ORIGINAL ARTICLE

Carcinoembryonic antigen level of draining venous blood as a predictor of recurrence in colorectal cancer patient

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Purpose: We designed this study to evaluate the efficacy of carcinoembryonic antigen in draining venous blood (vCEA) as a predictor of recurrence. **Methods:** Draining venous and supplying arterial bloods were collected separately during the operation of 82 colorectal cancer patients without distant metastasis from September 2004 to December 2006. Carcinoembryonic antigen was measured and assessed for the efficacy as a prognostic factor of recurrence using receiver operating characteristic (ROC) and Kaplan-Meier curves. **Results:** vCEA is a statistically significant factor that predicts recurrence (P = 0.032) and the optimal cut-off value for vCEA from ROC curve is 8.0 ng/mL. The recurrence-free survival between patients with vCEA levels >8 ng/mL and ≤8 ng/mL significantly differed (P < 0.001). The significance of vCEA as a predictor of recurrence gets higher when limited to patients without lymph node metastasis. The proper cut-off value for vCEA is 4.0 ng/mL if confined to patients without lymph node metastasis. The recurrence-free survival between the patients of vCEA levels >4 ng/mL and ≤4 ng/mL significantly differed (P < 0.001). Multivariate analysis revealed vCEA is an independent prognostic factor in patients without lymph node metastasis. **Conclusion:** vCEA is an independent prognostic factor of recurrence in colorectal cancer patients especially in patients without lymph node metastases.

Key Words: Colorectal neoplasms, Carcinoembryonic antigen, Prognosis, Recurrence

INTRODUCTION

Tumor node metastasis (TNM) staging system is a major prognostic factor of colorectal cancers. However, actual prognosis of the patients within the same TNM stages may be different [1]. Therefore, various molecular markers have been investigated as minor prognostic factors to compensate for the defects of TNM staging. One of the most important minor prognostic factors is carcinoembryonic antigen (CEA). Serum CEA have been used to detect recurrence after surgery and to assess the effect of treatment [2-6]. CEA is a membranous protein expressed

normally in adult and fetal intestine and usually over expressed in colorectal, breast and lung cancer [7]. CEA is a protein for cellular aggregation and is related to invasion and metastasis in colorectal cancer [8,9]. But, there are debates on the efficacy of prognostic value of preoperative CEA levels [10-12].

CEA levels in the peripheral blood are influenced by many pathological and physiological factors including metabolism by the liver. Therefore, CEA in the draining venous blood (vCEA) has been known to reflect the antigens expressed in the tumors better than that in the peripheral blood [13]. And there are some reports saying that vCEAs are more useful to predict the prognosis of colorectal cancers [14,15]. But more evidences are needed to determine vCEA as a prognostic factor of colorectal cancer. Therefore, we performed this study in order to evaluate the possibility of vCEA as a prognostic factor of colorectal cancer.

METHODS

One hundred and seventy-three colorectal cancer patients were operated with curative intent at Government Seoul National University Boramae Medical Center from September 2004 to December 2006. Among them, ninety-one patients were excluded due to various reasons. Forty-one patients who were operated on in laparoscopic surgery were excluded due to the technical difficulties of blood sampling. The decision for laparoscopic surgery was made as follows; Patients with tumors of T4 lesion in the preoperative computed tomography scan, with tumors in the lower third of rectum and with intestinal adhesions by previous abdominal operation were chosen to undergo open surgery (these patients were included in this study). And because the public medical insurance of Korea did not cover the costs for the instruments of laparoscopic surgery during the study period, the laparoscopic surgeries were performed only in the patients who wanted and were willing to pay the additional costs for laparoscopic instruments. The other 22 of the 91 patients were excluded due to distant metastases as metastatic lesion affects the CEA level of draining veins. Another 28 of the 91

patients were excluded by failure in blood sampling due to small diameter of artery or vein, failure in separation of vein from artery, inconspicuous middle colic vessels in transverse colon cancer patients, and emergency operations. Therefore, eighty-two patients out of 173 were enrolled in this study.

Samplings of blood were done before the ligation of the feeding artery and draining vein. After dissection of the colon, the draining vein was clamped at its most proximal part and sampling was done in the congested distal vein. The vein was cut and ligated. Afterwards, arterial blood was sampled by puncture of the feeding artery, then the artery was cut and ligated. The feeding artery and draining vein were ileocolic artery and vein for right colon cancers, middle colic artery and vein for transverse colon cancer, and inferior mesenteric artery and vein for left colon cancer, sigmoid colon cancer and rectal cancers. In patients with colon cancer around the hepatic flexure, the vessels were chosen between ileocolic and middle colic vessels by the prominence of lymph node enlargement.

Immunoradiometric assay was used to measure CEA. Statistical analyses were done by receiver operating characteristic (ROC) and Kaplan-Meier survival curves, Student's t-test and Cox regression model in the SPSS ver. 14.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Demographics and distribution of CEA

There were 46 male and 36 female patients and the mean age was 66.4 years (range, 36 to 90 years). There were 19 right colon cancers, 3 transverse colon cancers and 60 left colon cancers including sigmoid and rectal cancers. Three patients had *in situ* carcinomas, nine patients had TNM stage I cancers, 32 patients had TNM stage II, and 38 had TNM stage III. Mean follow up duration was 36.6 months and 22 patients had distant recurrences. Local recurrence was not detected during the follow up period. The range of CEA from the feeding arteries (aCEA) were from <1.0 ng/mL to 51.8 ng/mL and the range of vCEA was from <1.0 ng/mL to 51.5 ng/mL. Fourteen aCEAs and ten vCEAs were under 1.0 ng/mL. On the assumption that

CEA value under 1.0 ng/mL equals to 0.5 ng/mL, mean and standard deviation of aCEAs were 5.92 ng/mL and 9.10 ng/mL each, and mean and standard deviation of vCEAs were 7.32 ng/mL and 9.98 ng/mL each. The mean of vCEA was higher than that of aCEA (P = 0.001).

aCEA and vCEA as prognostic factors

ROC curves of aCEA, vCEA and vaCEA (vCEA-aCEA) were made on the basis of specificities and sensitivities of recurrence (Fig. 1A). While aCEA and vaCEA were in-eligible as prognostic factors by a significance of 0.245 and 0.162, respectively, vCEA was identified as a significant prognostic factor of recurrence by the P-value of 0.032 and by the area under the curve of 0.656.

The appropriate cut-off value of vCEA for optimizing sensitivity and specificity in predicting recurrence on the basis of ROC curve was 8.0 ng/mL. Recurrence rates of the groups with 61 patients of vCEA ≤8.0 ng/mL and with 21 patients of vCEA >8.0 ng/mL were 16.4% and 57.15% each. The sensitivity was 54.5% and specificity was 85.0% for the cut-off value of 8.0 ng/mL.

Kaplan-Meier survival curve confirmed the significant difference in disease-free survivals between the groups of vCEA ≤8.0 ng/mL and of vCEA >8.0 ng/mL (Fig. 1B).
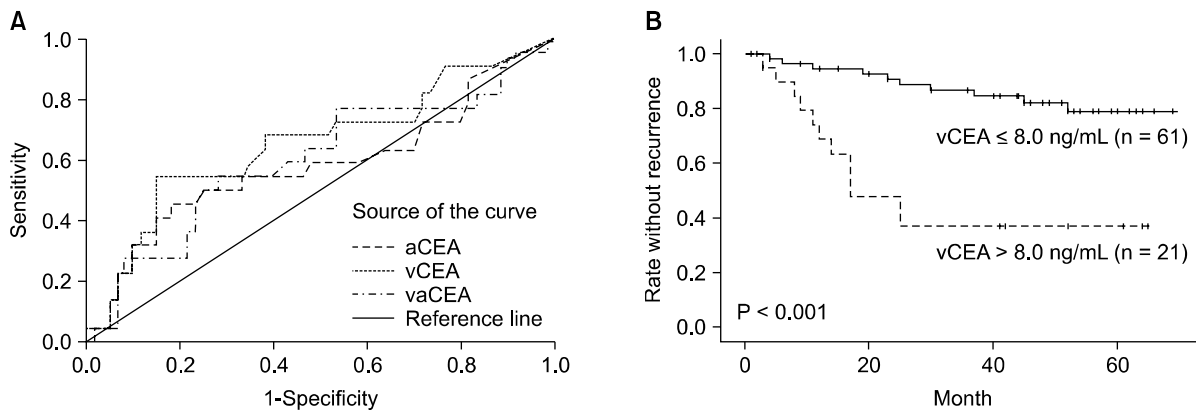

Fig. 1. Efficacy of vCEA as a predictor of recurrence. ROC curve shows that vCEA is a statistically significant factor that predicts recurrence (A). There are significant differences in recurrence-free survival between high vCEA group and low vCEA group (B). aCEA, feeding anterial blood CEA; vCEA, draining venous blood CEA; vaCEA, difference between vCEA and aCEA (vCEA-aCEA); ROC, receiver operating characteristic.
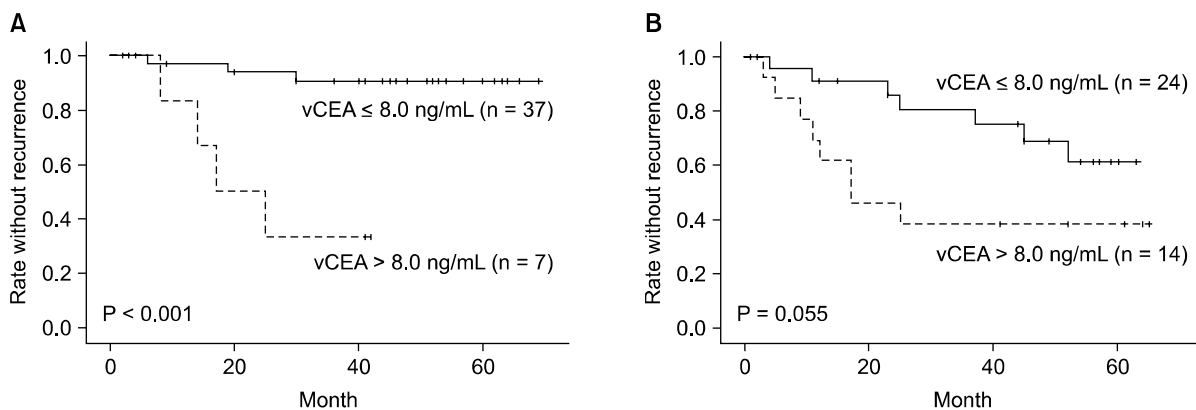

Fig. 2. Recurrence-free survivals between high vCEA group and low vCEA group stratified by lymph node (LN) metastasis. There are significant differences in recurrence-free survival between two groups if the patient has no LN metastasis (A), but not if the patient has LN metastasis (B). vCEA, draining venous blood CEA.
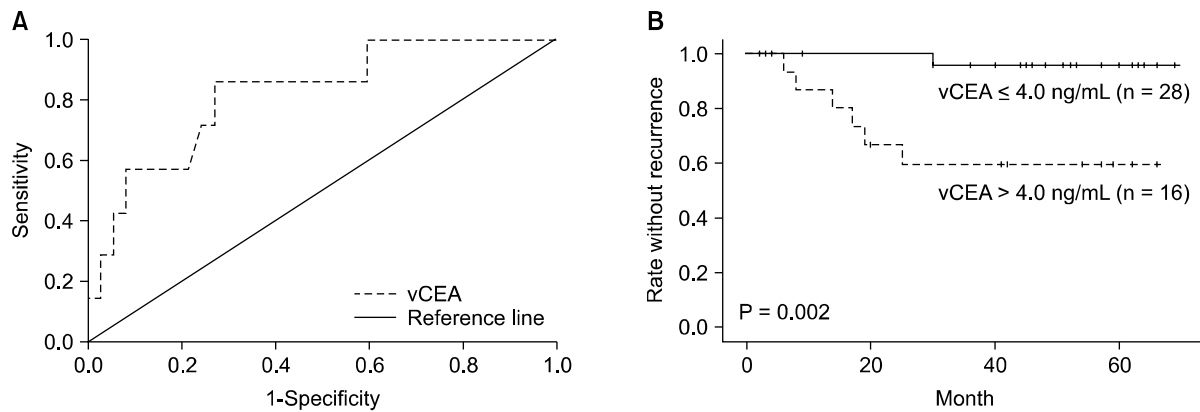
Fig. 3. The efficacy of vCEA as a predictor of recurrence in patients without lymph node (LN) metastasis. ROC curve shows that vCEA is a more reliable prognostic factor when confined to patients without LN metastasis (A). There are significant differences in recurrence-free survival between high vCEA group and low vCEA group (B). vCEA, draining venous blood CEA; ROC, receiver operating characteristic.

Table 1. Clinicopathological factors and recurrence rate

Variable	No.	Recurrence rate (%)	P-value
Age (yr)			0.872
<65	31	25.8	
≥65	51	27.5	
Sex			0.411
Male	46	30.4	
Female	36	22.2	
Location			0.260
A	19	21.1	
T	3	66.6	
D, S, R	60	26.7	
Tumor diameter (cm)			0.325
<5	41	22.0	
≥5	41	31.7	
T category			0.041
T0 + T1 + T2	12	8.3	
T3 + T4	70	30.0	
N category			0.019
Negative	44	15.9	
Positive	38	39.5	
Vascular/neural invasion			0.005
Negative	39	12.8	
Positive	43	39.5	
vCEA (ng/mL)			0.002
≤8.0	61	16.4	
>8.0	21	57.1	

A, ascending colon; T, transverse colon; D, S, R, descending colon, sigmoid colon and rectum; CEA, carcinoembryonic antigen.

vCEA as a prognostic factor in patients without lymph node metastasis

If the patients were separated by the lymph node meta-stasis, the cut-off level of vCEA (8.0 ng/mL) still made a statistically significant difference of disease-free survival in patients without lymph node metastases but did not make a significant difference in patients with lymph node metastases (Fig. 2).

If ROC curves were refined for the 44 patients without lymph node metastasis, the efficacy of vCEA as a prognostic factor was improved by a P-value of 0.008 and area under the curve of 0.820 (Fig. 3A). The optimal value of vCEA was 4.0 ng/mL with a sensitivity of 85.7% and a specificity of 73.0%. Also Kaplan-Meier curve confirmed the efficacy of a new cut-off value of vCEA of 4.0 ng/mL by a significant difference in disease-free survivals in patients without lymph node metastases (P = 0.002) (Fig. 3B).

Multivariate analysis with other prognostic factors

Known prognostic factors and demographic parameters as well as vCEA were evaluated for relation to prognosis in all 82 patients. Age, sex, location of tumor and size of tumor were not related to recurrence. Meanwhile, T stage, N stage, neurovascular invasion and vCEA were related to tumor recurrence in univariate analysis (Table 1). The same parameters were also evaluated in patients without lymph node metastasis (Table 2), age, sex, and location and size of tumors, as well as T stage and neurovascular invasions not related to prognosis, while the vCEA made significant difference in recurrence rate between the groups of >4.0 ng/mL and ≤4 ng/mL.

Table 2. Clinicopathological factors and recurrence rate (without lymph node metastasis)

	No.	Recurrence rate (%)	P-value
Age (yr)			0.175
<65	15	6.7	
≥65	29	20.7	
Sex			0.481
Male	26	19.2	
Female	18	11.1	
Location			0.380
A	10	30.0	
T	1	0.0	
D, S, R	33	12.1	
Tumor diameter (cm)			0.785
<5	21	14.3	
≥5	23	17.4	
T category			0.412
T0 + T1 + T2	12	8.3	
T3 + T4	32	18.8	
Vascular/neural invasion			0.192
Negative	30	10.0	
Positive	14	28.6	
vCEA (ng/mL)			0.018
≤4.0	28	3.6	
>4.0	16	37.5	

A, ascending colon; T, transverse colon; D, S, R, descending colon, sigmoid colon and rectum; CEA, carcinoembryonic antigen.

Table 3. Clinicopathological factors and hazard ratio (without lymph node metastasis)-multivariate analysis

	Hazard ratio (95% CI)	P-value
T category (T3 + T4 vs. T0 + T1 + T2)	0.278 (0.015-5.211)	0.392
Tumor diameter (cm) (≥5 vs. <5)	4.387 (0.646-29.787)	0.130
Vascular/neural invasion (positive vs. negative)	7.305 (0.924-57.783)	0.059
vCEA (ng/mL) (>4.0 vs. ≤4.0)	16.928 (1.735-165.116)	0.015

CI, confidence interval.

Multivariate analysis with major clinico-pathological factors was done for the prognostic significance in the patients without lymph node metastasis (Table 3). T stage and tumor size were not related to prognosis while neuro-vascular invasion was near significant with a P-value of 0.059 and with a hazard ratio of 7.305. vCEA was the only significantly independent prognostic factor with a hazard ratio of 16.928.

DISCUSSION

Since the isolation of the antigen by Gold and Freedman [16] in 1965, CEA from peripheral vein (pCEA) has been widely utilized for detection of recurrence and evaluation of treatment. However, pCEA is influenced by various pathological and physiological factors such as production in cancer cells, secretion from cancer cells to surrounding tissues and lymphatics, metabolism in the liver and dilu-

tion within the blood [17,18]. Meanwhile, vCEA can be more useful than pCEA to predict recurrence or prognosis because it is less influenced by such physiological factors [13,14].

Some reported that preoperative pCEA would be an independent prognostic factor for recurrence after surgical resection in colorectal cancer patients [8,12,19-22] and others reported that preoperative pCEA would be a prognostic factor for survival [23,24]. In this study, we investigated whether the CEA from feeding artery and draining vein, sampled before the removal of tumor during operation, could be a prognostic factor. Mean vCEA was higher than that of aCEA (vCEA, 7.32; aCEA, 5.92) probably because vCEA reflects the antigens of primary tumors better than aCEA and because vCEA is not diluted by blood from other sites.

ROC curve has been used since World War II and is a useful tool to evaluate the diagnostic efficacy of a signal. ROC curve is a graphical plot of sensitivity and 1-specificity in each cut-off value of a signal and the area under the ROC curve (AUC) is a parameter indicating the usefulness of the signal in discriminating the true from the false. Evaluation of the vCEA, aCEA and vaCEA for the efficacy as a prognostic factor by ROC curve proved that aCEA, from the systemic circulation, is not a useful prognostic factor while only vCEA, from the local circulation of tumor, is a significant prognostic factor of recurrence.

In previous studies, recurrences and survivals were evaluated in comparative groups of normal pCEA levels and elevated pCEA levels. But the normal reference levels were arbitrary in each study making it difficult to have

consistent results and to compare results from diverse studies [21]. In this study, the ROC curve indicated the cut-off value of vCEA as 8.0 ng/mL or 4.0 ng/mL, which were higher or lower than the normal reference value in our hospital (<5 ng/mL). These differences originated from the different source of blood which was either of cancer patients or normal population, and which was peripheral blood or draining venous blood.

Patients with higher vCEA levels than 8.0 ng/mL showed significant difference in disease-free survival compared to patients with lower vCEA level. This suggests the possibility of more occult metastasis or more disseminating conditions at or around the time of operation. According to Lloyd et al. [25] disseminated tumor cells existed in 32.8% of stage I and II patients and prognosis was poor when the disseminated tumor cells were detected in washings after tumor resection. All of these imply that there are possibilities of residual tumor cells after curative resection and that the current staging system alone is not sufficient to determine treatment level.

The value of CEA as a prognostic factor in each stage has long been evaluated. In some studies, preoperative high pCEA had a poor prognosis in only Duke's stage C [23,26]. In other studies, preoperative pCEA made a difference in disease-free survival of stage II patients only [27]. In our study, vCEA was significantly related to the prognosis of patients without lymph node metastasis, but did not relate to those with lymph node metastasis. vCEA is expected to contribute to hematogenous metastasis because it reflects the antigens drained to the vein and portal circulation. And in patients without lymph node metastasis, the difference between patients with and without hematogenous metastasis is more evident, but in patients with lymph node metastasis the difference reduces. This correlates well to the results of this study that vCEA is more useful in patients without lymph node metastasis.

vCEA was an independent prognostic factor of recurrence when multivariate analysis was done with major pathological parameters in patients without lymph node metastasis. The small number (44) of patients without lymph node metastasis made the range of 95% confidence interval wide and additional data from more patients are needed for more confident conclusions.

The two limitations of this study are that relatively more patients of T4 tumors might have been included in this study due to excluding laparoscopic surgery and that we had small number of patients. Although these are not detrimental to this study and although we could get statistical significance with these patients, we need confirmation of the results with more patients and with new methods making blood sampling possible during laparoscopic surgery.

Adjuvant chemotherapy is recommended as standard treatment in stage III colorectal cancer or more but there are still debates on the necessity of adjuvant chemotherapy in stage II patients. The current recommendation of adjuvant chemotherapy for stage II patients by American Society of Clinical Oncology falls on poorly differentiated cancer, T4 cancer, tumor perforation and small number of lymph node retrieval [28]. It is very important to find high-risk patients for recurrence among stage II patients and we expect the vCEA level as another candidate for prognostic factors in stage II patients.

vCEA is a candidate of independent prognostic factors of recurrence in colorectal cancer patients without lymph node metastasis, although additional studies with more patients are necessary.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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