regimen and toxicity

5- FU 1750mg/5days/2weeks continuous i.a. x 6 (1993-1996) no. of patients 49	5- FU 1500mg/24hr/week continuous i.a. x 8 (1996-1997) no. of patients 41
Catheter occlusion 14 Nausea/appetite loss 2	Catheter occlusion 1 Nausea/appetite loss 2 Stomatitis 1
33 patients completed	37 patients completed
Nausea/ appetite loss 8 Abdominal pain 3 Liver dysfunction 2	Nausea/ appetite loss 6 Abdominal pain 1 Liver dysfunction 0

TABLE 2 Various Characteristics of the Two Groups

		HAIC group (n=70)	Non-HAIC group (n=62)
Sex	Male	47	37
	Female	23	25
Age	Average	60.1	60.5
	Range	29-83	29-82
Location	Colon	40	26
	Rectum	30	36
Depth of tumor	Within proper muscle	10	8
	Beyond proper muscle	60	54
Location of positive lymph nodes	Proximal	47	33
	Distal	23	29
Pathology	Well	34	14
	Moderately	26	30
	Poorly	2	2
	Mucinous	8	6
Tumor size (mm)	Average	48.2	54.7
	Range	12-130	15-120
Serum CEA level (ng/mL)	Average	9.5	7.9
	Range	0.5-69	0.6-69
Median follow-up period (month)		42.2	48.5

RESULTS

Of the 90 patients who received adjuvant hepatic arterial infusion therapy, 20 were excluded because the total chemotherapy dosage of at least 7g of 5-FU could not be completed due to 15 cases of catheter occlusion, 4 of nausea and appetite loss, and 1 of stomatitis (**Table 1**). Therefore, analysis was performed using the 70 patients who formed the HAIC group. Toxic effects occurred in 14 patients (20%); nausea, appetite loss, or both developed in 12 patients, abdominal pain developed in 4 patients, and liver dysfunction developed in 2 patients. None of these toxic effects exceeded grade 2 of the WHO criteria. No diarrhea and bone-marrow suppression was observed in this study (**Table 1**). The background characteristics of the patients are presented in **Table 2**, and show no significant differences between the HAIC group and the non-HAIC group in terms of sex, age, location of tumor, depth of tumor, locations of positive lymph nodes, tumor pathology, tumor size, serum CEA level or median follow-up period.

There were recurrences in 18 of the 70 patients in the HAIC group and in 19 of the 62 in the non-HAIC group (**Table 3**). In the HAIC group, the site of initial recurrence was the liver in 5 patients, the lung in 7, the brain in 1, local tissues in 4 patients and peritoneal dissemination in one. Nine of these 18 patients died of recurrent colorectal cancer. In three of the 5 patients who had liver metastases, metastatic lesions were resected. One of them still survives after 54 months. Five of the seven patients with lung metastases underwent surgery, and six of the seven are still alive. The cases with brain metastasis and peritoneal dissemination were all inoperable, and 2 of the patients were dead within 24 months.

Resection of the tumors was performed in 2 of the 4 cases of local recurrence, and the patients survive at this present time. The cumulative overall 5-year survival rate of the HAIC group was 84.1%, which was significantly higher than that of the non-HAIC group (65.2%, p=0.0369) (**Figure 1**). The cumulative 5-year liver metastasis-free ratio shows no statistical difference between the HAIC group (92.7%) and the non-

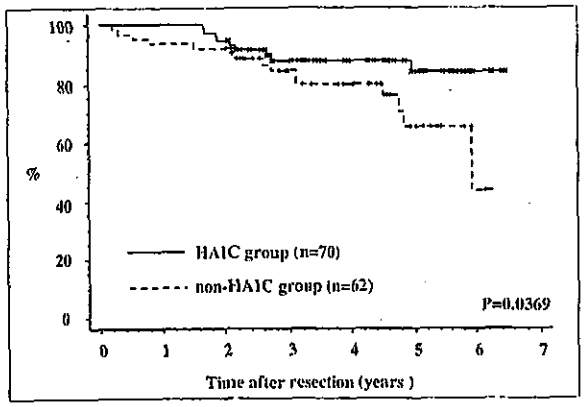

FIGURE 1 Survival curves after curative resection of Dukes C colorectal cancer.

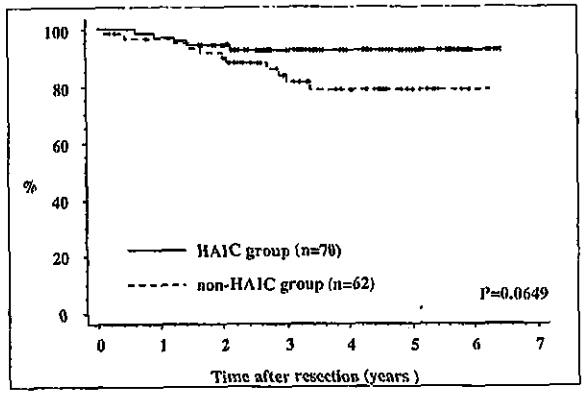

FIGURE 2 Liver metastasis-free curves after curative resection of Dukes C colorectal cancer.

TABLE 3 Location of First Recurrence and Treatment in the Two Groups

		HAIC group (n=70)	Non-HAIC group (n=62)
Location	Liver	5	11
	Lung	7	3
	Brain	1	0
	Local	4	3
	Bone	0	1
	Dissemination	1	1
	Total	18 (25.7%)	19 (30.6%)
Treatment	Surgery	10	9
	Radiation	5	6
	Chemotherapy	0	1
	No treatment	2	2

HAIC group (78.6%) (**Figure 2**). On the other hand, in the cases positive for distant lymph node metastasis, one of the risk factors of liver metastasis of colorectal carcinoma, the cumulative 5-year liver metastasis-free ratio of the HAIC group (91.7%) was significantly higher than that of the non-HAIC group (58.6%, $p=0.0268$) (**Figure 3**).

DISCUSSION

Approximately 50% of the patients who underwent curative resection for primary colorectal cancer died of metachronous liver metastasis. Reducing the number of such metastases would result in improved survival (11). Most of the many attempts made to do so in previous prospective, randomized trials of adjuvant systemic chemotherapy (6-8) have failed to reduce the development of liver metastasis. It has been suggested that one reason for the lack of success of adjuvant systemic chemotherapy was that sufficiently high doses of drug could not be given because of systemic side effects. Previous researchers have described portal vein infusion of cytotoxic agents at the time of surgery and during the postoperative period as an effective method of preventing metachronous liver metastasis (3,4). However, a large randomized trial of portal vein infusion of fluorouracil and heparin revealed that it had no significant impact on survival (5). Even metastases too small for detection by the naked eye receive a blood supply from newly developed arterioles (12). Ridge *et al.* (13) demonstrated that portal infusion chemotherapy could not maximize drug delivery to hepatic metastases. Daly *et al.* (14) reported significant improvement of tumor response after hepatic artery infusion compared with portal vein infusion. These findings suggest that adjuvant chemotherapy via the hepatic artery is more effective. At the time of initial surgery, micrometastases may already exist in the liver, and if a circulating tumor embolus has caused micrometastasis, portal infusion adjuvant chemotherapy is probably not very effective. Hepatic regional infusion of anticancer drugs achieves high local and low systemic drug concentrations, with the potential for an increased local response rate and decreased systemic toxicity, because the administration of drugs into the hepatic artery may maximize the concentration of the drug in the liver, at least during the first pass through this organ (9,10) and because hepatic extraction and metabolism of the drug will in turn decrease systemic exposure (11).

In this study, the initial regimen was to infuse 5-FU over a five-day period. Because this protocol resulted in many cases of catheter occlusion and the regimen completion rate was only 71.4%, we were obliged to change the protocol to a 24-hour infusion method in 1996. The widely accepted method for effective HAIC administration of 5-FU has been the 5 or 7-day continuous infusion method. However, in 1996, Arai *et al.*

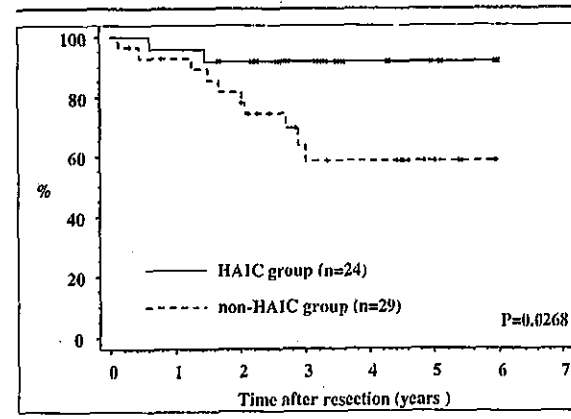

FIGURE 3 Liver metastasis-free curves in distal lymph node positive cases.

(15) reported that a weekly high-dose 5-FU infusion was effective for treating unresectable liver metastases. To treat ambulatory patients safely and to avoid catheter complications at the same time, we decided to alter the drug delivery protocol to the 24-hour infusion method.

In both protocols of this study, systemic toxicity was relatively mild and no patients developed chemical hepatitis or biliary sclerosis. The mean total dose of 5-FU administrated in this course of HAIC was 11.2g and not more than 15g. We assessed the effects of HAIC against patients to whom 7g or more of 5-FU was given, and concluded that 12g of 5-FU could be given without presenting toxicity.

In this study, we found there was an impact on survival after HAIC as a result of decreased liver metastases, especially in the cases positive for distant lymph node metastases. This is a significant finding, because these cases had high potential of developing recurrent tumors in the liver, ultimately leading to death, whereas HAIC effectively prevented recurrence. In several randomized studies, other investigators have reported that HAIC after resection of liver metastases prevented liver recurrences and improved patient outcome (16-18). Based on these reports, we can state that HAIC administered after local resection of tumor in Dukes C colorectal cancer patients had a prophylactic effect on hepatic metastases.

As is shown in **Table 3**, the effect of HAIC against extrahepatic metastases was limited. The total incidence of recurrence was almost the same in the two groups (25.7% *vs.* 30.6%) because of the increase of lung metastases in the HAIC group. This suggests the low systemic drug concentration produced during HAI would have little or no therapeutic effect on extrahepatic metastases. Thus, a combination of systemic and regional regimen is necessary for the effect of adjuvant chemotherapy to include other sites as well as the liver.

In conclusion, HAIC is an effective procedure for preventing metachronous liver metastasis and improving the prognosis in cases of advanced colorectal cancer, and this protocol we used in this pilot study can be safely employed in a prospective randomized study.

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Original article

Preoperative probability model for predicting overall survival after resection of pulmonary metastases from colorectal cancer

Y. Kanemitsu, T. Kato, T. Hirai and K. Yasui

Department of Gastroenterological Surgery, The Aichi Cancer Centre, Nagoya, Japan
Correspondence to: Dr Y. Kanemitsu, Department of Gastroenterological Surgery, The Aichi Cancer Centre, 1-1 Kanokoden, Chikusa-ku, Nagoya, 464-8681 Japan (e-mail: ykanemit@aichi-cc.jp)

Background: The aim of this study was identify readily available factors most helpful in predicting survival and to develop a prognostic nomogram for patients with pulmonary metastases from colorectal cancer who are candidates for thoracotomy.
Methods: Pretreatment data on 313 patients with metastases who underwent thoracotomy were analysed. Fourteen preoperative clinical and pathological variables were used to develop a probability model, in which their association with 3-year survival was tested. A nomogram to predict median, 1- and 3-year survival was constructed and validated internally using the concordance index (c-index). The nomogram was then validated with an external data set.
Results: Five variables were identified as independent predictors of 3-year survival: prethoracotomy carcinoembryonic antigen level, number of pulmonary tumours, presence of hilar or mediastinal tumour-infiltrated lymph nodes, histology of the primary tumour and presence of extrathoracic disease. The nomogram was well calibrated for predicting 3-year overall survival. The internal validated c-index of the nomogram was 0·72. Applied to another data set, the external validated c-index was 0·66.
Conclusion: This model has moderate predictive ability to discriminate between patients who are likely to survive after thoracotomy for pulmonary metastases from colorectal cancer.

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Introduction

Thomford *et al.*[1] reported criteria for resection of pulmonary metastases in 1965 that were widely accepted. However, in recent years these indications have been extended. Several studies have reported 5- and 10-year survival after resection of solitary pulmonary metastases arising from colorectal cancer[2–8]. However, resection of multiple or bilateral metastases remains controversial. The results of surgical resection compare favourably with those of chemotherapy for pulmonary metastatic colorectal cancer, and support the view that, in selected patients, surgery is the most effective therapy as it offers the potential for long-term survival. However, the number of reported cases is small and analyses of prognostic factors have shown important discrepancies, suggesting strict selection criteria or selective reporting that may have introduced a serious bias.

Patients with pulmonary metastases from colorectal cancer have a narrow range of therapeutic alternatives. The decision of whether to recommend surgery for a particular individual depends on the clinical presentation and estimated treatment benefits. An appropriate treatment policy should include an estimate of the baseline risk. This can be achieved by use of a risk model that integrates relevant prognostic features. The ability to assess the prognosis before therapy is important when counselling patients about their long-term outlook and in guiding treatment decisions. Such a predictive model is not currently available for patients with pulmonary metastases from colorectal cancer.

The present study is based on an analysis of the largest known series of resections of colorectal cancer metastatic to the lung. The aims were to identify readily available preoperative factors most helpful in predicting survival of patients with pulmonary metastases from colorectal cancer and to develop a prognostic nomogram that could be used routinely to predict the probability of surgical treatment failure in patients undergoing thoracotomy.

Patients and methods

Study population

The study was based on summarized medical records collected between 1980 and 1998 as part of a joint study undertaken by active members of an organization conducting government-supported cancer research and working at 11 major medical centres throughout Japan. Patients were eligible for surgical treatment of pulmonary metastases if there was no extrapulmonary metastatic disease at the time of thoracotomy, they were fit for pulmonary resection with a prospect of good postoperative quality of life, the primary tumour was controlled, and complete resection of the metastatic tumour was anticipated.

Three hundred and twenty-seven patients who underwent pulmonary resection for metastatic colorectal cancer were identified. Fourteen patients with missing follow-up data were omitted from the analysis. The remaining 313 patients were eligible for the study. All patients had a pathologically proven primary adenocarcinoma. Pulmonary resections included lobectomy (137 patients), partial resection (132), segmentectomy (38) and pneumonectomy (six). Limited resection was the preferred option in most institutions. Hilar or mediastinal lymph nodes were dissected or sampled in 184 patients. Liver metastases were found in 59 patients (18·8 per cent), and in 15 patients metastatic hepatic spread was identified at diagnosis of the primary tumour. All liver metastases detected up to the time of diagnosis of lung metastases could be resected with clear resection margins. Including these patients, but excluding those with other organs directly invaded by pulmonary tumour, a total of 84 patients were defined as having extrathoracic disease.

The endpoint of the study was survival time. Death from any cause was considered an event. The rationale for constructing a probability model was to identify patients whose prognosis was so poor that, even with lung resection, surgery was not appropriate. Patients who were still alive at last follow-up (with or without disease) were censored. Survival duration was measured from the date of first resection of pulmonary metastases to death or to the date of the last known follow-up evaluation. The median follow-up time was 29 (range 1–168) months.

The following data were retrieved from the patients' case report forms: age at thoracotomy, sex, primary site, histology of the primary tumour, tumour (T) stage of the primary cancer, node (N) stage of the primary cancer, number and distribution of pulmonary metastases, size of the largest pulmonary metastasis, presence of hilar or mediastinal tumour-infiltrated lymph nodes, presence of extrathoracic disease (including liver metastases) before or at the time of pulmonary metastasectomy, prethoracotomy carcinoembryonic antigen (CEA) level, interval between resection of the colorectal primary tumour and pulmonary resection (disease-free interval), and use of adjuvant chemoradiotherapy. Selection of potential predictive variables was based on a review of the literature, clinical experience and discussion. Although all patients had lung resection, some also received adjuvant chemotherapy or radiotherapy. Adjuvant use reflected the policy of individual institutions but was included as it represented a possible major confounding variable. Pathological features of the thoractomy specimen and data on surgical margins were not included as this information was not available before operation. All variables used to characterize pulmonary metastases (distribution and number of metastases, size of the largest metastasis and presence of extrathoracic disease) are routinely measured during preoperative investigations. Most CEA measurements were made using a sandwich antigen–antibody technique. To keep the model as simple and widely applicable as possible, recently developed markers, and those with less well demonstrated predictive value, not routinely measured in every patient, were not included in the analysis.

Information from the same institutions on a further 560 patients with pulmonary metastases from colorectal cancer, who had been medically diagnosed as having inoperable cancer and received any treatment except thoracotomy, was also obtained. These results helped to build the prognostic model by estimating the survival benefits of surgical treatment between 1 and 3 years after thoracotomy.

Statistical analysis

Univariable and multivariable Cox proportional hazards regression analyses were used to evaluate the relationships between baseline characteristics and death at 3 years after thoracotomy. All variables were entered at the multivariable stage, irrespective of the results of the univariable analyses. Univariate screening, in which only significant variables are entered into a subsequent multivariate regression model, is a forward stepwise variable selection technique in which non-significant variables from the first step are not reanalysed in later steps[9]. This approach may be suboptimal for maximizing predictive accuracy[10]. Whether any of the factors retained in the final model violated the assumption of proportional hazards was assessed, and then the univariable relationship of each factor with the outcome was determined by using restricted cubic spline functions[10] for continuous variables that showed non-linear relationships. The final

multivariable model was constructed by a backward elimination method to determine the significant predictors in the model (elimination criterion $P > 0.050$). Using the coefficients of the multivariable model, a nomogram was then developed to predict the probabilities of an individual patient with pulmonary metastases from colorectal cancer surviving at 1 and 3 years after thoracotomy. All decisions regarding the coding of the nomogram variables were made before modelling, because making these decisions afterwards can have detrimental effects on the predictive ability of the model[10].

The amount of missing data varied from 1·3 per cent for patient sex to 19·2 per cent for the prethoracotomy CEA level. As the exclusion of patients with missing data might lead to biased risk estimates[10], this was partly corrected for by performing all regression analyses on a data set that included imputed predictive variables. The iterative imputation technique applied estimated the missing value for a given predictor using a method for simultaneous imputation and transformation of predictor variables based on the concepts of maximum generalized variance and canonical variables[11]. However, for comparison, a data set consisting of only complete records was also modelled.

The discriminatory accuracy of the multivariable models was evaluated using Harrell's concordance c statistics (c-index) as an index of model performance[12]. The c-index estimates the probability of concordance between predicted and observed outcomes in rank order, and is closely related to the area under the receiver–operator characteristic (ROC) curve[13]. It expresses how well the model is able to discriminate between patients who survive and those who do not. Higher values indicate better discrimination; a value of 0·5 indicates no predictive discrimination, whereas 1·0 indicates perfect separation of patients with different outcomes[12].

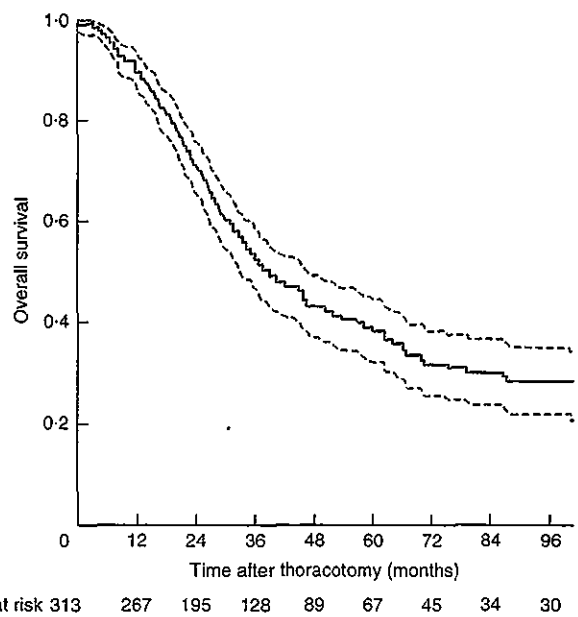

Fig. 1 Overall survival of patients in the derivation data set who underwent thoracotomy. Dotted lines represent 95 per cent confidence intervals

Table 1 Description of the derivation date set ($n = 313$)

	No. of patients
Age at thoracotomy (years)*	61 (26–83) (60)
Sex ratio (F : M)	130 : 183
Primary site	
Colon	126 (40·3)
Rectum	187 (59·7)
Histology of primary tumour	
Well differentiated	150 (47·9)
Moderately differentiated	129 (41·2)
Mucinous or poorly differentiated	34 (10·9)
Tumour stage	
Tis	3 (1·0)
T1	17 (5·4)
T2	26 (8·3)
T3	174 (55·6)
T4	93 (29·7)
Metastatic lymph nodes (primary lesion)	
No	124 (39·6)
Yes	189 (60·4)
Distribution of pulmonary lesions	
Ipsilateral	236 (75·4)
Bilateral	77 (24·6)
No. of pulmonary tumours*	1 (1–29) (2·1)
Size of largest pulmonary tumour (cm)*	2·5 (1–37) (3·0)
Hilar or mediastinal tumour-infiltrated lymph nodes	
No	274 (87·5)
Yes	39 (12·5)
Extrathoracic disease	
No	229 (73·2)
Yes	84 (26·8)
Prethoracotomy CEA level (ng/ml)*	6·7 (0·6–555·0) (19·4)
Interval between primary and pulmonary resection (months)*	30·6 (– 4·6 to 111·7) (35·9)
Adjuvant chemoradiotherapy	
No	228 (72·8)
Yes	85 (27·2)
Patient status	
Alive	134 (42·8)
Died from cause other than CRC	13 (4·2)
Died from unknown cause	21 (6·7)
Died from CRC	141 (45·0)
Died from treatment complications	4 (1·3)

Values in parentheses are percentages unless otherwise indicated; *values are median (range) (mean). CEA, carcinoembryonic antigen; CRC, colorectal cancer.

The models developed in the study population were further evaluated with respect to calibration by bootstrapping techniques: 200 bootstrap samples were drawn, with replacement, to estimate the extent to which the predictive accuracy of the models based on the entire population was overoptimistic[14]. A calibration graph was obtained by plotting the observed *versus* predicted probabilities. External validity of the nomogram was assessed using an external data set contributed by a further 72 hospitals participating in the Japanese Research Society for Cancer of the Colon and Rectum (JRSCCR)[15]. Of the 421 patients enrolled in the JRSCCR population, 64 were eliminated from this analysis because they had missing follow-up data; this left a modelling sample of 357 patients. In this JRSCCR population, nomogram predictions were assessed for discriminatory ability by quantifying the c-index, and predictions were assessed for calibration accuracy by plotting actual survival against predicted survival probabilities for patients stratified by predicted risk.

All analyses were performed using S-Plus® software version 2000 Professional Edition (Math Soft, Seattle, Washington, USA), together with packages of S-Plus® functions called Design and Hmisc[11]. All P values were obtained from two-sided statistical tests.

Results

Descriptive statistics for all predictor variables after imputing are listed in *Table 1*. Actual overall survival rates were 90·4 (95 per cent confidence interval (c.i.) 87·1 to 93·7) per cent at 1 year, 53·0 (95 per cent c.i. 47·0 to 59·0)

Table 2 Univariable predictors of death by 3 years after thoracotomy

	Wald χ^2	df	P	Hazard ratio
Prethoracotomy CEA level	46·2	3	<0·001	
No. of pulmonary tumours	31·6	2	<0·001	
Histology of primary tumour	11·2	2	0·004	
Mucinous or poorly differentiated				0·70 (0·41, 1·20)
Moderately differentiated				1·51 (1·11, 2·06)
Well differentiated				1·00
Extrathoracic disease	10·9	1	0·001	
Yes				1·73 (1·25, 2·39)
No				1·00
Metastatic lymph nodes (primary lesion)	5·9	1	0·015	
No				0·68 (0·50, 0·93)
Yes				1·00
Hilar or mediastinal tumour-infiltrated lymph nodes	5·4	1	0·021	
Yes				1·61 (1·08, 2·42)
No				1·00
Distribution of pulmonary lesions	5·1	1	0·023	
Bilateral				1·46 (1·05, 2·03)
Ipsilateral				1·00
Adjuvant chemoradiotherapy	4·4	1	0·037	
Yes				0·69 (0·49, 0·98)
No				1·00
Tumour stage	5·5	4	0·243	
Tis				2·04 (0·65, 6·44)
T1				1·09 (0·58, 2·04)
T2				0·56 (0·30, 1·05)
T3				1·00
T4				0·88 (0·63, 1·24)
Interval between primary and pulmonary resection	3·8	3	0·282	
Age at thoracotomy	1·9	3	0·587	
Primary site	1·0	1	0·329	
Colon				0·86 (0·63, 1·17)
Rectum				1·00
Size of largest pulmonary tumour	0·3	3	0·970	
Sex	0·0	1	0·936	
F				0·99 (0·73, 1·33)
M				1·00

Values in parentheses are 95 per cent confidence intervals. Hazard ratios are not presented for continuous variables because the data are transformed by restricted cubic spline function with three or four knots. CEA, carcinoembryonic antigen.

per cent at 3 years and 38·3 (95 per cent c.i. 32·1 to 44·5) per cent at 5 years (*Fig. 1*). One hundred and seventy-nine patients had died at the time of the last follow-up in December 1998, giving a median survival of 38·4 months. The 1-, 3- and 5-year survival rates of the 560 patients with pulmonary metastases from colorectal cancer who did not undergo thoracotomy were 58·6 (95 per cent c.i. 54·5 to 62·8), 8·5 (95 per cent c.i. 6·1 to 11·0) and 1·9 (95 per cent c.i. 0·6 to 3·2) per cent respectively (46 patients censored), and their median survival time was 14·4 months.

Univariable analyses

Table 2 shows the univariable relationships between baseline characteristics and outcomes. Of the 14 variables tested, eight were independently associated with outcome. The number of pulmonary metastases and prethoracotomy CEA level were strongly associated with death by 3 years. Other important risk factors were histology of the primary tumour, presence of extrathoracic disease, lymph node metastasis of the primary tumour, presence of hilar or mediastinal tumour-infiltrated lymph nodes, distribution of pulmonary lesions and adjuvant chemoradiotherapy.

Multivariable model

Five of the outcome predictors with significant associations in the univariable analyses remained important in the multivariable model (*Table 3*). After correction for other determinants, prethoracotomy CEA level showed the strongest relationship with death at 3 years; the number of pulmonary metastases was the next strongest predictor.

Table 3 Multivariable predictors of death by 3 years after thoracotomy

	Wald χ^2	d.f.	*P*	Hazard ratio
Prethoracotomy CEA level	42·1	3	<0·001	
No. of pulmonary tumours	10·5	2	0·005	
Hilar or mediastinal tumour-infiltrated lymph nodes	10·2	1	0·001	
Yes				2·18 (1·35, 3·52)
No				1·00
Histology of primary tumour	7·3	2	0·003	
Mucinous or poorly differentiated				0·73 (0·40, 1·33)
Moderately differentiated				1·52 (1·07, 2·17)
Well differentiated				1·00
Extrathoracic disease	5·3	1	0·021	
Yes				1·55 (1·07, 2·26)
No				1·00
Adjuvant chemoradiotherapy	3·3	1	0·068	
Yes				0·71 (0·49, 1·03)
No				1·00
Age at thoracotomy	6·0	3	0·113	
Size of largest pulmonary tumour	5·3	3	0·153	
Tumour stage	4·6	4	0·327	
Tis				1·02 (0·27, 3·79)
T1				1·88 (0·94, 3·75)
T2				0·90 (0·46, 1·78)
T3				1·00
T4				0·84 (0·56, 1·24)
Sex	2·9	1	0·086	
F				0·73 (0·51, 1·05)
M				1·00
Interval between primary and pulmonary resection	2·4	3	0·501	
Distribution of pulmonary lesions	0·9	1	0·338	
Bilateral				0·76 (0·44, 1·33)
Ipsilateral				1·00
Primary site	0·5	1	0·462	
Colon				0·87 (0·59, 1·27)
Rectum				1·00
Metastatic lymph nodes (primary lesion)	0·0	1	0·947	
No				1·01 (0·69, 1·50)
Yes				1·00

Values in parentheses are 95 per cent confidence intervals. Hazard ratios are not presented for continuous variables because the data are transformed by restricted cubic spline function with three or four knots. CEA, carcinoembryonic antigen.

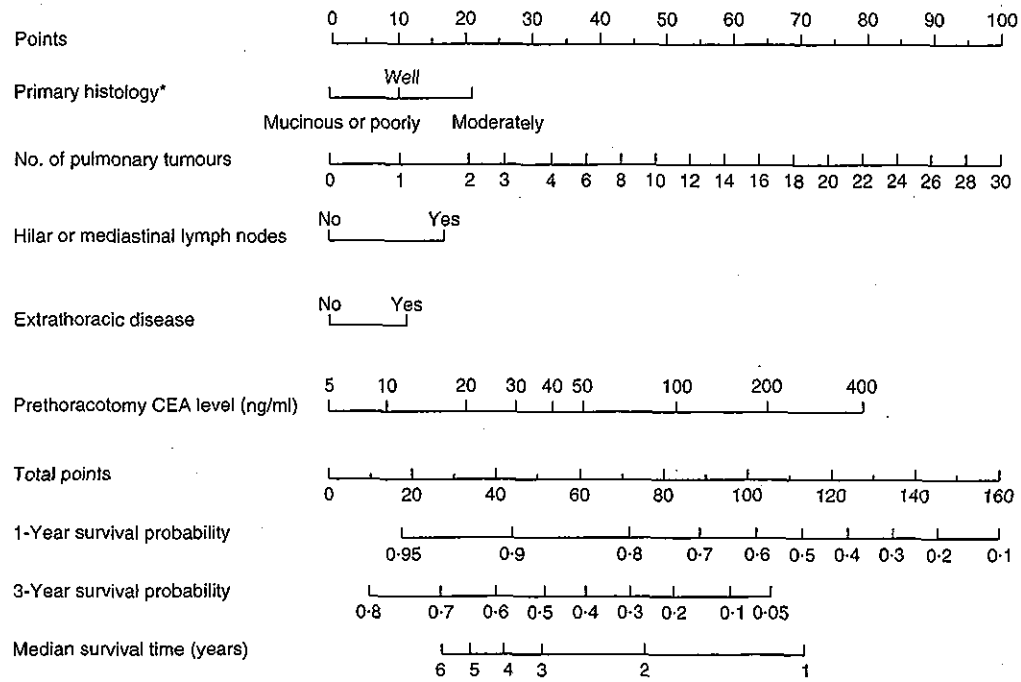

Fig. 2 Preoperative nomogram for survival based on 313 patients with pulmonary metastases from colorectal cancer treated with thoracotomy. *Well, moderately, or mucinous or poorly differentiated tumours. CEA, carcinoembryonic antigen

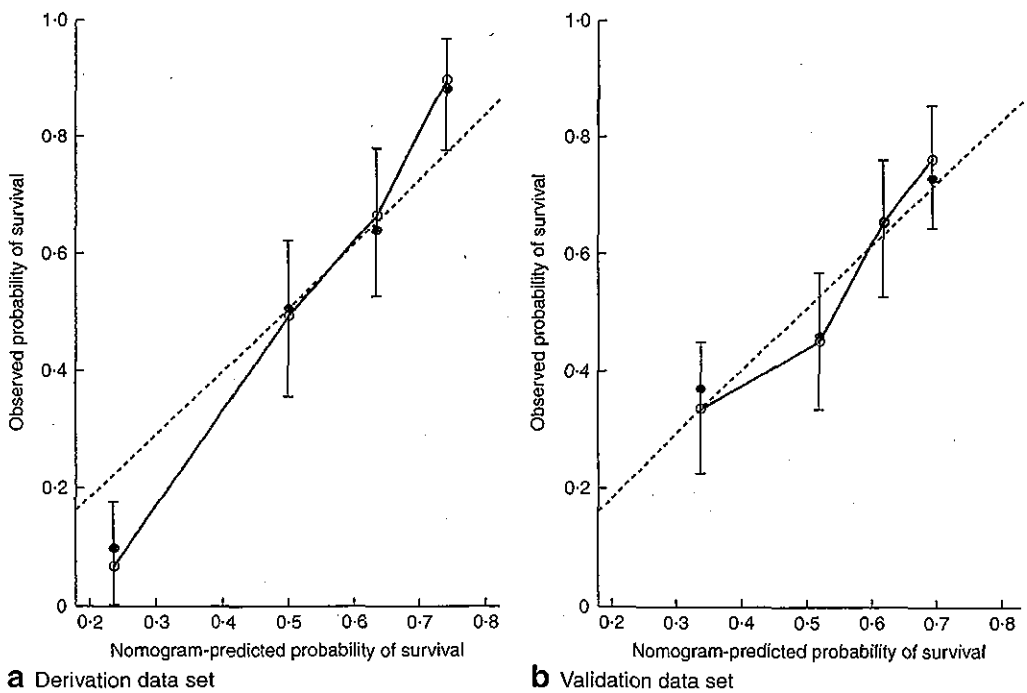

Fig. 3 a Calibration of the nomogram in the derivation data set. Horizontal axis is nomogram-predicted probability of remaining alive. Vertical axis is actual survival estimated at 3 years by Kaplan–Meier method. Dotted line indicates the reference line on which an ideal nomogram would lie. Solid line indicates performance of the present nomogram. ○, Subcohorts of database; ●, bootstrap-corrected estimate of nomogram's performance with 200 resamples. Bars indicate 95 per cent confidence intervals. b Comparison of nomogram-predicted and observed 3-year survival after thoracotomy in the validation data set

Other important risk factors were presence of hilar or mediastinal tumour-infiltrated lymph nodes, histology of the primary tumour and presence of extrathoracic disease.

A nomogram incorporating each of these five clinical predictors was constructed according to the Cox model (*Fig. 2*). No violation of the proportional hazards assumption was evident ($P = 0{\cdot}060$) and none of the ten two-way interactions was statistically significant ($P = 0{\cdot}323$). To use the nomogram, determine the points assigned on the 0–100 scale for each predictor and add them together. Locate this value on the 'total points' axis with a vertical ruler and follow the ruler down to read off any predicted values of interest. For example, for a patient with a total of 72 points, the nomogram predicts an 80 (95 per cent c.i. 70 to 90) per cent chance of avoiding death at 1 year and a 30 (95 per cent c.i. 20 to 40) per cent chance at 3 years.

The c-index for the nomogram was 0·72, reflecting moderate predictive ability to discriminate between patients who did and did not die after thoracotomy. Analyses that included non-imputed values continued to favour the derivation data set (228 patients) for prethoracotomy CEA level ($P < 0{\cdot}001$), number of pulmonary metastases ($P < 0{\cdot}001$) and presence of extrathoracic disease ($P = 0{\cdot}022$), with a trend for hilar or mediastinal lymph nodes ($P = 0{\cdot}075$). When bootstrap validation was performed, the non-imputed model also achieved a c-index of 0·72. The analyses were repeated when missing variables were not imputed, and verified that the results were virtually unchanged. *Fig. 3a* shows the calibration graph for the nomogram, in which the 3-year survival probabilities predicted by the nomogram are plotted against the corresponding observed survival rates obtained by the Kaplan–Meier method. This illustration suggests good calibration of the nomogram.

External validation

The survival of JRSCCR patients was very similar to that in the derivation set as shown. Actual overall survival rates were 89·8 (95 per cent c.i. 86·6 to 93·0) per cent at 1 year, 54·1 (95 per cent c.i. 48·7 to 59·5) per cent at 3 years and 39·9 (95 per cent c.i. 34·4 to 45·3) per cent at 5 years. The c-index for this validation cohort was 0·66. The nomogram also predicted better than chance for the external data set. Calibration plots suggested that the nomogram was well calibrated for all predictions (*Fig. 3b*). Patient survival, stratified by quartiles of the nomogram-predicted median survival times, is shown in *Fig. 4*. The observed survival differed significantly between patients in the different quartiles ($P < 0{\cdot}001$). *Table 4* shows details of each quartile of median survival in the validation data set.

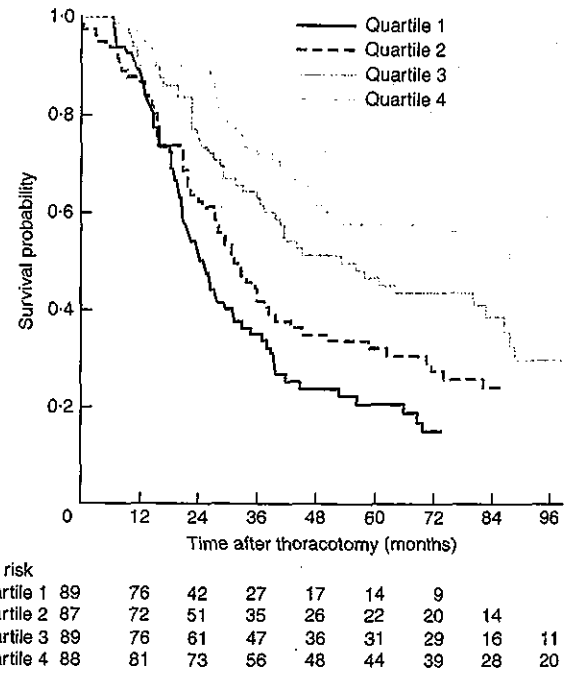

Fig. 4 Survival curves for patients in the validation set stratified by quartiles of the nomogram-predicted median survival time

Table 4 Risk group stratification based on nomogram predictions of median survival

Quartile	Nomogram prediction of survival (months)	
	Median	95% c.i.
1	24·7 (14·4–32·4)	20·7 to 31·1
2	32·7 (32·4–43·5)	26·0 to 43·4
3	57·3 (43·5–62·6)	38·5 to 80·4
4	90·0 (62·6–111·0)	54·6 to 104·5

Values in parentheses are ranges. The 95% per cent confidence intervals (c.i.) for the median nomogram predictions were obtained by bootstrapping.

Discussion

The intention of this study was to incorporate several readily available factors into a prognostic nomogram, with rigorous model validation. As in previous studies, the number of pulmonary metastases[16,17], presence of extrathoracic disease[18], presence of hilar or mediastinal tumour-infiltrated lymph nodes[3] and CEA level[16–19] were established as independent predictors of death after thoracotomy. A multivariable analysis of prognostic factors was then undertaken with the aim of developing a probability model for use at the time of presentation to assess the chance of survival.

With regard to the number of pulmonary metastases, which was established as one of the important prognostic factors in this study, many authors have reported a favourable survival rate for patients with a solitary pulmonary metastasis[2,4,7,17] and a poor prognosis for those with two or more pulmonary metastases[2]. However, McAfee *et al.*[4] observed a 5-year survival rate after surgery of 25 per cent for patients with multiple metastases. In the present study the 5-year survival rate for 132 patients with two or more pulmonary metastases was 32·4 (95 per cent c.i. 22·3 to 42·4) per cent after thoractomy and 38 patients have survived for more than 3 years. Therefore multiple pulmonary metastases *per se* should not be considered a contraindication to operation if surgery is technically feasible.

The prethoracotomy CEA level was the most important independent prognostic factor in this study. Previous studies have also shown that a raised serum level of CEA before thoracotomy is associated with a poor prognosis in patients with pulmonary metastases from colorectal cancer[16–19]. In these studies the CEA level was dichotomized according to an arbitrary cut-off value of 5 ng/ml. According to their prognostic criteria, thoracotomy seemed warranted for patients with pulmonary metastases and a normal CEA level, but the situation was less clear for patients with a raised CEA level. Ideally, the predictor should be a continuous variable to maximize the amount of information that it can convey[20].

The present model has some limitations. First, the nomogram is not perfectly accurate. When subjected to internal validation, the area under the ROC curve (c-index) for the nomogram was 0·72, and internal calibration showed that the predictions reasonably approximated actual survival probabilities. In the external validation analysis, the nomogram achieved a c-index of 0·66, suggesting adequate discrimination. Furthermore, this model readily enabled patients with pulmonary metastases to be stratified into four risk groups based on nomogram-predicted median survival times, and is worth using in a clinical setting. Second, the nomogram predicted death after thoracotomy to a maximum of 3 years. It is possible for a patient to die from cancer after 3 years, so the nomogram does not provide a true probability of cure after surgery. However, knowledge of the patient's chance of survival in the short to medium term is important to both medical staff and to the individual facing a terminal illness[21]. A third limitation of this study is that the nomogram was developed in a population of patients who were subsequently treated by thoracotomy, so is applicable only to potential candidates for surgery, rather than all patients diagnosed with pulmonary metastases from colorectal cancer. It would be most appropriate to apply the nomogram as the last step in the decision-making process, after the patient and physician have decided upon thoracotomy as the treatment choice. Thoracotomy seems warranted for patients with a high probability of survival at 3 years after operation. However, the risk of overtreating patients with a low life expectancy of between 1 and 3 years after thoractomy should be avoided as most patients who did not become candidates for thoracotomy during this period went on to die.

The unique aspects of the nomogram are that it produces a point estimate indicating the probability of death for each patient with a given combination of predictors. Although this point estimate represents the best single-number estimate, it does not provide any sense of the degree of uncertainty surrounding it. A rough estimate of that uncertainty is provided by the 95 per cent confidence interval. The vertical bars in *Fig. 3* indicate 95 per cent confidence intervals based on the bootstrap analysis. In general, the performance of the nomogram appears to be within 10 per cent of the actual outcome. Thus, patients whose values fall within 10 per cent of either side of the percentage predicted from the nomogram might be expected to be alive at 1 and 3 years after thoracotomy.

This model may provide the most accurate preoperative prediction of survival after thoracotomy for individual patients with pulmonary metastases from colorectal cancer. Although these predictions are not perfect, use of the nomogram may contribute to treatment planning and routine care of such patients.

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"On Table" Positioning for Optimal Access for Cancer Excision in the Lower Rectum

Koutarou Maeda, M.D., Ph.D., Morito Maruta, M.D., Harunobu Sato, M.D., Koji Masumori, M.D., Hiroyuki Aoyama, M.D.

Department of Surgery, Fujita Health University School of Medicine, 1-98 Kutsukake, 470-1192 Toyoake, Aichi, Japan

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Abstract. Poor visualization and restricted access often make tumor lesions in the lower rectum difficult to excise, particularly in a narrow male pelvis. The aim of this study was therefore to study whether (and if so to what extent) different positions of the patient on the operating table might improve accessibility. Twenty consecutive patients (men and women) undergoing laparotomy with surgery of the lower rectum were studied. The geometric configuration of the pelvis was studied and compared on lateral radiographs obtained at the operating table in each of four positions. Compared with the conventional lithotomy position, the thighs-flat" position caused significant extension movement of the lumbosacral joint. Augmentation of the lumbar lordosis widened the pelvic view and enabled a more vertical view of the lower rectum (27.5 degrees in lithotomy position, 13.0 degrees in the thighs-flat position). Insertion of a "lumbar pad" contributed further to the augmentation (7 degrees). When compared on radiographic studies, the thighs-flat position is preferable to the conventional lithotomy position in terms of facilitating low rectal surgery by improving both visibility and accessibility to the pelvic cavity.

Positioning the patient for operations on the rectum is controversial [1]. The lithotomy or lithotomy-Trendelenberg position with or without a sacral rest or the supine, prone, or jack-knife position have been tried, with these modifications having been widely adopted for rectal surgery in many institutions [2–9]. Lloyd-Davies and Lond reported that "the best exposure of the pelvic cavity is obtained when the thighs are extended" [9]. We have reconsidered the concept and after some modification found that "the thighs-flat" position with or without a "lumbar pad" is a more rational position than the traditional lithotomy position because of the visibility and access from the abdomen. To our knowledge, no studies have compared the various positions in terms of better visibility. This trial therefore aimed to determine the positions that are most advantageous from the geometric point of view in the pelvis for better visibility of the lower rectum.

Materials and Methods

Altogether, 20 consecutive patients, including 11 men of median age 56 years (range 36–66 years) and 9 women of median age 59 years (range 48–68 years) who were undergoing excision of a rectal carcinoma/low anterior resection (LAR), Hartmann's operation, or abdominoperineal excision (APR) were entered into the study. They were randomly (using sealed envelopes) divided into two groups (10 patients each) according to the positions studied. Five male patients were included in a group of 10 patients and 6 male patients in another group. There was no past history of lumbar or back pain or diseases of the lumbar vertebra in these patients. Informed consent to participate in the study was obtained from all patients before the operation.

After induction of anesthesia the patient was placed in position. A specially designed operating table that allowed automatic control of the leg supports was used. Changing the angle of the leg supports could be easily and carefully performed when flexion of the thighs was needed. Special care was taken to avoid improper pressure on the legs.

Four positions were studied: position I (lithotomy position), position II (thighs-flat position), position I with a sacral pad, and position II with a lumbar pad (Fig. 1). The angle between the body and the thighs was measured by a graduator and set at 140 degrees for the lithotomy position and 180 degrees for the thighs-flat position. A cushion pillow was used as a sacral pad or a lumbar pad and was placed underneath the second to third sacral vertebrae or the fourth lumbar vertebra, respectively, to elevate the sacrum or to increase the "swayback." Potential complications related to positioning were studied after the operation.

Contrast medium was introduced into the rectum. A metal bolt was placed on the skin of a cranial margin of the pubic bone after each position was established. Three plain radiographs with a lateral view of the pelvis were obtained after placing each patient in each position. Positions I, I with a sacral pad, and II were studied in one group; and positions I, II, and II with a lumbar pad were studied in another group. Geometric measurements of the patients in the various positions were performed on the radiographs, as demonstrated in Figure 1. The upper margin of the anal canal was defined as the lowest point of the rectum demonstrated by the medium to be tapering on the radiograph. The site of the metal bolt was defined as the cranial margin of the pubic bone.

Angle A was measured with a pelvic tilt to the operating table.

Correspondence to: Koutarou Maeda, M.D., Ph.D., e-mail: kmaeda@fujita-hu.ac.jp

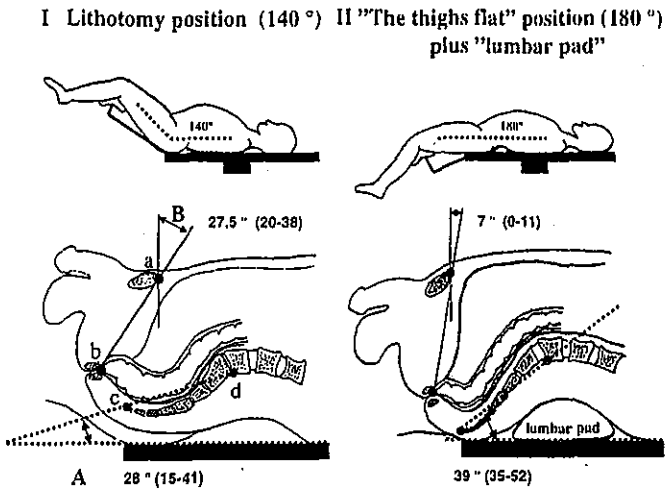

Fig. 1. Positions I and II with a "lumbar pad" and radiologic assessment. Angle between the thighs and body was set to 140 or 180 degrees in the lithotomy position or "the thighs-flat" position. A: angle between the horizontal line and the line from c to d; B: angle between the vertical line and the line from a to b; a: cranial margin of the pubic bone; b: lowest point of the rectum demonstrated tapering on medium; c: tip of the coccyx; d: posterocaudal edge of the fifth lumbar vertebra.

The tip of the coccyx and the posterocaudal edge of the fifth lumbar vertebral body were used as landmarks for measuring the pelvic tilt, as shown on Figure 1. Angle B was measured between a vertical line and a line from the cranial margin of the pubic bone to the upper margin of the anal canal. It is thought that making angle B as small as possible improves visualization and pelvic exposure [10].

Radiologic parameters were compared among the positions studied in each group ($n = 10$). Angle B was compared among the positions according to gender and for the gender in each position as well. Data from both groups ($n = 20$) were used to compare positions I with II.

The paired t-test was used to determine the statistical significance of radiologic parameters among positions, and the nonpaired t-test was used to compare men and women. Differences were considered significant at $p < 0.05$.

Results

The rectum and the metal bolt were well demonstrated on radiographs, and the upper limit of the anal canal and cranial margin of the pubic bone were clarified in all 60 films. Therefore angle B could be evaluated in all films. The posterocaudal edge of the fifth lumbar vertebral body was not clarified in 12 films as it was beyond the field of the radiograph. The tip of the coccyx was not identified in 35 films because the radiographic conditions were too strong for visualizing the soft coccyx. Finally, angle A could be evaluated in 24 films.

The pelvic tilt to the table (angle A) became significantly greater in the thighs-flat position (position II or II with a lumbar pad) than in the lithotomy position (position I or I with a sacral pad) ($p < 0.04$) with a further increase of the angle by adding a lumbar pad ($p = 0.014$), as shown on Table 1. However no significant difference was confirmed in angle A by adding a sacral pad to the lithotomy position, which might be due to the small samples obtained.

The median angle B was 27.5 degrees in position I (range 20–38 degrees), 35 degrees in position I with a sacral pad (range 25–43 degrees), 13 degrees in position II (range 4–29 degrees), and 7 degrees in position II with a lumbar pad (range 0–11 degrees); it was significantly smaller in the thighs-flat position (position II or II with a lumbar pad) than in the lithotomy position (position I or I with a sacral pad) ($p < 0.0001$), as shown in Table 1. Furthermore, angle B was further reduced by adding a lumbar pad to the thighs-flat position ($p < 0.0001$). Angle B became significantly greater by adding a sacral pad to the lithotomy position ($p = 0.0056$).

Even when compared by gender, as shown on Table 2, angle B was significantly smaller in the thighs-flat position (position II or II with a lumbar pad) than in the lithotomy position (position I or I with a sacral pad) (men, $p < 0.002$; women, $p < 0.0024$). Furthermore, angle B became significantly smaller by adding a lumbar pad to the thighs-flat position for each gender (men, $p = 0.0019$; women, $p = 0.0022$). In contrast, angle B became greater significantly by adding a sacral pad to the lithotomy position in male patients ($p = 0.013$); the difference was not significant in female patients ($p = 0.13$).

When comparing the positions, as shown on Table 2, angle B was smaller in female patients than in male patients in positions I, I with a sacral pad, and II, although statistical significances were not confirmed. Potential complications, lumbago, and back pain related to positioning were not observed after surgery.

Discussion

The thighs-flat position studied here has been used as a standard position for surgery of the rectum in our unit for more than 10 years. By increasing the "swayback" a lumbar pad added to the lithotomy position has previously been reported to be advantageous for improving the visibility of the lower rectum [10]. A lumbar pad used in addition to the thighs-flat position was therefore included in the study. There were no potential complications or symptoms related to the positioning.

The pubic bone is usually the main restricting factor, interfering with access to the distal pelvis; and it often causes imprecise, inadequate dissection. The articular processes of the vertebral site posteriorly and intervertebral space open up anteriorly with increasing lumbar lordosis [11, 12]. Therefore the posterocaudal edge of the fifth lumbar vertebral body was used as a point working like a fulcrum, which is well documented on radiographs assessing the movement of the pelvis in the present study. In a previous study, which compared abdominal visibility using the lithotomy position with or without a lumbar pad, reducing angle B improved visibility in the lower rectum [10]. Extension movement of the lumbosacral joint with an increased lumbar lordosis occurred in the thighs-flat position compared with the lithotomy position, as shown by a steeper pelvic tilt with a reduction in angle B, which reflected the opening up of the pelvic view and enabled more vertical visualization of the lower rectum from the abdomen. The lumbar lordosis was increased further by adding a lumbar pad to the thighs-flat position, thereby improving access to and visibility of the pelvis as reflected by the increased angle A (Fig. 1).

The advantage of the thighs-flat position was described by Lloid-Davies and Lond in 1939 from their experience as "the best exposure of the abdomen is obtained when the thighs are extended" [9]. The results of the present study supports this statement, although the wide range of angle B was clarified even in the thighs-flat position. Therefore the position chosen is best individualized by adding

Table 1. Radiologic evaluation of angles A and B.

Parameter	Position I	I with "sacral pad"	II	II with "lumbar pad"	p (paired t-test)
Angle A (degrees)	28 (15–41) (n = 7)	24 (19–28) (n = 3)	35 (26–48) (n = 9)	39 (35–52) (n = 5)	< 0.04*
Angle B (degrees)	27.5 (20–38) (n = 20)	35 (25–43) (n = 20)	13 (4–29) (n = 10)	7 (0–11) (n = 10)	< 0.006**

Values are shown as the median and range of all cases.
*p = 0.039 position I vs. II; *p < 0.02 position II vs. I with a "sacral pad" and II with a "lumbar pad."
**p < 0.006 between each position

Table 2. Radiologic evaluation of angle B according to gender.

Gender	Position I	I with "sacral pad"	II	II with "lumbar pad"	p (paired t-test)
Male	29 (20–38) (n = 11)	35 (34–43) (n = 5)	15 (4–29) (n = 11)	8 (0–11) (n = 6)	< 0.014*
Female	26 (20–34) (n = 9)	30 (25–37) (n = 5)	11 (6–18) (n = 9)	8 (2–9) (n = 4)	< 0.0024**
p (nonpaired t-test)	0.22	0.12	0.17	0.92	

Values are shown as the median and range of all cases.
*p = 0.013 position I vs. I with a "sacral pad"; *p < 0.002 between the other positions;
**p < 0.0024 between each position except position I vs. I with a "sacral pad" (p = 0.13)

a lumbar pad to the thighs-flat position in patients with a deep rectum (i.e., a rectum situated caudally far below the upper margin of the pubic bone). However, extreme positions may not be needed to expose the upper rectum.

To improve the perineal approach, a sacral rest is often recommended for LAR or APR [2, 3, 5, 7, 8]. The center of a sacral rest is placed underneath the promontorium, with the main aim of allowing the buttocks to project over the edge of the table [8]. Lloyd-Davies and Lond [9] noted that the best exposure of the perineum was obtained when the thighs were flexed in the lithotomy position. However, by separating the legs widely [8], access to the perineum is still adequate even in the thighs-flat position. The thighs-flat position with a lumbar pad used in the present study is similar to this abducted thighs-Trendelenburg position [8].

When exposure is not enough for the perineal procedure with the thighs-flat position, the thighs can be easily flexed using a special table. If an operating table that allows easy control of the angles of the leg supports is not available, it is recommended that the thighs be slightly flexed enough to allow insertion of the circular stapler through the anus or simultaneous access to the anal canal and the pelvis for rectal resection. The thighs-flat position is preferred during the pelvic dissection with an additional head-down tilt during the perineal phase of the operation. As an alternative, a staged approach might be used for an operation that requires a bilateral approach with the thighs-flat position for the abdominal phase, followed by the Lloyd-Davies position for the perineal phase.

Résumé. Une mauvaise visualisation et un accès *restreint* rendent souvent difficile l'excision des tumeurs du bas rectum, particulièrement chez l'homme à bassin étroit. Le but de cette étude a donc été de déterminer si, et à quel degré, les postions différentes du patient sur la table d'opération pourrait améliorer cette accessibilité. Vingt patients consécutifs, hommes et femmes, ayant eu une *laparotomie pour lésion* du bas rectum ont été inclus. La configuration géométrique du pelvis a été comparée sur les clichés radiologiques latéraux pris sur la table d'opération dans quatre positions différentes. Comparée à la position de lithotomie conventionnelle, la position « cuisses à plat » provoquait d'une extension importante de l'articulation lombosacrée. Une exagération de la lordose lombaire a amélioré la vision du pelvis et a permis une meilleure vision verticale du bas rectum (27.5 degrés en position de lithotomie, 13 degrés en position «cuisses à plat»). L'insertion d'un billot lombaire a augmenté cette amélioration (7 degrés). Selon les résultats de cette étude radiologique, la position «cuisses à plat» est préférable à la position de lithotomie classique car elle améliore la chirurgie du bas rectum en *augmentant la visibilité et* l'accessibilité à la cavité pelvienne.

Resumen. La pobre visualización y el acceso restringido frecuentemente dificultan la resección de tumores del recto distal, especialmente en la angosta pelvis *masculina*. El *propósito* del presente estudio fue analizar hasta qué punto las diferentes posiciones del paciente en la mesa de cirugía podrían mejorar el acceso. Veinte pacientes consecutivos masculinos/femeninos sometidos a laparotomía para cirugía sobre el recto distal fueron investigados. Se hizo el estudio de la configuración geométrica de la pelvis y se comparó con radiografías laterales tomadas con el paciente sobre la mesa en cada una de las cuatro diferentes posiciones. En comparación con la posición convencional de litotomía, la posición de "muslos en posición plana" resultó en un significativo movimiento de extensión de la articulación lumbosacra. El aumento de la lordosis lumbar amplió la visión pélvica y permitió una visión más vertical del recto distal (27.5 grados en litotomía, 13 grados en la *posición de "muslos en posición* plana"). La inserción de un soporte lumbar contribuyó más al aumento (7 grados). La posición de "muslos en posición plana" es preferible sobre la posición de litotomía convencional por cuanto facilita la cirugía rectal baja al mejorar tanto la visibilidad como el acceso a la cavidad pélvica, según se deriva de los *estudios radiográficos*.

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Application of RT-PCR to clinical diagnosis of micrometastasis of colorectal cancer: A translational research study*

OSAMU TAKAYAMA[1], HIROFUMI YAMAMOTO[1], KIMIMASA IKEDA[2], HIDEYUKI ISHIDA[3], TAKESHI KATO[4], MASAKI OKUYAMA[2], TOSHIYUKI KANOU[5], MUTSUMI FUKUNAGA[6], SHUSEI TOMINAGA[7], SHUNJI MORITA[8], YUJIRO FUJIE[1], HIROKI FUKUNAGA[1], MASAKAZU IKENAGA[1], MASATAKA IKEDA[1], MASAYUKI OHUE[1], MITSUGU SEKIMOTO[1], NOBUTERU KIKKAWA[4] and MORITO MONDEN[1]

[1]Department of Surgery and Clinical Oncology, Graduate School of Medicine, Osaka University; [2]Department of Surgery, Toyonaka Municipal Hospital; [3]Department of Surgery, Sakai Municipal Hospital; [4]Department of Surgery, Minoh City Hospital; [5]Department of Surgery, NTT West Osaka Hospital, Osaka; [6]Department of Surgery, Kansai Rosai Hospital, Hyogo; [7]Department of Surgery, Ikeda Municipal Hospital; [8]Department of Surgery, Yao Municipal Hospital, Osaka, Japan

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Abstract. We previously reported in a retrospective study that CEA-based RT-PCR was useful for predicting the prognosis of patients with node-negative colorectal cancer. RT-PCR is well established for laboratory use, but many issues remain to be resolved prior to its clinical application. In addition to the false positive rate of RT-PCR, we addressed several issues, including the timing of lymph node sampling, stability of RNA after surgery, and reproducibility of results. After appropriate modification, including development of a tissue sampling kit, a multi-institutional clinical study was commenced prospectively from November 2001, and 100 patients were enrolled for examination of micrometastasis. RNA was stable in lymph nodes for up to 3 h after surgical resection. This range of sampling time was acceptable to the surgeons. RNA was well preserved in RNA later™ at -20°C for 3 weeks. Dilutions of MKN45 and LoVo cells served as positive controls for conventional PCR since these controls were found to be highly stable and ensured reproducibility. Moreover, simultaneous use of quantitative PCR (Light Cycler™) ensured double confirmation of the results. Our clinical study showed that the quality of RNA was excellent or good in most samples (98 of 100; 98%). Twenty-four of 98 (24.5%) cases were judged to be micrometastasis-positive. In conclusion, the current translational research study established a clinically feasible RT-PCR system for micrometastasis. Our system could potentially be useful as a clinical tool.

Correspondence to: Dr Hirofumi Yamamoto, Department of Surgery and Clinical Oncology, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita City, Osaka 565-0871, Japan
E-mail: kobunyam@surg2.med.osaka-u.ac.jp

*Multicenter Clinical Study Group of Osaka, Colorectal Cancer Division

Abbreviations: cDNA, complementary DNA; CEA, carcinoembryonic antigen; CK20, cytokeratin 20; CRC, colorectal cancer; LN, lymph node; PBGD, porphobilinogen deaminase; RT-PCR, reverse transcriptase-polymerase chain reaction

Key words: colorectal cancer, micrometastasis, carcinoembryonic antigen, real-time PCR, translational research

Introduction

Reverse transcriptase-polymerase chain reaction (RT-PCR) is a powerful method for detection of micrometastases of colon cancer cells. It has been reported that 29.6-100% of node-negative (N0) patients with colorectal cancer (CRC) had micrometastasis in lymph nodes (LN) by RT-PCR (1-6). Liefers *et al* showed that carcinoembryonic antigen (CEA)-based RT-PCR-positive cases had a significantly poorer prognosis than RT-PCR-negative cases in a series of 26 stage II CRCs (1). We previously showed that RT-PCR using CEA or cytokeratin 20 (CK20) as a genetic marker was helpful to predict the rapid recurrence of CRC patients (2). Moreover, our retrospective study using paraffin blocks showed that CEA-based RT-PCR-positive stage II CRCs had a significantly poorer prognosis than CEA RT-PCR-negative CRCs during more than 5 years of follow-up (3). These findings suggest that RT-PCR may be a useful tool to predict clinical outcome and help in selecting appropriate treatment for node-negative CRC patients.

At present, CEA-based RT-PCR is well established for laboratory use, but much remains to be resolved prior to its practical application to clinical use. Firstly, false positivity has been a fundamental problem associated with the PCR technique. This issue was avoidable by establishment of PCR

conditions with reasonable sensitivity, as described in our earlier studies (2,3). Thus, amplification of PCR for CEA transcript was restricted to 35 cycles by the appearance of a few false positive samples in normal control LNs.

Secondly, the stability of RNA in LN must be assessed. Surgeons usually collect LNs from resected specimens several hours after surgery. It is probable that RNA might be impaired during this period since RNA from certain tissues undergoes degradation very quickly. This is a critical issue because if degradation of RNA in LN occurs extremely rapidly, diagnosis of micrometastasis by RT-PCR is practically impossible in most hospitals. Moreover, in cost-benefit terms, it would be better to perform RNA extraction and RT-PCR solely in node-negative CRC patients, not in node-positive patients. For this purpose, RNA in LNs must be well preserved until the pathological report is available, which usually takes about 1-2 weeks.

Thirdly, particular attention has to be taken to avoid contamination of tumor cells during LN sampling procedures. Elimination of RNase in the environment is also a strict requirement (7). However, it would be a practical difficulty at most hospitals to prepare tools specialized for such a purpose, for example, RNase-free forceps, scissors and blades. Therefore, certain strategies are necessary to facilitate this procedure. In addition, to establish molecular detection using RNA sampling as a generally applicable technique, demonstration of LN sampling for educational purposes is also important during the first sampling opportunities.

Finally, the greatest hurdle to overcome with the RT-PCR method is reproducibility, especially when very minimal transcript is present (1,8). This difficult problem must be absolutely resolved because clinical treatment could differ solely on the basis of positive or negative results of RT-PCR.

As described above, many issues remain to be resolved before RT-PCR can be clinically applied for the diagnosis of micrometastasis. Here, to advance our basic research (2,3) toward a clinical stage, we conducted a translational research study and established a micrometastasis detection system using RT-PCR that can be applied for clinical use.

Materials and methods

Hospital groups. The clinical study was performed with the collaboration of the Department of Surgery and Clinical Oncology, Graduate School of Medicine, Osaka University, and its related hospitals (n=17) from November 2001 to February 2003. The list of participating hospitals and co-operative physicians is shown in the Appendix. To obtain LN samples from CRC patients, the project was approved by the ethics committee at each hospital, and informed consent was obtained from the patients.

Surgical specimens. Within 3 h of surgical resection, all lymph nodes were collected, cut into halves using the disposable tissue sampling kit without cross-contamination from the main CRC tumor. Half of each node was fixed in 10% buffered formalin and embedded in paraffin for routine histopathological examination. The other half was preserved in a tube containing RNA later (Ambion, Austin, TX) (10-12) at -20°C until RNA extraction.

RNA extraction. Tissue specimens were minced with a disposable homogenizer (IEDA™, Tokyo, Japan) in TRIzol Reagent (Invitrogen, Carlsbad, CA). RNA extraction was performed as described previously (2,9). Purified RNA was quantified and assessed for purity by UV spectrophotometry. For assessment of RNA quality, 1 μg of RNA from each LN was electrophoresed on a 0.8% agarose mini-gel and ribosomal RNAs and the extent of degradation was visualized with ethidium bromide.

Conventional RT-PCR. Conventional RT-PCR was performed as described previously (2,3). Briefly, complementary DNA (cDNA) was generated using avian myeloblastosis virus reverse transcriptase using the procedure provided by the supplier (Promega, Madison, WI). Reverse transcription was performed at 42°C for 60 min, followed by heating at 95°C for 10 min. The primer sequences for PCR amplification of CEA (2,3) and porphobilinogen deaminase (PBGD) (13,14) were as follows: CEA (forward), 5'-TCTGGAACTTCTCCT GGTCTCTCAGCTGG; and CEA (reverse), 5'-TGTAGCTG TTGCAAATGCTTTAAGGAAGAAGC. PBGD (forward), 5'-TGTCTGGTAACGGCAATGCGGCTGCAAC; and PBGD (reverse), 5'-TCAATGTTGCCACCACACTGTCCG TCT. The size of amplicons for CEA and PBGD was 160 and 120 bp, respectively.

PCR was performed with a GenAmp® PCR System 9600 (Perkin-Elmer Cetus, Foster City, CA). PCR amplification was performed in a 25-μl reaction mixture containing 2 μl of cDNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.8 mM deoxynucleotide triphosphatase, 0.2 μM each primer and 1 unit of Taq DNA polymerase (AmpliTaq Gold; Roche Molecular Systems, Pleasanton, CA). PCR conditions were set as follows: a) for CEA; one cycle of denaturing at 95°C for 12 min, followed by 35 cycles at 95°C for 1 min and 72°C for 1.5 min before a final extension at 72°C for 10 min, and b) for PBGD; one cycle of denaturing at 95°C for 12 min, followed by 40 cycles at 95°C for 1 min, 62°C for 1 min and 72°C for 1.5 min before a final extension at 72°C for 10 min. The reaction mixture (10 μl) was electrophoresed on a 2% agarose gel and visualized with ethidium bromide.

Cell lines and cell culture conditions. The human gastric cancer cell line MKN45 was obtained from Health Science Research Resources Bank (Tokyo, Japan) (15). The LoVo, HT29 and DLD1 human colon carcinoma cell lines were obtained from the American Type Culture Collection (16). They were grown in Dulbecco's modified Eagle's medium plus 10% fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin, in 5% CO_2 at 37°C.

Cell production of control panel using CEA-expressing cells. RNA (1 μg) from four different cancer cell lines (MKN45, LoVo, HT29, and DLD1) was subjected to RT reaction. Each cDNA was mixed with cDNA prepared from rat spleen to make 10-fold serial dilutions of $1x10^{-1}$ to $1x10^{-5}$. PCR was performed using CEA primers and PBGD primers.

Quantitative PCR assay with Light Cycler. Fluorescence PCR was performed using the Light Cycler (Roche Diagnostics, Mannheim, Germany) in a 10-μl PCR reaction containing