RESEARCH ARTICLE

Serum Carbohydrate Antigen 19-9 as an Indicator of Liver Metastasis in Colorectal Carcinoma Cases

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Abstract

Purpose: The liver is the organ to which colorectal carcinomas (CRCs) most commonly metastasize, and surgical resection has been established as the most effective and potentially curative treatment for CRC with liver metastasis (LM). Therefore, surveillance of LM is vital for improvement of prognosis of CRC patients. In this study, we aimed to explore the potential value of carbohydrate antigen 19-9 (CA 19-9), carcinoembryonic antigen (CEA), and marker enzymes in indicating LM with CRC. Methods: Three groups of eligible patients with metastatic cancers were retrospectively included: CRC patients with LM (CRC-LM) or without LM (CRC-NLM), and non-CRC patients with LM (NCRC-LM). All metastatic lesions were identified by CT or MRI. Data on characteristics of the patients, the primary site, the locations of metastasis, CA 19-9, CEA, and biochemical parameters were collected for analysis. Results: A total of 493 patients were retrospectively included. More alcohol consumption was found in CRC-LM than CRC-NLM. Some biochemical enzymes were found to be significantly higher in groups with LM than without (CRC-LM or NCRC-LM v.s CRC-NLM). Both CEA and CA 19-9 were much higher in CRC-LM than CRC-NLM or NCRC-LM. For CRC patients, CA 19-9, γ-glutamyl transpeptidase, CEA and alcohol consumption were identified as independent factors associated with LM. Conclusion: Our analysis suggested the CA 19-9 might be a potential valuable indicator for LM of CRC in the clinic.

Keywords: Colorectal carcinoma - liver metastasis - CA 19-9 - CEA - γ-glutamyl transpeptidase

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Introduction

Colorectal cancer (CRC) is one of the most common malignancies in the world, which leads to the third highest tumor-related mortality (Gillams and Lees, 2009; Van Tilborg et al., 2011). Liver is the most common organ that CRC metastasize to and liver metastasis (LM) can be detected in about 25% patients at initial diagnosis of CRC (Min et al., 2002). What's more, about 50% of CRC patients finally developed liver metastasis within 3 years after primary tumor resection (Assumpcao et al., 2008). During the past decade, much progress has been made in the treatment of liver lesion of CRC patients, and surgical resection has been established as the most effective and potentially curative treatment for CRC with liver metastasis (Koike et al., 2000; Jaeck et al., 2004). Therefore, to monitor and treat liver metastasis is vital for prognosis of CRC patients.

Imageology is the principal method to monitor and diagnose CRC metastasis. Otherwise, tumor markers have their advantages of convenience, economy and no-ray damage, and are also commonly used in the follow-up. Some tumor markers, such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9), were shown their significance in the diagnosis and follow-up of CRC by many scholars (Nicolini et al., 1995; Lumachi et al., 2012; Petrioli et al., 2012). CEA, a glycolsyl-phosphatidylinositol (GPI) anchored glycoprotein, is most commonly used in diagnosis and follow-up for CRC (Chevinsky, 1991; Locker et al., 2006). CEA was reported to be elevated in approximately 80% to 90% of CRC and thought to be a contribution of malignant characteristics and metastasis of the tumor (Gangopadhyay et al., 1998; Holt et al., 2006). Usually, CEA is considered as an important marker to monitor recurrence or metastasis of CRC. CA 19-9, which is called sialyl Lewis a (sLa), is also another alternative marker for CRC (Kannagi et al., 1988; Levy et al., 2008). The elevation of CA 19-9 demonstrated a significantly higher frequency of metastasis and a distinctly lower survival rate, so it seemed to be an adverse prognostic factor for CRC patients (Wang et al., 2002). It is commonly accepted that CA19-9 is used as a marker of hematogenous metastasis and a predictor of prognosis in CRC (Takada et al., 1993). However, the significance of

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the elevated CA19-9 in CRC remains to be clarified. For example, the increase of CA 19-9 was reported as a risk factor for extrahepatic metastasis in CRC patients with liver metastasis (Sasaki et al., 2005). For CRC patients with normal CEA, CA 19-9 was a valuable prognostic factor and might help predict lung metastasis (Lin et al., 2012). Elevated CA 19-9 was also reported to be related with the peritoneal metastasis of CRC (Yang et al., 2004). Furthermore, both CEA and CA19-9 were found to be independent and significant predictors for overall survival in unresectable CRC liver metastasis (Mitsuyama et al., 2012). In this study, we aimed to explore the value of CA 19-9, CEA and some biochemical enzymes in indicating liver metastasis of CRC.

Materials and Methods

Study Population

Data on patients with metastatic cancers treated in West China Hospital of Sichuan University from January 2009 to March 2012 were retrospectively reviewed. The protocol of the study was approved by the ethic committee of West China Hospital. Eligible patients should have: histologic or cytologic diagnosis of solid cancers; metastatic lesions identified by computed tomography (CT) or magnetic resonance imaging (MRI); the metastatic lesions were newly diagnosed without prior antitumor treatments or at least one year after postoperative adjuvant chemoradiotherapy; complete data of medical history and blood parameter test including CEA, CA 19-9, alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT), hydroxybutyrate dehydrogenase (HBDH) and lactate dehydrogenase (LDH) within two weeks before or after the diagnosis of metastasis; the metastatic CRC were divided into two groups, liver metastasis with or without other lesions (CRC-LM) or metastatic lesions without liver metastasis (CRC-NLM); if solid cancers other than CRC patients, liver metastasis were only allowed to include (with or without other lesions, NCRC-LM). Patients were excluded if they had: any primary hepatobiliary or pancreatic diseases, including inflammation, obstruction, calculi, primary tumor and so on; multiple organ failure; or dual primary tumor.

Data Collection

Data on age, gender, the status of smoking or alcohol consumption, the primary site of disease, the locations of metastasis, prior treatment, biochemical parameters (including ALP, GGT, HBDH and LDH) and tumor markers (CEA and CA 19-9) were collected. Metastatic lesions in liver or other locations were identified by CT or MRI. The results of biochemical parameters and tumor markers were collected from the reports of the Biochemistry Report Laboratory Medicine Department and Clinical Immunology Laboratory of West China Hospital. The normal reference values of CEA and CA 19-9 were < 3.4 ng/mL and < 22 U/mL respectively. If the values were higher than 1000 ng/mL (CEA) or 1000 U/mL (CA19-9), they were recorded as "> 1000 ng/mL" or "> 1000 U/mL" respectively. If the value of CA19-9 was lower than 0.6 U/mL, it was recorded as "< 0.6 U/mL".

Table 1. Patient Characteristics, Biochemical Parameters and Tumor Markers

Parameters	No. of patients			*P**
	CRC-LM (n=181)	CRC-NLM (n=136)	NCRC-LM (n=176)	< 0.05
Age (medians [range])	58.3 [23-88]	58.4 [17-95]	58.8 [18-99]	None
Sex				
Male	110	85	118	
Female	71	51	58	None
Smoking				
Yes	72	45	83	
No	109	91	93	(3)
Alcohol consumption				
yes	68	32	55	
no	113	104	121	(1)
Primary site of disease				
colon	82	51	-	-
rectum	99	85	-	-
lung	-	-	88	-
stomach	-	-	52	-
others	-	-	36	-
The sites of metastasis				
liver	181	-	176	-
any other sites	69	136	116	-
Prior treatment				
surgery	66	68	37	-
chemotherapy	35	43	20	-
radiotherapy	6	14	7	-
None	115	68	139	None
Lab. Tests (Median [range],U/L)				
LDH	217.00 [33-2150]	176.00 [40-944]	214.00 [32-4909]	(1)(3)
ALP	93.67 [0.94-1191]	70.25 [5.09-271]	99.00 [5.37-1855]	(1)(3)
GGT	46.67 [5-1809]	17.67 [3-284]	37.33 [5-1194]	(1)(3)
HBDH	167.00 [84-1564]	141.00 [14-686]	170.67 [93-3837]	(1)(3)
Tumor Marks:				
CEA				
Median (ng/mL)	36.9	5.9	5.2	(1)(2)
>3.4ng/mL (%)	89.5	69.9	56.8	(1)(2)(3)
CA 19-9				
Median (U/mL)	96.1	16.4	12.5	(1)(2)
>22U/mL (%)	79.6	39	28.4	(1)(2)(3)

*(1), CRC-LM v.s CRC-NLM; (2), CRC-NLM v.s NCRC-LM; (3), CRC-LM v.s NCRC-LM; None, no difference between these three groups; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; HBDH, hydroxybutyrate dehydrogenase; CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9

Statistics Analysis

The normality test was carried out on all the data gathered. Rank-sum test and Chi-square statistics was used to explore the comparison of CA 19-9, CEA, and so on. Logistic regression was utilized for the most effective factor then. Sensitivity, specificity, accuracy were calculated for diagnostic tests. Kappa test was adopted to examine the consistency. In addition, ROC curve was used to reveal the cut-off point in diagnosis of liver metastasis. SPSS 17.0 was for statistical analysis, and the statistical significance was set as $P < 0.05$.

Results

Patient Characteristics

Four hundred and ninety-three patients were included for analysis (181 CRC-LM, 136 CRC-NLM, and 176 NCRC-LM, respectively). The characteristics of the patients in each group were shown in Table 1. More smoking patients were found in NCRC-LM than those in CRC-NLM, otherwise, more alcohol consumption was found in CRC-LM than CRC-NLM. No significant differences were observed in the distribution of sex, age or prior treatments among the 3 groups. All of the included biochemical parameters were found to have significant

Table 2. Multivariate Analysis for Liver Metastasis in CRC Patients

Factors	*P*	OR(95%CI)
Age		
<60 v.s ≥60	0.201	1.450(0.821-2.561)
Sex		
Male v.s Female	0.079	1.839(0.932-2.561)
Smoking		
yes v.s no	0.707	0.851(0.367-1.974)
Alcohol consumption		
yes v.s no	0.028	2.564(1.106-5.944)
Primary site of disease		
colon v.s rectum	0.836	0.943(0.540-1.646)
LDH (<220U/L)		
normal v.s elevated	0.205	1.489(0.805-2.754)
ALP (<138U/L)		
normal v.s elevated	0.354	0.589(0.193-1.804)
GGT (<46U/L)		
normal v.s elevated	0.000	4.388(1.984-9.706)
HBDH (<182U/L)		
normal v.s elevated	0.909	1.083(0.278-4.219)
CEA (<3.4ng/mL)		
normal v.s elevated	0.048	2.055(1.007-4.193)
CA 19-9 (<22U/mL)		
normal v.s elevated	0.000	4.873(2.750-8.634)

LDH, lactate dehydrogenase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; HBDH, hydroxybutyrate dehydrogenase; CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9

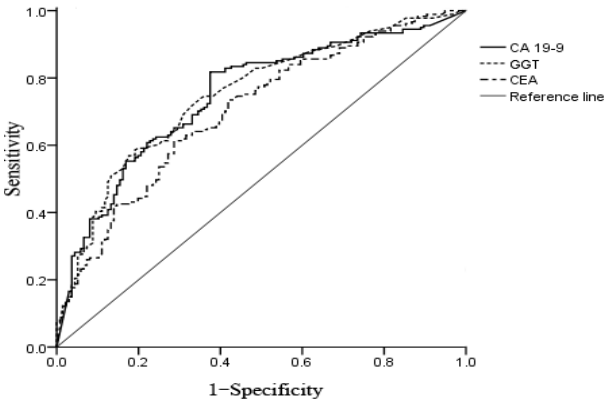

Figure 1. ROC Curves of CA 19-9, GGT and CEA in Diagnosis of Liver Metastasis of CRC

differences between groups with and without liver lesions (CRC-LM or NCRC-LM v.s CRC-NLM). Both CEA and CA 19-9 were much higher in CRC-LM than those in CRC-NLM or NCRC-LM ($P < 0.01$), no significant differences in CEA and CA 19-9 were found between CRC-NLM and NCRC-LM; if evaluating the proportion of abnormal CEA and CA 19-9, there were significant differences among the 3 groups.

Multivariate Analysis for Liver Metastasis of CRC

There were totally 317 CRC patients included in this study (CRC-LM and CRC-NLM). Potential associated factors with liver metastasis were evaluated by logistic regression (Table 2). CA 19-9, GGT, CEA and alcohol consumption were identified as associated factors with liver metastasis of CRC. Of those, CA 19-9 and GGT showed strong association with liver metastasis with odds ratio (OR) of 4.873 and 4.388 respectively ($P < 0.01$).

Table 3. Consistency Analysis of Diagnosing LM of CRC by Serum CA 19-9, GGT, CEA with CT

	CA 19-9 (U/mL)		GGT (U/L)		CEA (ng/mL)	
	>22	>22.22	>46	>35.5	>3.4	>19.57
SEN	81.80%	81.80%	49.70%	58.60%	89.50%	61.30%
SPE	61.00%	62.50%	88.10%	80.90%	30.10%	71.30%
DI	1.428	1.443	1.378	1.395	1.196	1.326
ACC	72.90%	73.50%	66.10%	68.10%	64.00%	65.60%
PV(+)	73.60%	74.40%	83.30%	80.40%	63.00%	74.00%
PV(-)	71.60%	72.00%	56.40%	59.50%	31.70%	58.10%
FPR	18.20%	18.20%	50.30%	41.40%	10.50%	38.70%
FNR	39%	38.50%	11.90%	19.10%	69.90%	28.70%
LR(+)	2.10	2.15	4.18	3.07	1.28	2.14
LR(-)	0.298	0.291	0.571	0.512	0.349	0.543
κ(P)	0.436	0.450	0.352	0.378	0.221	0.317
	(0.000)	(0.000)	(0.047)	(0.049)	(0.000)	(0.000)
OR	7.023	7.475	6.484	5.979	3.68	3.944
YI	0.428	0.443	0.378	0.495	0.196	0.326

LDH, lactate dehydrogenase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; HBDH, hydroxybutyrate dehydrogenase; CEA, carcinoembryonic antigen; CA 19-9: carbohydrate antigen 19-9. SEN, Sensitivity; SPE, Specificity; DI, Diagnostic Index; ACC, Accuracy; PV(+), Positive Predictive Value; PV(-), Negative Predictive Value; FPR, False Positive Rate; FNR, False Negative Rate; LR(+), Positive Likelihood Ratio; LR(-), Negative Likelihood Ratio; κ, Kappa value; OR, Odds Ratio; YI, Youden` s Index

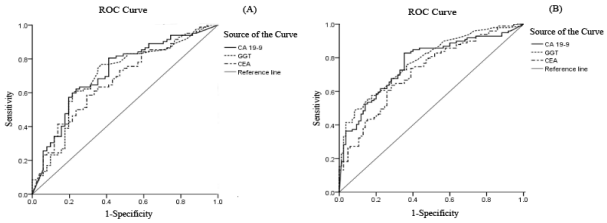

Figure 2. ROC Curves of CA 19-9, GGT and CEA in Colon (A) or Rectal (B) Carcinomas with Liver Metastasis

The Value of CA 19-9, GGT and CEA in Diagnosis of Liver Metastasis of CRC

Receiver operating characteristic (ROC) curves were used to analysis the features of CA 19-9, GGT and CEA in diagnosis of liver metastasis of CRC. The area under the curve (AUC) of CA 19-9, GGT and CEA is 0.747, 0.797 and 0.701 respectively, as shown in Figure 1. The cut-off points of CA 19-9, GGT and CEA was 22.22 U/mL, 35.50 U/L and 19.57 ng/mL, respectively. Sensitivity, specificity were calculated to compare the effect of CA 19-9, GGT and CEA on diagnosis of liver metastasis of CRC (Table 3). 22.22 U/mL as cut-off point of CA 19-9 showed a specificity of 62.5% with the highest sensitivity of 81.8%; 35.5 U/L of GGT showed the highest specificity of 80.9%, but with a lower sensitivity of 58.6%; 19.57 ng/mL of CEA showed a specificity of 71.3% and a lower sensitivity of 61.3%. Normal reference values of CA 19-9, GGT and CEA taken as cut-off points to evaluate sensitivity and specificity were also shown in the Table 3.

CA 19-9, GGT and CEA in Diagnosis of Liver Metastasis in Colon Cancer or Rectal Cancer

To observe if there were differences of CA 19-9, GGT and CEA in diagnosis of liver metastasis in colon cancer (CC) or rectal cancer (RC), the patients with liver metastasis of CC (CC-LM, n = 82) were compared with the patients with liver metastasis of RC (RC-LM, n = 99),

as shown in Figure 2. Interestingly, each of the CA 19-9, GGT and CEA seemed to have a better diagnostic value in RC-LM than that in CC-LM, with the AUC of 0.764 (95%CI, 0.695-0.833), 0.779 (95%CI, 0.714-0.845) and 0.718 (95%CI, 0.644-0.791) for RC-LM, and the AUC of 0.718 (95%CI, 0.627-0.808), 0.698 (95%CI, 0.604-0.792) and 0.676 (95%CI, 0.583-0.767) for CC-LM, respectively.

Discussion

Liver is the most common organ that CRC metastasize to (Min et al., 2002). During the past 10+ years, hepatic resection has been developed as the most effective and potentially curative treatment for patients with CRC liver metastasis (Koike et al., 2000; Jaeck et al., 2004). Unfortunately, only 10% - 20% of the patients are directly amenable to surgery (Adam, 2003). Thus, follow-up and early detection of liver metastasis are important for the prognosis of these patients. CT or MRI is commonly recommended methods to follow-up, otherwise, the methods are inconvenient, costly and/or radiation injury. According to our experience, more than 2/3 Chinese CRC patients didn't follow the doctor's follow-up guide possibly because of those reasons above, thus, easy and economical ways are urgent for these patients.

Firstly, in this study, we compared the characteristics of CRC patients with or without liver metastasis. To avoid non-specific characteristics of liver metastasis, we included a group of non-CRC patients with liver metastasis (NCRC-LM). We found that more smoking patients were found in NCRC-LM than those in CRC-NLM. This might be explained that there were many lung cancer patients included in NCRC-LM. As known to all, lung cancer is a disease closely associated with smoking (Moolgavkar et al., 2012; Rosenberg et al., 2012). Otherwise, alcohol consumption was found different between CRC groups with and without liver metastasis. Also, it identified as an independent risk factor for liver metastasis in CRC in this study. Similar result was previously reported by Maeda et al. (1998). Liver injury, effects on platelets, suppression of natural killer cells, and/or elevation of the blood gastrin level in response to alcohol consumption were possible interpretation for liver metastasis of CRC (Maeda et al., 1998). However, the mechanisms would be complex and need to be further elucidated. In this analysis, biochemical parameters, such as LDH, ALP, GGT and HBDH were found to be significantly higher in the liver metastasis groups (CRC-LM and NCRC-LM) than the non-liver metastasis group (CRC-NLM). It seems reasonable that the metastasis lesions in liver may affect the metabolism and enzymic activities of liver, that is to say, the changes of the biochemical parameters were not only found in liver metastasis of CRC, but also in that of other malignancies. The phenomenon observed in this study was also in accordance with other previous papers (Obrador et al., 2002; Simic et al., 2007; Prabasheela et al., 2012).

Both CA 19-9 and CEA were found to be higher in CRC-LM than in CRC-NLM or NCRC-LM, the proportions of abnormal CA 19-9 or CEA in CRC-LM were also higher than those in CRC-NLM or NCRC-LM. Importantly, in multivariate analysis, CA 19-9 and CEA were also identified as independent risk factors with liver metastasis of CRC respectively.

CA 19-9 was clarified as a risk factor strongly associated with liver metastasis of CRC, and ROC analysis also showed a cut-off point of 22.22 U/mL with a sensitivity of 81.8% and a specificity of 62.5%. The cut-off point of 22.22 U/mL was approximate to the normal upper limit of CA 19-9 (22 U/mL), which may have practical clinical significance. Previous studies showed that the increase of CA 19-9 had discordant results in evaluating metastasis sites of CRC. For example, the increase in CA 19-9 was reported as a risk factor for liver metastasis (Nakamori et al., 1993), extrahepatic metastasis, lung metastasis, or peritoneal metastasis of CRC respectively (Yang et al., 2004; Sasaki et al., 2005; Lin et al., 2012). Otherwise, our research primarily focused on CA 19-9 in serum for diagnosis of liver metastasis of CRC with good control groups and relatively larger sample size, the results in our study that CA 19-9 was an indicating marker of liver metastasis of CRC was receivable. Furthermore, CA 19-9 was reported to play a role in cancer invasion by enhancing cell adhesion and promoting angiogenesis indirectly (Ballehaninna and Chamberlain, 2012). It also helped cancer cells locate in liver by interacting with E-selectin (Brodt et al., 1997; Sato et al., 2010). These may help us to understand the results found in this study.

CEA was widely accepted as a significant prognostic factor and an indicator of recurrence or therapeutic effect in patients with CRC. But commonly, it is not considered as a specific marker for liver metastasis. In our analysis, it did be slightly associated with liver metastasis, otherwise, in ROC analysis, 19.57 ng/mL was identified as the cut-off point showing optimal sensitivity and specificity, which was much higher than the normal upper limit of CEA (3.4 ng/mL). That is to say, a much higher CEA should be considered to suggest liver metastasis of CRC, which might limit its clinical practice in this situation.

GGT was also identified as a potent independent risk factor for liver metastasis of CRC. Similar results were found in renal cell carcinoma, malignant melanoma and so on (Obrador et al., 2002; Simic et al., 2007). Otherwise, the cut-off point of ROC was 35.5 U/L in this analysis, which was lower than the normal upper limit value (46 U/L) of GGT. Furthermore, it was not a specific factor for liver metastasis of CRC, as previously discussed. These suggested that the clinical significant of GGT to indicate liver metastasis of CRC was frustrated.

In our study, CA 19-9, GGT and CEA were also analyzed by using ROC in CC-LM and RC-LM subgroups, we found there was a tendency of better diagnostic value of these parameters for RC-LM than CC-LM. The result was interesting but need larger sample analysis and basic research to address the problem.

It should be pointed out that our study was a retrospective analysis, prospective observation should be conducted to provide further verification. It's also important to note that the tumor markers could not replace imageological methods in the follow-up of CRC, we also recommend both imageological methods and tumor makers as routine during the follow-up. For those the imageological methods are not available, tumor markers,

such as CA 19-9, should be included to decide whether further examination should be complete.

In conclusion, CA 19-9, GGT and CEA were identified as independent risk factors for liver metastasis of CRC. Of them, CA 19-9 showed an optimal sensitivity and specificity with practical clinical significance.

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