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Biochemical liver function tests parameters do not indicate any difference in the degree of hepatotoxicity in patients with metastatic colorectal carcinoma treated with conventional anticancer drugs regardless the use of bevacizumab

Biohemijski parametri funkcije jetre ne ukazuju na razliku u stepenu hepatotoksičnog efekta konvencionalnih citostatika bez obzira na korišćenje bevacizumaba kod bolesnika sa metastatskim kolorektalnim karcinomom

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Abstract

Background/Aim. Colorectal carcinoma (CRC) is one the most frequent malignant disease with early liver metastasis. It requires the timely use of anticancer drugs. Current treatment of metastatic CRC consists of conventional anticancer drugs use, but they cause liver damage which is manifested by disorder in biochemical liver function parameters. The addition of one of monoclonal antibodies, e.g. bevacizumab improves their therapeutic effect, but its influence on caused biochemical disturbances is not completely known. Therefore the aim of this study was to compare the level of liver function test parameters in patients treated with conventional anticancer drugs with parameters in patients additionally treated with bevacizumab. **Methods**. The study was performed on the two groups of adult patients with liver metastatic CRC assigned according to the treatment protocol. One group of the patients (n = 44) was treated with FOLFOX4 (the group 1), and the other one (n = 52) with bevacizumab added to FOLFOX4 treatment protocol (the group 2). Depending on the response of patients, the duration of treatment varied from 2 to 6 months. Standard liver function tests were performed before and after the completion of the treatment. **Results**. Initial values of some biochemical function test parameters [alkaline phosphatase (ALP) in the group 1 of patients, gamma-glutamyl transferase (GGT) and lactate dehydrogenase (LDH) in both groups] were increased in relation to the normal reference values, with some intergroup differences ($p = 0.001$). Biochemical disturbances of liver function tests in the group of patients treated with conventional anticancer drugs were due to not only their metastases but also due to the hepatotoxic effect of drugs used. After the treatment, significant differences in biochemical liver tests parameters were found in aspartate aminotransferase (AST), alanine aminotransferase (ALP), GGT and LDH, being lower in the group 2 (patients additionally treated by bevacizumab) (p values were: 0.002 for AST; 0.001 for ALP and GGT; 0.000 for LDH). The levels of the other studied parameters, alanine aminotransferase (ALT) bilirubin, and proteins did not differ significantly between groups both pre- or post-treatment. **Conclusion**. Both, metastatic CRC and treatment with the conventional anticancer drugs induce significant disturbances of several liver function parameters. The addition of bevacizumab to the conventional anticancer drugs did not affect these disturbances.

Keywords:
colorectal neoplasms; neoplasm metastases; antineoplastic combined chemotherapy protocols; antibodies, monoclonal; bevacizumab; liver function tests.

Apstrakt

Uvod/Cilj. Kolorektalni karcinom (CRC) jedno je od najčešćih malignih oboljenja. U trenutku dijagnostikovanja kod većine obolelih otkrivaju metastaze na jetri. Zbog toga je pravovremena upotreba hemioterapeutika od velikog značaja za njihovo lečenje. Lečenje metastatskog CRC zasniva se na upotrebi konvencionalnih citostatika, koji oštećuju tkivo jetre što se manifestuje poremećajem vrednosti biohemijskih parametra kojima se prati funkcija jetre. Dodatak nekog od monoklonskih antitela, npr. bevacizumaba, konvencionalnim citostaticima, poboljšava njihov terapijski efekat, dok je njegov uticaj na prouzrokovani poremećaj vrednosti biohemijskih parametara nepoznat. Shodno tome, cilj istraživanja bio je da se ispita kako dodavanje bevacizumaba konvencionalnim ci-

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tostaticima utiče na vrednost biohemijskih parametara korišćenih za praćenje funkcije jetre. **Metode**. U istraživanje su bili uključeni odrasli bolesnici sa metastatskim CRC podeljenu u dve grupe na osnovu hemioterapijskog protokola koji su primali. Jedna grupa bolesnika (n = 44) lečena je po FOLFOX4 protokolu, dok je druga grupa (n = 52) lečena kombinacijom FOLFOX4 protokola i bevacizumaba. U zavisnosti od efekta primenjene terapije, bolesnici su lečeni od 2 do 6 meseci. Pre i posle završenog lečenja rađeni su kompletni testovi funkcije jetre. **Rezultati**. Početne vrednosti određenih biohemijskih parametara [alkalne fosfataze (ALP) u grupi 1, a gama-glutamil transferaze (GGT) i laktat dehidrogenaze (LDH) u obe grupe bolesnika bile su iznad gornje granice referentnih vrednosti, uz postojanje statistički značajne razlike između grupa (p = 0.001)]. Poremećaj vrednosti biohemijskih parametara kod bolesnika koji su lečeni konvencionalnim citostaticima posledica je ne samo metastatskih promena već i toksičnog efekta hemioterapije. Nakon sprovedenog lečenja, statistički značajno su se razlikovale vrednosti aspartat aminotransferaze (AST), ALP, GGT i LDH (p vrednosti bile su redom: 0.002 za AST; 0.001 za ALP i GGT; 0.000 za LDH). Vrednosti pomenutih parametara bile su niže kod bolesnika u grupi 2 koja je uz konvencionalne citostatike primala i bevacizumab. Suprotno tome, poređenjem vrednosti alanin aminotransferaze (ALT), ukupnog bilirubina i ukupnih proteina na početku i kraju lečenja nije utvrđena statistički značajna razlika između grupa. **Zaključak**. I metastatski CRC i lečenje konvencionalnim citostaticima dovode do značajnih poremećaja vrednosti nekih od biohemijskih parametara kojima se prati funkcija jetre. Dodatak bevacizumaba konvencionalnim citostaticima ne utiče na ove poremećaje.

Ključne reči:
kolorektalne neoplazme; neoplazme, metastaze; lečenje kombinovanjem antineoplastika, protokoli; antitela, monoklonska; bevacizumab; jetra, funkcijski testovi.

Introduction

Colorectal carcinoma (CRC), with an annual incidence of one million cases worldwide, is the third most frequent one among all malignant tumors. After lung and prostatic carcinomas in men and breast carcinoma in women, CRC is the most frequent cause of death [1, 2]. The primary site of CRC hematogenic metastases is liver [2]. At diagnosis, liver metastases are present in about 25% of patients (synchronous metastases), while in the one third of patients metastases are developed during the course of follow-up (metachronous metastases) [3, 4]. Aside this, liver metastases in patients died from CRC are found in 70% of cases, being thus considered as the main cause of mortality in these patients [3]. Because of that, the treatment of liver metastatic CRC (mCRC) is of the great importance and its success is dependent on their resectability.

Metastases are fully resectable in less than 10% of patients and can be removed surgically without prior use of chemotherapy [5]. However, there are far more patients with potential resectable or nonresectable metastases in which surgical removement is possible only after the use of neoadjuvant treatment.

The data show that the use of such treatment leads to the improvement of resectability in about 30% of patients with nonresectable liver metastasis, and thus to the increase of expected 5-year survival of these patients up to 25% [5].

In patients with mCRC, liver function is impaired not only as a consequence of the impact of metastases on healthy liver tissue but also as a result of the direct hepatotoxic effect of conventional anticancer drugs used for their treatment [6–8].

There are only a few papers dealing with the changes of liver biochemical parameters in oncologic patients. In one of them, Field et al. [7] presented only qualitative, but not quantitative results of these changes. In another one, King et al. [6] described the hepatotoxic effect of chemotherapy depending on the treatment protocol manifested by increased values of some liver biochemical tests.

In both mentioned papers, it was also pointed out that there are many comorbid factors (e.g. obesity, personal history of viral hepatitis, sex and age) which contributed to these disturbances.

The results of several clinical studies have shown that the addition of bevacizumab to conventional anticancer drugs [5-fluorouracil (5-FU), oxaliplatin, irinotecan, capecitabine)] greatly contributed to their therapeutic effect [9–16]. However, there were no data on its influence on the increased values of liver function tests parameters.

Methods

The research was designed as a retrospective study in which 96 patients with liver mCRC treated with FOLFOX4/FOLFOX4+Bevacizumab [FOLFOX: FOL – folinic acid (leucovorin; F – fluorouracil (5-FU); OX – oxaliplatin] as a neoadjuvant chemotherapy protocol were enrolled. Treatment was conducted at the Institute for Radiology and Oncology in Belgrade, Serbia, in the period from January 2009 to December 2014. Data were collected from patients' medical history documents.

According to the treatment protocol patients were divided into two groups: the group 1 (n = 44) was treated with FOLFOX-4, and the group 2 (n = 52) with bevacizumab added to FOLFOX-4 treatment protocol. Used chemotherapy protocols were in accordance with National Comprehensive Cancer Network recommendations for colorectal carcinoma treatment (NCCN) [17].

The group 1 of patients received FOLFOX4, which consisted of a 2-hour infusion of leucovorin (20 mg/m^2) followed by 5-FU *iv* bolus (400 mg/m^2) and 22-hour infusion (600 mg/m^2) for 2 consecutive days, with oxaliplatin (135 mg/m^2) as a 2-hour infusion on day 1. Besides this, patients from the group 2 additionally received bevacizumab on the first day of the therapy in a dose of 5 mg/kg. The duration of bevacizumab treatment was determined by a physician. The treatment was conducted on every two weeks, except in a case of high grade of toxicity when it was postponed until patient's recovery. Patients from both groups received at least four cycles of chemotherapy. Treatment response was determined after every fourth cycle until the end of the therapy. Patients response to therapy was evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 as a complete or partial response,

stable disease, and progressive disease. The evaluation was performed by a surgeon, oncologist, pathologist and radiologist who did not take part in the study.

Preoperatively, in order to confirm the diagnosis, examinations such as clinical, endoscopic and radiologic ones [abdominal ultrasound, chest x-ray, multislice computerized tomography (MSCT) of the abdomen] were performed in all the patients.

Only the patients who received FOLFOX4/ FOLFOX4 + bevacizumab for potentially resectable liver metastases as a first line treatment protocol were included in the study. Other inclusion criteria were: Eastern Cooperative Oncology Group (ECOG) performance status score 0-2, age 18 to 80 years, normal function of the bone marrow [white blood cells (WBC) > 4×10^9/L; platelet count > 100×10^9/L)], liver (upper limit of the normal range < 1.5 × ULN), and kidney (serum creatinine concentration < 1.5 × upper limit of the normal range (ULN) function, no previous other malignant disease except cervical carcinoma *in situ* and no contraindications for the drugs administration. Patients were followed-up until the end of the treatment or until the disease progression and switch to another treatment protocol.

The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. Approval of the protocol was obtained from institutional Ethics Committee.

The biochemical parameters of liver function [aspartate aminotransferase (AST); alanine aminotransferase (ALT); alkaline phosphatase (ALP); gamma glutamyl transferase (GGT); Lactate dehydrogenase (LDH) relevant to determine the chemotherapy hepatotoxic effects were determined before and after the completion of the treatment. Parameters were measured in serum utilizing commercial biochemical tests on the biochemical analyzer Advia 1800.

The intent-to-treat (ITT) patient population included all patients who participated in the study. The usual descriptive statistic parameters were used in statistical analysis of the obtained results (median with interquartile range 25–75 percentiles). Values of the analyzed parameters had no normal distribution. For dependent or independent non-parametric characteristics Wilcoxon test and Mann-Whitney *U*-test were performed. Commercially available statistical software package SPSS version 17.0, 2008 was used for statistical analysis.

Results

After completed treatment, a complete response was accomplished in only 3 (3.13%) patients, partial response in 38 (39.58%), stable disease in 23 (23.96%) and progression of the disease was observed in 32 (33.33%) patients. Complete response was accomplished only in the group 2 of patients. Out of 38 patients with partial response, 30 (78.95%) patients belonged to the group 2. Stabilization of the disease was almost equally represented in both groups of patients while progression of the disease was more common for patients in the group 1.

Results of studied biochemical parameters are presented in Table 1 and Figures 1–6. The results in both groups are given before and after the treatment.

In the group 1 initial values of ALP, GGT and LDH were above ULN in 53.5%, 45% and 33% of patients respectively (not shown). At the same time, in the group 2 differences in values of GGT and LDH were found in 24% and 21.1% of patients, respectively (not shown).

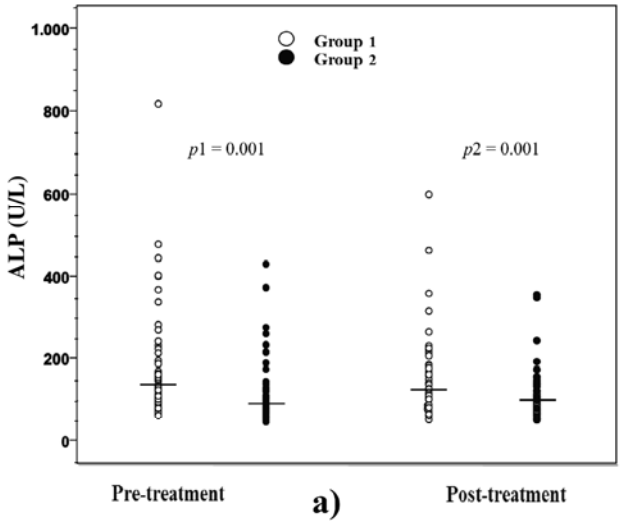
The intragroup pre- and post-treatment values of tested biochemical parameters found in the group 1 showed that conventional anticancer agents led to the statistically significant increase in serum levels of AST ($p = 0.002$) and bilirubin ($p = 0.001$).

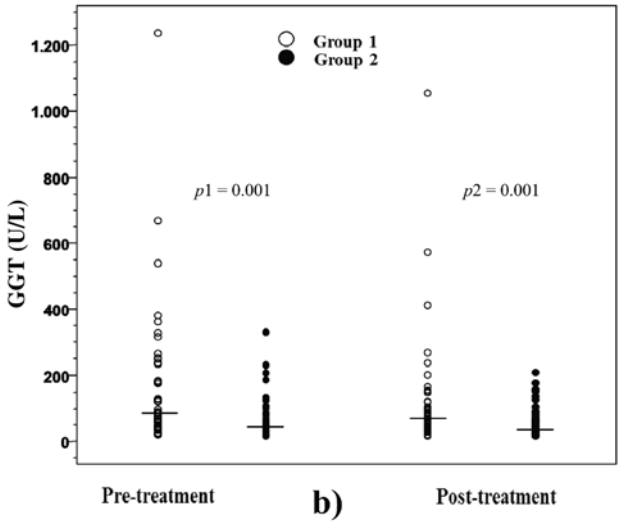
Table 1

Pre- and post-treatment values of biochemical parameters of liver function tests in patients treated with conventional anticancer (the group 1) and with their combination with bevacizumab (the group 2)

Parameters	ULN	Pre- treatment, median (IQR)		Post-treatment, median (IQR)	
		Group 1	Group 2	Group 1	Group 2
AST (U/L)	40	24.00	21.00	33.00	25.00**
		17.25–35.75	17.25–26	25.25–46.75	20.25–34.25
ALT (U/L)	40	23.00	20.00	26.00	25.00
		17.25–37.25	14.00–28.75	21.00–33.25	18.00–38
ALP (U/L)	141	134.00	94.50**	129.00	99.00**
		93.25–228.00	72.00–126.25	105.00–187.50	76.50–133.50
GGT (U/L)	60	87.00	47.00***	76.00	38.50**
		44.25–236.75	30.00–97.00	47.00–149.00	23.25–70.25
LDH (U/L)	460	449.00	350.50***	475.50	380.50***
		347.00–955.25	287.75–490.25	394.25–724	327.25–439.00
Bil (μmol/L)	20.5	8.75	8.00	10.70	10.10
		7.00–11.55	6.82–11.17	8.67–15.17	7.57–13.60
Prot (U/L)	82	73.00	73.50	71	72.00
		70.00–76.75	72.00–77.00	68.00–73.75	70.00–74.00

***$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ intergroup pre- and post-treatment comparison; ULN – upper limit of normal; IQR – interquartile range; AST – aspartate aminotransferase; ALT – alanine aminotransferase; ALP – alkaline phosphatase; GGT – gamma glutamyl transferase; LDH – lactate dehydrogenase; Bil – bilirubin; Prot – proteins.**





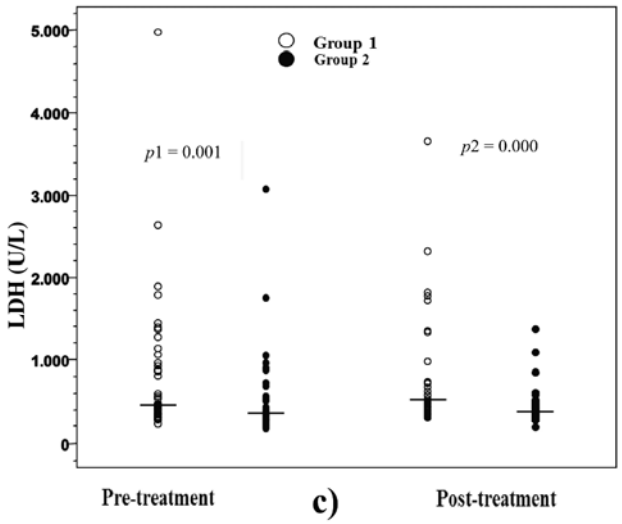
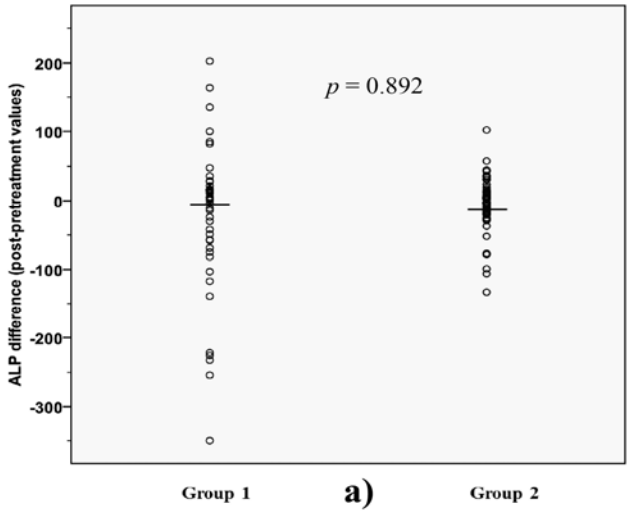
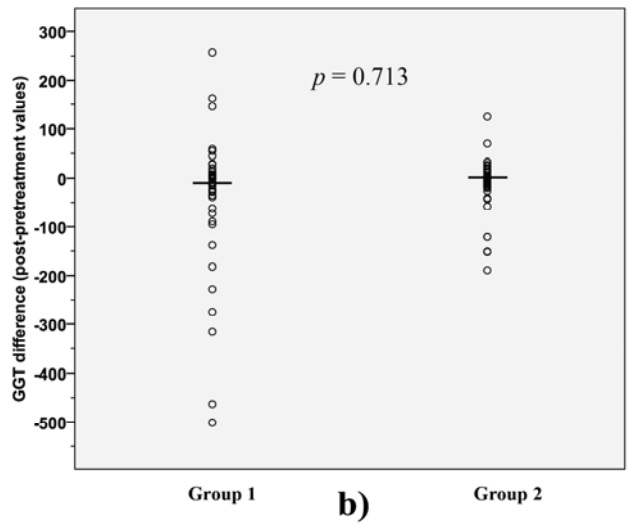


Fig. 1 – Intergroup comparison of pre- and post-treatment values: a) alkaline phosphatase (ALP); b) gama glutamyl transferase (GGT); c) lactate dehydrogenase (LDH). Group 1 – treatment with conventional anticancer drugs (FOLFOX4 protocol) alone; group 2 – treatment with combination of conventional anticancer drugs (FOLFOX4 protocol) and bevacizumab.





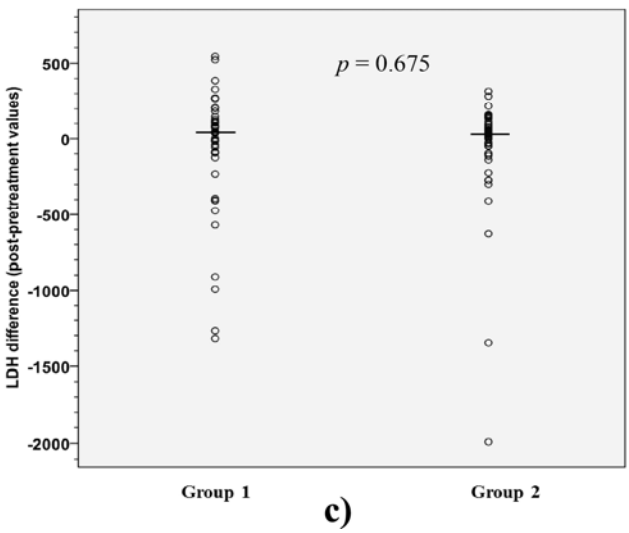


Fig. 2 – Intergroup comparison post- and pre-treatment value difference in: a) alkaline phosphatase (ALP); b) gama glutamyl transferase (GGT); c) lactate dehydrogenase (LDH). Group 1 – treatment with conventional anticancer drugs (FOLFOX4 protocol) alone; group 2 – treatment with combination of conventional anticancer drugs (FOLFOX4 protocol) and bevacizumab.

In the group 2 of patients, statistically significant increase in serum levels after the treatment was found in three parameters: AST, ALT and bilirubin ($p = 0.001$; $p = 0.001$; $p = 0.006$ respectively).

In both groups of patients amount of serum proteins after the treatment was statistically significantly decreased (p values for the group 1 and 2 were 0.043 and 0.005).

The analysis of intergroup (group 1 : group 2) pre-treatment results showed statistically significant difference ($p = 0.001$) in serum levels of ALP, GGT and LDH with the higher initial values registered in the group 1 (Table 1).

Comparison of post-treatment results in the group 1 in relation to the results in the group 2, showed statistically significantly lower values of some parameters in the group 2: AST ($p = 0.002$); ALP ($p = 0.001$); GGT ($p = 0.001$) and LDH ($p = 0.000$) (Table 1).

Results of the analysis of post and pre-treatment value difference between groups showed no statistically significant difference in values of the eight tested biochemical parameters.

In order to be easy-comprehended, the absolute values of statistically significant results obtained after intergroup comparison of pre- and post-treatment values are shown in Figures 1 a–c.

Intergroup comparisons of post- and pre-treatment value difference are shown in Figures 2 a–c.

Figures 1 a–c show that pre- and post-treatment ALP, GGT and LDH statistically significantly differ between two groups of patients, with a pronounced variability of values in the group 1 of patients.

Figures 2 a–c show no statistically significant difference when post- and pre-treatment value differences in serum levels of ALP, GGT and LDH were compared between two groups of patients.

Discussion

The results of the study showed that the treatment of mCRC patients with conventional anticancer agents led to the increase of values of several liver function tests parameters. As a consequence of the disease, these results were also initially increased in relation to the ULN. At the same time, the addition of bevacizumab to conventional anticancer treatment did not lead to a statistically significant decrease of those values.

The influence of mCRC on liver function tests

The unfavorable effects of liver colorectal metastasis on its biochemical parameters are dual: space occupying and energy consumption. The occupied space compresses nearby liver tissue with simultaneous "steal" of the energy required for normal liver function. This is so more pronounced because malignant cells are fast divided and thus consume a large amount of nutritional compounds. This leads to the disturbances of ion pumps followed by the leak of intracellular enzymes and increase of their blood values [18, 19].

Results of the study showed that the pre-treatment values of standard liver function tests parameters, such as ALP, GGT and LDH were significantly increased in relation to their ULN. This itself points out that liver metastases of CRC exert a hepatotoxic effect on liver cells.

The influence of conventional anticancer drugs on liver function tests

Many anticancer drugs, including 5-FU and oxaliplatin exert a direct hepatotoxic effect at least partly by producing free radicals [20–24]. They in turn damage lipoprotein membranes of liver cells which release and thus increase the values of corresponding enzymes in systemic circulation [5]. As a result of anticancer treatment, a hepatotoxic effect and mCRC as a basic disease, liver function tests parameters values were further additionally aggravated. If one compares the therapeutic and hepatotoxic effect of anticancer used drugs the conclusion is that there is a disparity between those two effects. Namely, 5-FU and oxaliplatin lead to temporarily stabilization of the disease but at the same time to the further increase of liver function tests parameters values (Table 1).

The influence of bevacizumab on conventional anticancer agents hepatotoxic effect

Bevacizumab is one of the newer monoclonal antibodies used as an additional treatment to current anticancer drugs in patients with mCRC. The results of so far clinical studies show that bevacizumab increased the therapeutic effect of 5-FU and oxaliplatin leading to the significant clinical improvement [9–16]. These findings were confirmed in our study, where the largest number of patients with complete and partial responses belong to the group of patients additionally treated with bevacizumab. However, there were no data about its influence on disturbances of liver function tests parameters induced by conventional anticancer drugs.

In this respect, results of our study showed that bevacizumab added to conventional anticancer treatment did not remarkably decrease the disturbed values of biochemical liver function tests parameters caused by these drugs (Table 1). These results do not correlate to the clinical improvement in patients treated with combined use of these drugs which provides more complete and partial remissions compared with those achieved with conventional anticancer drugs given alone. In other words, used as an addition to conventional anticancer treatment, bevacizumab leads only to the significant clinical improvement but not to the decrease of the hepatotoxic effect of these drugs.

Conclusion

The results of the study showed that out of seven tested biochemical liver function tests parameters, liver metastases of CRC led to the significant increase in serum values of ALP, GGT and LDH. Conventional anticancer drugs (5-FU and oxaliplatin) exerted the hepatotoxic effect in these patients, leading to the further significant increase of serum values of the mentioned parameters. The addition of bevacizumab to conventional anticancer drugs did not abate their hepatotoxic effect, in term of decreasing the values of monitored biochemical parameters.

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