JBUON 2013; 18(2): 391-397
ISSN: 1107-0625 • www.jbuon.com
E-mail: info@jbuon.com

ORIGINAL ARTICLE

D-dimer is a marker of response to chemotherapy in patients with metastatic colorectal cancer

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Summary

Purpose: *D-dimer, LDH and tumor markers are usually overexpressed in colorectal carcinomas (CRC). Our purpose was to assess the prognostic role of D-dimer, lactate dehydrogenase (LDH), CEA, CA19-9 and CA72-4 in patients with metastatic CRC treated with XELOX chemotherapy.*

Methods: *Thirty-eight CRC patients who had evidence of distant metastasis were enrolled in the study and blood samples were taken before chemotherapy for estimation of the tumor markers CEA, CA19-9 and CA72-4, and for D-dimer and LDH. Patients were randomized into 3 groups: those with partial response (PR), stable disease (SD), and progressive disease (PD) according to their clinical and radiologic evaluation after 3 cycles of XELOX chemotherapy. All parameters were reevaluated after the 3rd cycle of chemotherapy.*

Results: *Eighteen patients (47.3%) achieved PR, 10 (26.3%) SD, and 10 (26.3%) showed PD. After 3 cycles of XELOX CEA (20.55 vs 11.97 ng/ml; p=0.002), LDH (357.50 vs 214.0 U/lt; p=0.001) and D-dimer (1.56 vs 1.17 μgFEU/ml; p=0.022) levels were significantly decreased in the PR group. D-dimer levels were also notably decreased (1.36 vs 0.77 μgFEU/ml; p=0.021) in the SD group. In the PD group a considerable increase was seen in CA 19-9 (119.5 vs 243.09 U/ml; p=0.025), CA 72-4 (5.18 vs 25.8 U/ml; p=0.036) and D-dimer levels (1.77 vs 1.88 μgFEU/ml; p=0.012).*

Conclusion: *This study demonstrated that D-dimer, LDH and tumor markers can be helpful in determining CRC prognosis in patients with metastatic disease. D-dimer, LDH and tumor markers provided unique prognostic information in advanced CRC patients.*

Key words: *CA19-9, CA 72-4, CEA, colorectal cancer, D-dimer, LDH, metastasis*

Introduction

LCRC is the third most common malignancy worldwide and the second most lethal cancer type in the developed world [1]. Lymph node metastasis is an important prognostic indicator for disease progression and is crucial for the determination of therapeutic strategy of CRC. Nevertheless, there is currently no useful serological marker for metastatic CRC, especially nodal metastasis [2,3]. Fibrin turnover in the tumor extracellular matrix (ECM) is essential for tumor angiogenesis and growth [4,5] Crosslinked fibrin in the ECM serves as a stable framework for endothelial cell migration during angiogenesis and tumor cell migration during invasion. D dimer, a fibrin degradation product, is produced when both intravascular and extravascular crosslinked fibrin is degraded by plasmin. Evidence for activation of both the coagulation and the fibrinolytic systems induced by tumor cells can be provided by the amount of fibrin split products, such as D-dimer, in the patient's plasma [6]. The extent of such an activation has been reported to correlate with tumor stage and prognosis in some malignancies, including CRC [7]. In another study, a significant correlation was found between plasma concentration of D-dimer and serum levels of tumor markers CEA and

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Received: 02/09/2012; Accepted: 14/10/2012

CA-125 [8]. CEA, CA19-9 and CA72-4 represent the currently most useful tumor markers for gastrointestinal malignancies. Elevated serum levels of CEA, CA19-9 and CA72-4 have been found in many patients with colorectal, gastric, biliary tract, and pancreatic carcinomas [9,10]. Some authors showed that CEA is most frequently evaluated as a predictor of prognosis for patients with CRC [11]. LDH is involved in the reversible transformation of pyruvate, the end product of glycolysis, to lactate. In cancer patients, serum LDH levels are often increased and high serum LDH levels have been linked with poor postoperative outcome, as well as failure of radiotherapy and chemotherapy in sarcomas, lymphomas, and carcinomas, including CRC [12].

Recently, systemic activation of hemostasis and fibrolysis has been reported to be correlated with clinical progression, low rates of response to chemotherapy, and poor prognosis in lung cancer [13]. Since the elevated D-dimer levels have been found in patients with CRC, and because fibrin degradation is important in tumor angiogenesis, the current study evaluated the correlations between quantitative D-dimer, LDH and tumor markers levels and response to chemotherapy.

Methods

Inclusion/exclusion criteria

This study included 38 patients with histologically verified metastatic CRC. The patients were enrolled in the study between 2005 and 2007. Inclusion criteria were male and female patients older than 18 years, ECOG performance score ≤ 2, life expectancy at least 3 months, having measurable lesions, having not received prior chemotherapy except adjuvant chemotherapy, and time to progression to metastatic disease after adjuvant therapy at least 12 months. All patients were examined by computed tomography scan of the thorax and abdomen. Patients who had tendency to deep venous thrombosis (e.g. serious wounds, venous stasis ulcers) were excluded from study. Also patients with clinically significant cardiovascular or peripheral vascular disease were excluded, as were those who had undergone a major surgical procedure ≤ 28 days before "Day 0". Recent or current use of oral and parenteral anticoagulants (except for the maintenance of central lines) or aspirin was not allowed. The study was approved by the Regional Scientific Ethical Committee. Written informed consent was obtained from each patient before enrollment in the study.

Estimated parameters

Blood samples were taken for LDH, D-dimer, CEA, CA19-9, and CA72-4 estimation before treatment and after the 3rd cycle of chemotherapy. LDH (Beckman Coulter, USA), D-dimer (VIDAS D-dimer, bioMerieux, Durham, NC, USA), CEA, CA 19-9 and CA 72-4 (Bayer Centaur, Leverkusen, Germany) concentrations were measured using ELISA technique.

Chemotherapy

All patients received XELOX combination chemotherapy as first line treatment for metastatic disease, which consisted of oxaliplatin 130 mg/m^2 on day 1 followed by oral capecitabine 2000 mg/m^2 on days 1-14 of a 21-day cycle. Patients were treated until disease progression or unacceptable toxicity. After the 3rd cycle of chemotherapy response was reevaluated clinically and radiologically by using CT scan of the thorax and abdomen according to RECIST criteria (EORTC version 2000) [14]. Patients were classified into 3 groups according to response: PR, SD and PD.

Statistics

Statistical analysis was performed using SPSS 15.0 package program. Data of angiogenesis-related factors levels were presented as mean ± SD or median with range. Kruskal-Wallis, Wilcoxon, 2-sample T test and Cox regression analysis were used. A p value <0.05 was considered statistically significant.

Results

Patient characteristics are shown in Table 1. A total of 38 patients had available blood samples for LDH, D-dimer, CEA, CA 19-9, and CA 72-4 analysis and correlation with disease prognosis. Eighteen patients (47.3%) achieved PR, 10 patients (26.3%) SD and 10 patients (26.3%) showed PD. LDH, D-dimer, CEA, CA 19-9, and CA 72-4 levels at baseline and after 3 cycles of chemotherapy are summarized in Table 2.

There was no statistical difference among the 3 groups for the initial baseline levels of LDH, D-dimer, CEA, CA 19-9, and CA 72-4 values. After 3 cycles of XELOX chemotherapy CEA, LDH and D-dimer levels were significantly decreased (p=0.002, p=0.001, and p=0.022, respectively) in the PR group. D-dimer levels were also notably decreased (p=0.021) in the SD group. However, other parameters were not influenced in this group. In the PD group a considerable increase was seen in CA 19-9 (p=0.025), CA 72-4 (p=0.036) and D-dimer levels (p=0.012). A positive correlation was found between CEA and D-dimer in the PD group after 3 cycles of chemotherapy (p=0.028).

On multivariate analysis, the high CA 72-4 levels had the highest hazard ratio (HR) when the

Table 1. Patient characteristics

Characteristics	*N*	*%*
Years, mean ±SD	59.55±12.21	
Body surface area (m^2)	1.68±0.18	
Sex		
Male	17	44.7
Female	21	55.3
ECOG performance status		
0	25	65.8
1	9	23.7
2	4	10.5
Pathology		
Adeno	32	84.2
Signet-ring	2	5.3
Mucinous	3	7.9
Anaplastic	1	2.6
Tumor localization		
Colon	27	71.1
Rectum	11	29.9
Adjuvant chemotherapy		
No	30	78.9
Yes	8	21.1
Adjuvant radiotherapy		
No	34	89.5
Yes	4	10.5
No. of metastases		
Single	33	86.9
Multiple	5	13.1
Metastatic localization		
Liver only	30	78.9
Lung only	3	7.9
Liver+lung	3	7.9
Brain+lung	1	2.6
Lung+thyroid+skin	1	2.6

initial baseline levels were examined (p=0.022, HR: 1.005, 95% confidence interval 1.001 to 1.009). On the other hand, after the 3rd cycle of chemotherapy, the high CA 72-4, D-dimer and LDH levels had prominent hazard ratios for overall survival [CA 72-4, p=0.003, HR:1.011 (1.004-1.019); D-dimer, p=0.053, HR: 1.011 (1.000-1.022); LDH, p=0.001, HR: 1.0003 (1.0001-1.0004)].

In subgroup analyses, LDH levels were significantly decreased (458 vs 261.25 U/lt; p=0.034) in patients with isolated liver metastasis in the PR group. LDH levels also declined (1137.87 vs 495.75 U/lt; p=0.128) in the SD group. On the other hand, LDH levels were increased (1065.7 vs 2419.11 U/lt; p=0.214) in the PD group (Table 3).

Discussion

The process of metastasis involves multiple tumor-host interactions. To survive, metastatic cancer cells firstly must leave the primary tumor, migrate into the lymphovascular system, and establish a new blood supply at their metastatic site. Fibrin remodeling is almost certainly involved in all steps of metastasis and has been proven to play a crucial role in new vessel formation [15,16]. Cross-linked fibrin in the ECM serves as a stable framework for endothelial cell migration during angiogenesis and tumor cell migration during invasion. Extracellular remodeling of fibrin is essential for angiogenesis in tumors [17] and activation of intravascular fibrin formation and degradation has been shown to occur in the plasma of breast cancer patients [18]. In addition, other indicators of fibrinolytic pathway activation, such as levels of plasminogen activator inhibitor and urokinase plasminogen activator, have been shown to have prognostic significance in patients with breast cancer [19]. Knockout mouse models have also revealed the importance of fibrin remodeling in tumor growth and metastasis. Mice that are deficient in plasminogen develop larger tumors, have more distant metastases, and have decreased life spans compared with mice with wild-type plasminogen [20].

Plasma D-dimer levels have been shown to be increased in patients with prostate cancer [21], CRC [22], lung cancer [23], ovarian malignancies and breast cancer [24]. Oya et al. [25] demonstrated that preoperative plasma D-dimer levels were higher in patients with larger tumors, deeper wall penetration, lymph node metastasis and lymphatic and venous invasion. Postoperative survival of patients with higher preoperative plasma D-dimer levels was significantly shorter than that of patients with lower plasma D-dimer levels [7]. Another research showed that preoperative plasma D-dimer levels correlated with the presence of vascular invasion [26].

The current study confirms previous studies that demonstrated up-regulated fibrinolytic activity in patients with metastatic disease. Our study represents the first attempt to look at a product of fibrin degradation (D-dimer) as a specific marker for response to chemotherapy in metastatic CRC patients who are treated with XELOX chemotherapy. D-dimer levels significantly decreased in the PR and SD groups, whereas opposite response was seen in the PD group. Finding a positive correlation between the D-dimer and CEA after 3 cycles of chemotherapy was another remarkable result of our study.

CEA, a tumor marker, is widely used as an indicator of disease progression or recurrence after resection of primary CRC. Nowadays, CEA level is considered as important as TNM stage [27,28].

Table 2. Level of cancer antigens, LDH and D-dimer during chemotherapy

Response		*Baseline level. Median (range)*	*After 3rd chemotherapy cycle. Median (range)*	*p-value*
Partial response	CEA (ng/ml)	20.55 (2.26-990)	11.97 (1.78-480)	0.002
	CA 19-9 (U/ml)	51.99 (0-768)	60.70 (0-541)	0.006
	CA 72-4 (U/ml)	5.86 (0.60-440)	5.89 (0.01-51.40)	0.071
	LDH (U/lt)	357.50 (184-1988)	214.00 (136-620)	0.001
	D-dimer (μg FEU/ml)	1.56 (0.06-4.16)	1.17 (0.02-4.79)	0.022
Stable disease	CEA (ng/ml)	103.15 (0.26-560)	81 (1.08-583)	0.139
	CA 19-9 (U/ml)	41.65 (0.81-710)	37 (0-765)	0.241
	CA 72-4 (U/ml)	13.50 (0.01-65)	9.71 (0.02-232)	0.878
	LDH (U/lt)	287 (220-4971)	242 (167-1749)	0.109
	D-dimer (μg FEU/ml)	1.36 (0.24-4.64)	0.77 (0.13-3.12)	0.021
Progressive disease	CEA (ng/ml)	74.28 (2.11-540)	109.95 (5.96-670)	0.093
	CA 19-9 (U/ml)	119.50 (0.90-785)	243.09 (59.60-890)	0.025
	CA 72-4 (U/ml)	5.18 (0.01-67.70)	25.80 (1.20-135.60)	0.036
	LDH (U/lt)	582.50 (139-3828)	658.50 (218-13147)	0.161
	D-dimer (μgFEU/ml)	1.77 (0.49-5.53)	1.88 (1.11-5.83)	0.012

Table 3. LDH levels during chemotherapy in patients with isolated liver metastasis among groups

Response	*LDH baseline (U/lt). Median (range)*	*p-value*	*LDH after the 3rd cycle of chemotherapy (U/lt). Median (range)*	*p-value*
Partial response	458 (184-1452)	0.345	261.25 (165-620)	0.036
Stable disease	1137.87 (175-4971)		495.75 (175-1749)	
Progressive disease	1065.7 (139-3828)		2419.11 (213-13147)	

In many studies, high preoperative CEA level was associated with advanced disease [29]. Sener et al. suggested that the preoperative level of serum CEA was an indicator of survival in patients with CRC, independent of the stage of disease at diagnosis [30]. In the present study, CEA values insignificantly increased when compared with the baseline in the PD group. However, remarkable decrease was seen in the PR and SD groups. Eventually our results seem to be consistent with the general literature that CEA is still the most reliable prognostic marker for the CRC.

CA19-9 is the carbohydrate determinant of a circulating antigen that functions as an adhesion molecule and plays a role in tumor progression [31]. Previous studies have shown that cancer cells expressing CA19-9 can adhere to endothelial cells through E-selectin. The attachment between cancer cells and endothelial cells is an important process in tumor metastasis [32]. High levels of CA19-9 also had a higher risk of lung metastasis, indicating that the prognostic value of CA19-9

is not restricted to primary CRC alone. Previous reports demonstrated that elevated preoperative serum levels of CEA and CA19-9 were predictive of increased mortality in CRC [33]. Increased levels of CEA and CA19-9 were also associated with venous and lymphatic spread [34]. CA72-4 was confirmed to have better sensitivity and specificity than CEA and CA19-9 [35]. However, very few studies on the prognostic value of CA72-4 in CRC have been reported in the literature, except some concerning gastric cancer [36-38]. Elevated preoperative serum levels of tumor markers CEA, CA 19-9, CA 242, CA 72-4 are related to poor outcome in patients with CRC. Dukes stage is the strongest prognostic factor, but tumor markers CEA and CA 72-4 are also independent prognostic factors [37]. In our research CA 19-9 levels were significantly decreased in the SD group but increased in the PD and PR groups. CA 72-4 levels were significantly increased only in the PD group. In conclusion, tumor markers alone are not sufficient enough to evaluate treatment response; therefore, they should be combined for determining the course of disease.

LDH catalyzes the reversible transformation of pyruvate to lactate under anaerobic conditions. The induction of anaerobic glycolysis is an important step for both normal cells and cancer cells that need to survive and maintain adenosine triphosphate resources in the absence of oxygen. Under such conditions, pyruvate, the end product of glycolysis, does not enter the Krebs cycle, but rather is converted to lactate by LDH. Upregulation of LDH ensures an efficient glycolytic metabolism while enabling tumor cells to become independent of an oxygen supply.

Generally, high concentrations of LDH are usually found in the liver, heart, erythrocytes, skeletal muscles, and kidneys. Consequently, diseases affecting these organs, such as cancers, have been reported to be associated with significant elevations in total serum LDH activity. Elevated serum LDH levels have also been associated with the presence of metastatic liver tumors in patients with CRC, melanoma, and cancers of unknown primary sites. Therefore, elevated levels of serum LDH have been hypothesized to indicate the presence of a hypoxic environment associated with tumor cells. Correspondingly, the oxygenation status of a tumor has been shown to be an important determinant of clinical effectiveness of radiotherapy and chemotherapy [39].

Except an old article, several studies demonstrated that LDH was a good prognostic factor for CRC [40]. High serum LDH levels is a common finding in human malignancies, including CRC; in a meta-analysis by Watine et al. serum LDH was one of the most important prognostic variables in CRC [41]. Recent studies confirm the adverse predictive role of serum LDH in the response of CRC to chemotherapy [42]. In patients with metastatic CRC elevated serum CEA and LDH levels have been reported as poor prognostic factors [42,43]. Also another study showed that lymph node involvement, CEA and LDH levels at diagnosis and tumor stage were significant predictors for overall survival [44].

In this study the highest value of LDH was in the PD group and the lowest in the SD group for the initial values. However, there was no statistical difference among groups. After 3 cycles of chemotherapy there was a significant decrease in the PR group, a non significant decrease in the SD group and an insignificant increase in the PD group. Therefore, LDH is a qualified marker to demonstrate clinical prognosis

Conclusion

The current study underscores the importance of the tumor microenvironment with respect to growth, metastases, and response to therapy. Despite the small number of patients in this study, the results clearly support a role for increased plasma D-dimer levels in predicting response to treatment and poor survival in CRC patients. It is hoped that D-dimer will serve as useful tool for monitoring CRC, especially in patients undergoing therapy that targets the host environment. Further studies evaluating the contribution of fibrin remodeling in cancer therapy are underway and should provide additional insight into potential therapeutic targets.

LDH seems to be more effective than the other tumor markers which are used in daily routine practice, particularly in patients with isolated liver metastases to evaluate the response to treatment. Tumor markers alone are not sufficient enough to evaluate treatment response. For that reason they should be combined for determining the course of the disease. CEA, CA 19-9 and CA 72-4 in conjunction with the use of LDH and D-dimer will contribute more efficiently in determining the prognosis of the disease.

Acknowledgment

This study was supported by a grant from the Erciyes University Research Foundation (Project: *TT-07-25*)

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