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Clinical effects of bevacizumab targeted treatment on advanced colorectal cancer with liver metastasis

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Abstract. – OBJECTIVE: In this study, the clinical effect of bevacizumab targeted treatment on advanced colorectal cancer with liver metastasis were studied.

PATIENTS AND METHODS: We consecutively selected 86 cases of patients with advanced colorectal cancer with liver metastasis, and divided them into the control group and observation group (n = 43 each). The FOLFOX chemotherapy scheme (oxaliplatin + fluorouracil + leucovorin) was given to the control group, FOLFOX plus bevacizumab treatment was given to the observation group.

RESULTS: It was found that the progression-free survival period and median survival period was significantly prolonged in the observation group, the survival rate was increased within 1 year and 2 years ($p < 0.05$), also the comparison of 3-year survival rates was not statistically significant ($p > 0.05$). Moreover, the overall effective rates in the observation group were significantly higher that the control group ($p < 0.05$). In terms of the complication occurrence rates, the difference was not statistically significant. However, the rise time (RT) and mean transit time (mTT) in the observation group significantly increased with the time ($p < 0.05$). The RT and mTT were unchanged ($p > 0.05$) in the control group; the RT and mTT at each time point of the observation group were significantly higher than the control group ($p < 0.05$).

CONCLUSIONS: The bevacizumab targeted treatment of advanced colorectal cancer with liver metastasis can improve the survival rate, prolong the survival period and cannot increase the complications, the CEUS quantitative indicator rise time and mean transit time were significantly changed, which can be used as an important indicator to predict responses.

Key Words:

Bevacizumab, Targeted treatment, Advanced colorectal cancer, Liver metastasis, FOLFOX and CEUS.

Introduction

The surgical risk of advanced colorectal cancer with liver metastasis (CRCLM) is very high, the effect is still controversial, and the chemotherapy has become the main method of treatment[1]. The targeted treatment carry out the intervention from the cancer gene, protein expression, receptor, cytokine, signal transduction pathway, etc., thereby, significantly inhibiting the tumor proliferation and metastasis and improving the patient effect[2]. The bevacizumab is the first new drugs inhibiting angiogenesis and playing a role in preventing cancers. Currently, it has been applied in a variety of malignant tumor in the clinic treatment, such as colorectal cancer, liver cancer, breast cancer, etc., the effect is significant and the complications are reduced[3,4]. However, to screen and assess the best benefiting population is the key to treatment. It can be evaluated from the morphology only by conventional imaging examinations after a month usually or even longer treatment such as CT, MRI, there is a greater lag[5]. The sensitivity and specificity of the tumor markers are poor, our center found that after CEUS quantitative analysis on the parameter changes on 7d, 14d, 30d and 60d after the treatment[6]. It is better correlated with the treatment effect and the drug may become an effective means for early predicting the treatment effects and assessing the prognosis.

Patients and Methods

Patients

We consecutively selected the patients diagnosed as CRCLM in the hospital from January 2012 to January 2014, without any surgical indication or refused surgical treatment, with

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chemotherapy indication and without contraindications. The inclusion criteria include (1). There were focuses that could be measured with ultrasound and CT, (2) There were at least two courses of chemotherapy, the chemotherapy had not been changed, (3) There were no other severe combined organ function diseases, it was expected that the survival period was more than 3 months, (4) The compliance was good and the clinical data were improved. The exclusion criteria were the chemotherapy drug allergies, newly discovered tumors during the study period, death or treatment interruption due to other causes, simultaneous participation in other studies and persons losing their follow-up patients.

The study has been approved by the Ethics Committee of our hospital and has obtained an informed consent of patients and their families. The patients were divided into the control group and the observation group according to the order of admission with 43 cases each. The control group included 25 males and 18 females with ages of 48 to 76 years and the mean of 62.5 ± 13.7 years. There were 20 cases with the primary lesion located in the sigmoid colon, 18 cases in the rectum, 3 cases in the right semi-colon, 2 cases in the left semi-colon. The primary lesion maximum diameter was 1.5-5.6 cm with the mean of 3.3 ± 1.4 cm. There were 10 cases with metastasis lesion in the left liver lobe, 33 cases in the right lobe. Further, there were 1 to 3 cases of metastasis lesions with the mean of 1.5 ± 0.6, the metastasis lesion maximum diameter was 0.5-3.7 cm, with the mean of 2.0 ± 0.6 cm. The observation group included 27 males and 16 females with ages of 46 to 77 years old with the mean of 62.3 ± 12.5 years. There were 23 cases with a primary lesion located in the sigmoid colon, 16 cases in the rectum, 3 cases in the right semi-colon, 1 case in the left semi-colon. The primary lesion maximum diameter was 1.0-5.5 cm with the mean of 3.2 ± 1.5 cm. There were 8 cases with liver metastases in the left lobe, 35 cases in the right lobe, 1 to 4 cases of metastasis lesions with the mean of 1.8 ± 0.7. The metastasis lesion maximum diameter was 1.0-3.5 cm, with the mean of 2.2 ± 0.7 cm. The baseline data of the two groups were comparable.

Chemotherapy Scheme

FOLFOX chemotherapy scheme includes oxaliplatin (85 mg/m^2 d1), leucovorin (400 mg/m^{2}d1), fluorouracil (400 mg/m^2 bolus d1), continuous infusion of fluorouracil (2400 mg/m^2) for 4-6h once every 2 weeks.

Intravenous infusion of bevacizumab includes 5 mg/kg for d1. Close monitoring was performed of blood routine examination, liver and kidney function, blood coagulation function, ECG and other vital signs, regular assessment of adverse reactions and observation after withdrawal if necessary.

Observation Indicators

The follow-up visit was carried out to January 2016 with an interval of 3 years. The progression-free survival periods, median survival periods, survival rates, overall effective rates, adverse drug reactions, use contrast-enhanced ultrasound (CEUS) for quantitative analysis of the parameter changes on the 7d, 14d, 30d and 60d before and after the treatment were compared. Wherein, the overall effective rate assessment used the mRECIST, provided that it will be complete response (CR) if the lesions are completely disappeared or the lesions are not strengthened for four weeks, but it will be partial response (PR) if the lesion enhanced area is reduced by > 30% and the time is no less than four weeks. It will be stable disease (SD) if the lesion enhanced area is increased by not more than 20% or reduced by not more than 30% and it will be a progressive disease (PD) if one or more lesions were increased by more than 20% or the new lesions occur, wherein the overall effective rate = (CR + PR + SD) / total population× 100%. The enhanced CT should assess the tumor cases before treatment, in the last month and two months.

The ultrasonography was Acuson Sequoia 512 type color ultrasound diagnosis apparatus (Siemens Company, Munich, Germany) with the probe of 4V1, the frequency of 3.5 MHz, the frame rate of 8 to 20 frames/sec, low mechanical index < 0.2 and equipped with the appropriate contrast pulse sequence imaging technology. All parameter conditions of the ultrasonic machines in the CEUS on the same patient were compared before and after targeted treatment remained unchanged. There were changes in the assessed parameters (mainly including the rise time and the mean transit time) on 7d, 14d, 30d and 60d before and after treatment, respectively. SonoVue (Bracco, Milan, Italy) was applied as the ultrasound contrast agent, 5 ml of saline solution was injected into the bottle to make suspension and 2.4 ml solution was extracted and injected into the median cubital vein according to the bolus injection standard requirements. Subsequently, 5ml of saline was injected for flushing. The enhanced

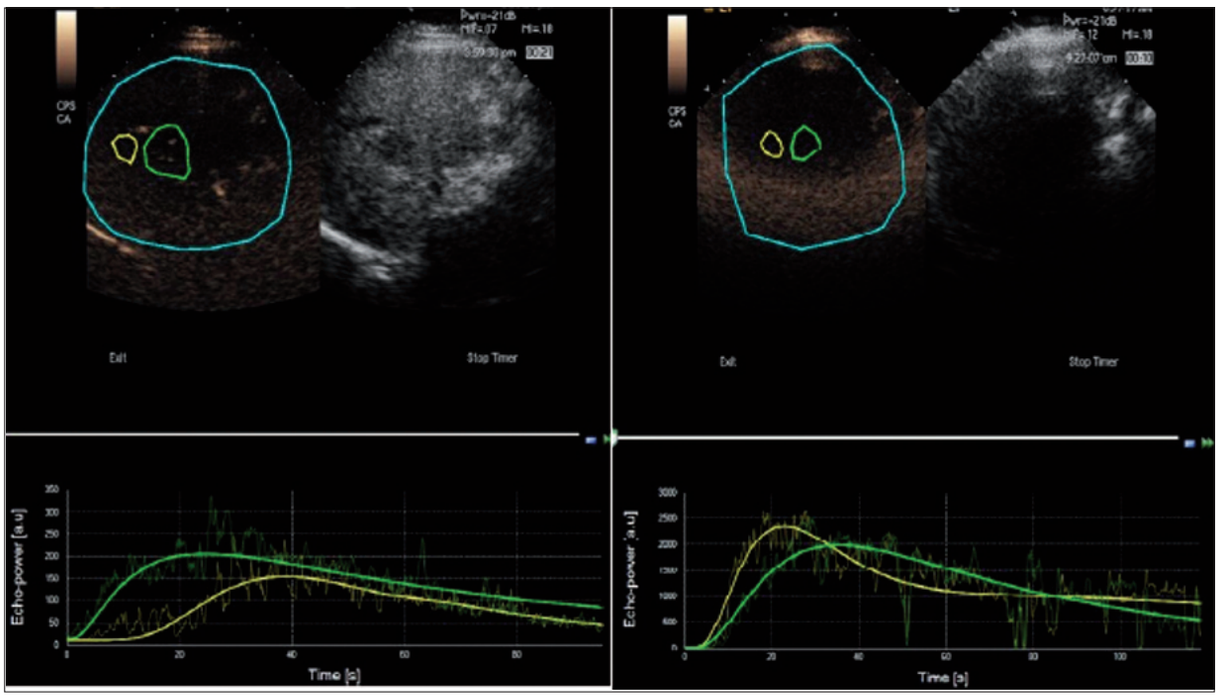

Figure 1. CEUS time enhancement curve image (the *left*: after chemotherapy, the *right*: on 3d after chemotherapy).

performance of the normal livers and liver lesions with contrast-enhanced ultrasound in the arterial, portal and delay periods for a total of 3 min were observed. The hand holding the probe of the sonographer in charge of contrast-enhanced ultrasound should not be moved as much as possible during recording the whole process of the contrast-enhanced ultrasound dynamic images and the patient should keep breathing as calm as possible, thereby reducing the impact of the breathing mobility. All contrast-enhanced ultrasound dynamic images were stored in DICOM format for CEUS quantitative parameter offline analysis. After angiography, the quantitative analysis software was used so that the time-intensity curve time could be obtained within the interest region of the tumor so as to carry out a quantitative analysis with the time-intensity curve on the hemodynamics.

Statistical Analysis

SPSS 20.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for data analysis. The measured data was expressed as mean ± standard deviation, the comparison between the two groups was carried out with *t*-test and the comparison of the measurement data was repeated using analysis of variance. The count data was expressed using the number of cases or the percentage; the comparison was carried out between the two groups using χ^2 test. The survival analysis was carried out by using Kaplan-Meier (KM) analysis and Log Rank test χ^2 method. The difference with $p < 0.05$ was considered as statistically significant.

Results

Comparison of Progression-free Survival Periods, Median Survival Periods and Survival Rates

The progression-free survival period and median survival period were significantly prolonged in the observation group, the survival rate was significantly increased within 1 year and 2 years ($p < 0.05$), and in terms of the comparison of the 3-year survival rate, the difference was not statistically significant ($p > 0.05$) (Table I and Figure 2).

Comparison of Overall Effective Rates and Adverse Drug Reactions

The overall effective rate in the observation group was significantly higher ($p < 0.05$); in terms of the comparison of the prevalence rates of complications, the difference was not statistically significant ($p > 0.05$) (Table II).

Table I. Comparison of progression-free survival periods, median survival periods and survival rates.

Group category	Case number	Progression-free survival period (month)	Median survival period (month)	One year's survival rate [rate (%)]	Two years' survival rate [rate (%)]	Three years' survival rate [rate (%)]
Control	43	3.8 ± 1.2	32.0 ± 0.4	28 (65.1)	20 (46.5)	15 (34.9)
Observation	43	6.2 ± 1.3	34.0 ± 0.6	36 (83.7)	29 (67.4)	20 (46.5)
t (χ^2)		7.628	28.579	3.909	3.842	1.204
p		0.009	0.000	0.048	0.050	0.272

Comparison of Parameters by CEUS Quantitative Analysis

The rise time (TR) and mean transit time (mTT) were significantly increased with the time in the observation group ($p < 0.05$). The RT and mTT were unchanged ($p > 0.05$) in the control group, whereas RT and mTT at each time point in the observation group were significantly higher ($p < 0.05$) as shown in Figure 3.

Discussion

Bevacizumab is an extract from the culture supernatant fluid of Chinese hamster ovary cell and is a kind of recombinant humanized IgG1 monoclonal antibody and its light chain variable area can include the murine components and be combined with VEGF, the heavy chain fixed area and most of the light chain area are humanized sections[7]. The main advantages for the bevacizumab playing the anti-tumor effect[8] include that the target point is directly exposed to the blood, thereby facilitating the direct action on the drug. The target point gene expression is stable and the drug resistance is not easily produced. There are downstream amplification effects, which can inhibit tumor metastasis and there are relatively few adverse reactions.

TREE test results showed that through the comparison of combined FOLFOX, b-FOL and CapeOx schemes respectively with bevacizumab and the single chemotherapy, the ORR was improved, the PFS and OS were prolonged[9]. ECOG 3200 test randomly group 829 cases of patients with advanced colorectal cancer, with the overall response rate (ORR) of 21.8% and 9.2% respectively in the bevacizumab group and the single chemotherapy group[10]. In 2006, the FDA of USA approved the application of bevacizumab in combination with FOLFOX4 as a second-line treatment scheme of metastatic colorectal cancer[11]. Through the study results, the progression-free survival period and median survival period were significantly prolonged in the observation group and the survival rate was signifi-

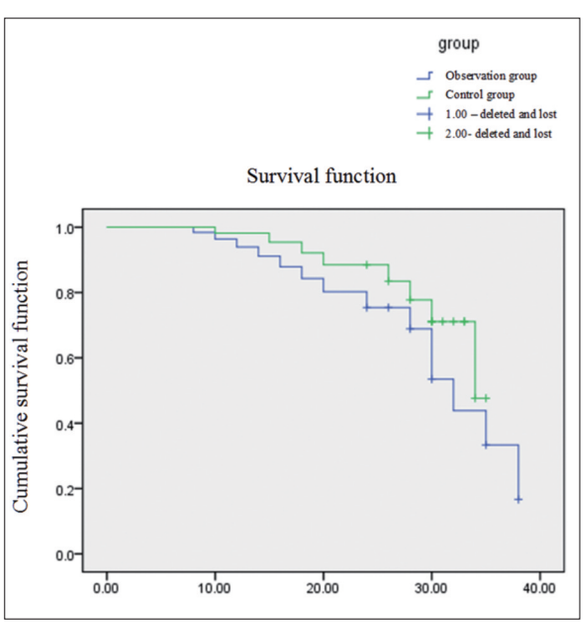

Figure 2. Analysis on median survival period with K-M method.

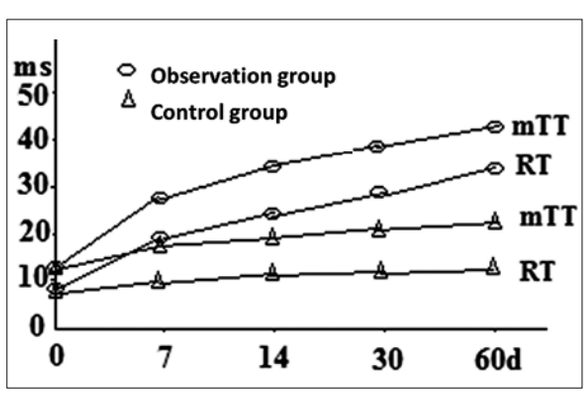

Figure 3. Parameters trend chart by CEUS quantitative analysis.

Table II. Comparison of overall effective rates and adverse drug reactions [rate (%)].

Group category	Case number	CR	PR	SD	PD	Overall effective rate	Bone marrow suppression	Gastrointestinal toxicity	Phlebophlogosis	Toxicity to nervous system	Others	Total complications
Control	43	1	5	18	19	24 (55.8)	1	2	1	1	1	6 (14.0)
Observation	43	5	8	20	10	33 (76.7)	1	3	2	1	1	8 (18.6)
t (χ^2)					4.214						0.341	
p					0.040						0.559	

cantly increased within 1 year and 2 years. In terms of the comparison of three-years survival rates, there was no difference was statistically significant. The overall effective rate was significantly higher in the observation group. In terms of the comparison of the complication prevalence rates, the difference was not statistically significant, promoting that bevacizumab could improve the recent survival rate of CRCLM. The reason for not improving the high and long-term survival rate may be that the CRCLM survival rate itself was not high[12], and the 3-year survival rates were less than 50%.

CEUS can be used in the quantitative assessment of changes in the arteries in a tumor blood vessel-enriched degree after the bevacizumab treatment of patients with primary liver cancer, which may be up to 3 d after treatment at most[13]. CEUS is known as non-invasive microvascular angiography with dynamic and real-time observation advantages, which can provide liver tumor and its background liver tissue micro-perfusion information. CEUS cannot only observe the tumor capillaries and real imaging characteristics of liver, kidney, spleen, pancreas and other abdominal organs, but can also observe the breast, thyroid, lymph node and other superficial tube tumor characteristics, be used for angiographic parameters quantitative analysis through special software and show a broad application prospect[14] in the assessment of tumor angiogenesis.

We found that the rise time (RT) and mean transit time (mTT) were increased significantly with the time in the observation group. The RT and mTT were unchanged in the control group, RT and mTT at each time point in the observation group were significantly higher. There were two reasons (1) before targeted treatment, there were a lot of new blood vessels and arterial-venous fistula traffic branches formed. The vascular density was significantly increased, the local blood flow perfusion speed was rapid in the tumor area during the radiography, the local contrast agent perfusion speed was rapid and significantly increased[15]; (2) for the tumor sensitive to the targeted treatment after treatment, the drugs had significant inhibitory and destruction effect on the tumor angiogenesis so that the total number of tumor blood vessels was reduced, the local vascular density was decreased, the blood supply was reduced[16]; (3) the tumor vascular targeted drug itself could also cause meningitis within the arteries of small blood vessels and periangiitis,

leading to vascular stenosis or even intravascular thrombosis formation, significant reduction of the blood flow and reduction of the slow blood flow velocity, thereby prolonging the rise time and mean transit time[17].

Conclusions

The bevacizumab targeted treatment on the advanced colorectal cancer with liver metastasis can improve the survival rate, prolong the survival period and not add complications. There was a significant change in the CEUS quantitative indicators rise time and mean transit time, which can be taken as an important indicator for reaction prediction.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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