INFLUENCE OF THE NUMBER OF PLATELETS AND HEMOGLOBIN CONCENTRATIONS IN PREDICTING THE DEVELOPMENT OF PROTEINURIA INDUCED BY THE ADMINISTRATION OF BEVACIZUMAB

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UTICAJ BROJA TROMBOCITA I KONCENTRACIJE HEMOGLOBINA U PREDVIĐANJU RAZVOJA PROTEINURIJE IZAZVANE PRIMENOM BEVACIZUMABA

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ABSTRACT

Bevacizumab neutralizes a key stimulation factor in tumour angiogenesis - vascular endothelial growth factor (VEGF). The origin of VEGF is related to platelets, and the basic stimulus for its production is hypoxia. By neutralizing VEGF, changes occur in the blood that are manifested at the platelet and haemoglobin levels. Study results indicate that proteinuria is a side effect of bevacizumab treatment. The aim of the present study was to examine the percentages of proteinuria in patients with metastatic colorectal cancer after the administration of bevacizumab. The correlation between the degree of proteinuria and changes in the number of platelets and concentration of haemoglobin in patients, as well as in relation to the age (< 60 i ≥ 60) and gender of the patients, was examined.

The study group of respondents included 20 patients with metastatic colorectal cancer (9 men and 11 women) ranging in age from 43 to 73 years. Proteinuria was tested using "Laboquick" test strips. The levels of platelets and haemoglobin were measured using an autoanalyser, the type Cell Dyn 3700 – Abbott, according to the protocol prescribed by The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

Statistically significant differences were recorded for the platelet measurements before and after therapy in the overall group of respondents ($p = 0.025$). Statistically significant decreases in platelet counts and increases in haemoglobin concentrations were also noted in women (Plt: $p = 0.0036$; Hb: $p = 0.0286$) and in patients < 60 years old (Plt: $p = 0.0270$; Hb: $p = 0.0553$). No significant differences were identified in men or in patients ≥ 60 years of age. Proteinuria was detected in one case in which demonstrating an increase in only the recorded number of platelets and a decrease in the level of haemoglobin.

Platelets and haemoglobin may serve as prognostic biomarkers of the risk of proteinuria. Based on the initial values of these parameters, we can predict the target group, in terms of gender and age, that may obtain the most benefit from bevacizumab therapy.

Key words: angiogenesis, bevacizumab, VEGF, platelets, haemoglobin.

SAŽETAK

Bevacizumab neutrališe ključni faktor stimulacije tumorske angiogeneze vaskularni endotelni faktor rasta (VEGF). Poreklo VEGF-a se vezuje za trombocite, a osnovni stimulans za njegovu produkciju je hipoksija. Neutralizacijom VEGF-a nastaju promene na krvnoj slici manifestovane promenama nivoa trombocita i hemoglobina. Rezultati studija ukazuju na proteinuriju kao neželjeni efekat primene bevacizumaba. Cilj studije je bio ispitati u kom procentu će se pojaviti proteinurija kod ispitanika sa metastatskim kolorektalnim karcinomom nakon primene bevacizumaba. Studija analizira da li postoji korelacija između stepena proteinurije i promena u broju trombocita i koncentraciji hemoglobina kod ispitanika kao i promene u odnosu na starosnu dob (< 60 i ≥ 60) i pol ispitanika.

Grupa ispitanika je obuhvatala 20 pacijenata sa metastatskim kolorektalnim karcinomom, 9 muškaraca i 11 žena, starosne dobi od 43 do 73 godina. Proteinurija je testirana "Laboquick" test trakama. Nivoi trombocita i hemoglobina su određene na autoanalizatoru tipa Cell Dyn 3700 – Abbott, po protokolu propisanom od strane Međunarodne federacije za kliničku hemiju i laboratorijsku medicinu (IFCC).

Evidentirane su statistički signifikantne razlike u nivoima izmjerenih trombocita pre i nakon terapije u ukupnoj test grupi ($p = 0,025$). Statistički značajne razlike, u smislu smanjenja broja trombocita i povećanja koncentracije hemoglobina, evidentirane su kod žena (Plt: $p = 0,0036$; Hb: $p = 0,0286$) i kod ispitanika < 60 godina (Plt: $p = 0,0270$; Hb: $p = 0,0553$). Nije bilo statističke značajnosti kod muškaraca i ispitanika ≥ 60 godina. Proteinurija se pojavila kod jednog pacijenta kod kojeg je jedino evidentirano povećanje broja trombocita i smanjenje nivoa hemoglobina.

Trombociti i hemoglobin mogu poslužiti kao prognostički biomarkeri koji mogu ukazati na rizik od pojave proteinurije. Na osnovu inicijalnih vrijednosti ovih parametara može se predvideti koja ciljna grupa pacijenata, u odnosu na pol i dob, može dobiti maksimalnu efikasnu korist od terapije bevacizumabom.

Ključne reči: angiogeneza, bevacizumab, VEGF, trombociti, hemoglobin.

INTRODUCTION

Angiogenesis is the process of creating new blood vessels from existing ones; it is manifested by the activation, proliferation and migration of endothelial cells and is rapidly activated in response to hypoxic or ischaemic conditions. It is of crucial importance during physiological and pathological processes that occur in humans. Angiogenesis is regulated by numerous paracrine growth factors that are divided into stimulators and inhibitors and that are in a constant equilibrium. In response to prevailing factors that cause stimulation, excessive angiogenesis occurs, resulting in pathological processes such as tumour growth, atherosclerosis, chronic inflammatory diseases (rheumatoid arthritis, Crohn's disease), diabetes, psoriasis, endometriosis and adiposity (1). Small tumours with a diameter of less than 2 mm represent so-called dormant tumours and provide nutrients via diffusion. Larger tumours generate their own blood vessels from existing ones in the host through an increase in the expression of growth factors. This elevated expression is further increased under conditions of hypoxia. The blood vessels of tumours are structurally different from those of healthy tissues. They have an irregular shape and inconsistent diameter, are tortuous and permeable, and they are not organized into final venules, arterioles and capillaries. As a result of the irregular structure of tumour blood vessels, the blood flow to the tumour is variable, often providing a sub-optimal supply of nutrients to tumour cells (2). VEGF (*vascular endothelial growth factor*) is a specific mitogen for the vascular endothelium and an important stimulator of angiogenesis (3). Angiogenesis that is conditioned by VEGF enables tumour metastasis. The basic stimulus for the production VEGF is hypoxia, which develops in the tumour (2). Because there is a correlation between anaemia and tumour hypoxia, we can expect an increased level of VEGF in the serum of anaemic patients. Thus, anaemia, i.e., a low level of haemoglobin, can stimulate angiogenesis (4).

Platelets are the main source of VEGF in the blood. VEGF is important in two processes in vertebrates: angiogenesis and haematopoiesis (5). VEGF increases the activity of haematopoietic stem cells and has the capacity to suppress stem cell apoptosis. In this way, platelet production is increased in the bone marrow in response to VEGF (6). Platelets contribute to angiogenesis by releasing VEGF from alpha granules after activation by ADP (7).

Angiogenesis plays a significant role in tumour growth and hence represents an attractive therapeutic goal. In clinical trials, more than 30 substances have been shown to inhibit angiogenesis by neutralizing VEGF, by inhibiting VEGF receptors or by inhibiting matrix degradation (8). The drugs approved by the FDA as anti-angiogenic agents are as follows: the bevacizumab and tyrosine kinase inhibitors sorafenib, sunitinib and pazopanib.

Bevacizumab is a humanized monoclonal IgG antibody (93% human, 7% murine) that was produced by recombinant DNK technology in Chinese hamster ovary cells. The

mechanism of action of bevacizumab is based on its binding to VEGF receptors on the surface of endothelial cells. By neutralizing VEGF activity, tumour vascularisation decreases, resulting in regression of the existing tumour blood cells, inhibition of new tumour vasculature and, consequently, a decrease in the tumour size (9). Bevacizumab also normalizes the tumour vasculature, reduces interstitial pressure in the tumour, and thus increases the flow into and effects of chemotherapeutic agents in the tumour (10). Bevacizumab has been approved by the FDA in combination with chemotherapy for the treatment of patients with metastatic colorectal, lung, breast and kidney cancer (11). The safety and effectiveness of the recommended dose of bevacizumab in combination with chemotherapy in metastatic cancer has been examined in randomized, active-controlled clinical studies. The results of these studies have demonstrated high rates of objective responses, overall survival and duration of survival without disease progression compared with chemotherapy. The most common adverse reactions were hypertension, proteinuria, gastrointestinal perforation, fistula, haemorrhage, thromboembolism, congestive heart failure, and hypersensitivity reactions. In clinical studies, proteinuria occurred in 0.7% to 38% patients who received bevacizumab. The severity of the proteinuria ranged from clinically asymptomatic, transient, trace proteinuria to nephritic syndrome. Proteinuria has been noted in all studies; it was not related to renal dysfunction and rarely necessitated permanent discontinuation of therapy (12).

Proteinuria includes the occurrence of protein in the urine in amounts that exceed physiological concentrations (150-200 mg/24 hours), which is indicative of a number of renal disorders. Proteinuria was clinically significant. Proteinuria is the cause of progressive glomerular dysfunction and progressive renal insufficiency. Renal diseases are chronic and progressive. Most renal diseases lead to gradual and irreversible damage to kidney function that results in a state of renal insufficiency (13). The terminal stage of kidney insufficiency inevitably leads to dialysis or kidney transplantation. The excessive filtration of serum proteins contributes directly to the progression of renal damage (14).

The main objective of this study was to analyse the percentage of proteinuria in patients with metastatic colorectal cancer after the administration of bevacizumab. We assessed whether there was any relationship between the degree of proteinuria and changes in platelet counts and haemoglobin concentrations in the whole group of patients and with respect to age (<60 and ≥ 60) and sex.

MATERIALS AND METHODS

Patients tested

This prospective non-randomized study included 20 patients from the Oncology Clinic, Clinical Center of

Montenegro, consisting of 9 men and 11 women aged 43 to 73 years. Before monitoring the results, whether the patients suffered from a chronic disease or received an accompanying treatment was considered in terms of the potential for proteinuria induction. The study was designed in accordance with the standards of good clinical practice. The criterion to include patients in the study was a set patho-histological diagnosis of colorectal cancer. The patients provided written consent to participate in the study. One patient was released from the study after one month. In 11 patients, the first line of chemotherapy was FOLFOX (5-fluorouracil/leukovorin/oxaliplatin), and in 9 patients, it was XELOX (capecitabine/oxaliplatin). In the second-line treatment for metastatic colorectal cancer, the patients received chemotherapy in combination with bevacizumab. Before starting the treatment with bevacizumab, the patients were tested for proteinuria. After introducing bevacizumab, the presence of protein in the urine was tested using a qualitative screening for protein in urine collected over 24 hours. Side effects that occurred during the study were documented on a form used to monitor side effects.

The indicated form is filled out by the doctor every other week. Security data were reported in accordance with Volume 9^a EU Clinical Trials. The protocol used for the clinical study describes the method that was used to

monitor side effects. The clinical study protocol describes the method used to monitor adverse events. The intensity ratio between the drug and the adverse effects and the outcomes of the adverse effects were assessed. Serious adverse events were any adverse event, regardless of the dose, that met at least one of the following criteria (15):

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those events that may not be immediately life-threatening but are clearly of major clinical significance. Such events may jeopardize the subject and may require intervention to prevent other serious outcomes noted above.

The stated study was approved by the Ethical Committee of the Clinical Center of Montenegro in which the stated protocol was applied, as well as the Agency for Medications and Medical Devices of Montenegro.

Biochemical analyses:

The platelets counts and haemoglobin concentration were performed separately for each patient during the first visit (initial visit before treatment with bevacizumab), during treatment (every two months) and after completion of the treatment (final visit). The final visit (fifth visit) was recorded at ten months after the date of bevacizumab initiation in all but six patients. During the study, five serious adverse events were recorded that were not associated with bevacizumab according to the physician:

- In two patients, disease progression occurred, leading to death.
- One patient was hospitalized due to ileus.
- One patient was hospitalized because of aggressive behaviour.
- One patient was hospitalized because of neurovegetative dystonia.

In addition to the adverse events mentioned above, events associated with the study drug bevacizumab were noted, such as hypertension and proteinuria. In three patients, an increase in blood pressure was detected, which was successfully controlled during the study with antihypertensive agents and did not result in discontinuation of therapy. After eight months of bevacizumab treatment, one patient presented proteinuria and haematuria. The patient was hospitalized in the Department of Nephrology, and further investigations were performed.

To obtain a more accurate diagnosis, the results were analysed for all 19 patients included in the study as well as for the 14 patients who completed the study. The platelet count and haemoglobin concentration were determined using a Cell Dyn 3700 autoanalyser from Abbott according to the protocol prescribed by IFCC.

Inicijalni pacijenta: _____ Broj pacijenta: _____

NEŽELJENI DOGAĐAJ	Intenzitet neželjenog događaja:	Odnos između lijeka i neželjenog događaja:	Ishod:
☐ blagi ☐ umjereni ☐ ozbiljan ☐ životno-ugrožavajući	☐ vjerovatan ☐ mogućan ☐ neznatan ☐ nepovezan	☐ oporavak ☐ u toku ☐ nepoznat ☐ smrt	☐ <i>Preduzeta radnja:</i>
☐ blagi ☐ umjereni ☐ ozbiljan ☐ životno-ugrožavajući	☐ vjerovatan ☐ mogućan ☐ neznatan ☐ nepovezan	☐ oporavak ☐ u toku ☐ nepoznat ☐ smrt	☐ <i>Preduzeta radnja:</i>
☐ blagi ☐ umjereni ☐ ozbiljan ☐ životno-ugrožavajući	☐ vjerovatan ☐ mogućan ☐ neznatan ☐ nepovezan	☐ oporavak ☐ u toku ☐ nepoznat ☐ smrt	☐ <i>Preduzeta radnja:</i>
☐ blagi ☐ umjereni ☐ ozbiljan ☐ životno-ugrožavajući	☐ vjerovatan ☐ mogućan ☐ neznatan ☐ nepovezan	☐ oporavak ☐ u toku ☐ nepoznat ☐ smrt	☐ <i>Preduzeta radnja:</i>

Figure 1. Form to monitor adverse effects

Protein quantification

Protein in the urine was quickly detected using “Labo-quick” test strips. If any shade of green colour developed, protein was present in the urine.

In cases with a positive identification of protein in the urine using the test strips, a quantitative assessment of the protein concentration was conducted using the biuret method. This method is based on a compound with at least two peptide bonds that react with copper salts under alkaline conditions to yield a purple product. The colour intensity is proportional to the protein concentration and was measured photometrically using a Humalyzer 2000 spectrophotometer at 546 nm. The measured parameter (R) represents the amount of protein in the urine. The amount of protein in the urine over 24 hours is expressed in g/l and was calculated according to the following formula:

$$\frac{R \bullet \text{diuresis}}{1000}$$

The amount of protein in urine over 24 hours was assessed for 3 days. A proteinuria grade was assigned to the samples. According to the toxicity criteria of the National Institute for Cancer version I and II (National Cancer Institute Common Toxicity Criteria version I or II), proteinuria comprises four grades: Grade1 (+) – protein amounts from 0.1 g to 1 g/24 h; Grade 2 (++ to +++) – protein amounts from 1 g to 3.5 g/24 h; Grade3 (++++ – protein amounts greater than 3.5 g/24 h; Grade 4 – nephric syndrome.

Statistical analysis

The collected laboratory data were entered into and converted using the SPSS programme.

RESULTS

The test results for the presence of proteinuria

Eight months after the initiation of bevacizumab, proteinuria and haematuria were recorded in one patient. The quantitative analysis of the protein concentrations was conducted over 3 days. The measured values were based on daily diuresis in patients and ranged from 2.91 g/L, 2.32 g/L and 2.52 g/L (proteinuria grade 2). After the presence of proteinuria was determined, the patient was admitted to the Nephrology Division, and treatment with bevacizumab was terminated, after which the protein levels in the urine decreased.

Test results for platelet counts and haemoglobin concentrations before and after treatment with bevacizumab

The platelet counts and haemoglobin concentrations were determined for each patient separately during the

Table 1 Differences in platelet counts and haemoglobin levels between the initial and final visit in the 19 patients who entered the study

INITIAL VISIT (IV)		FINAL VISIT (FV)		% differences IV and FV	
Measured values		Measured values		Hb (g/L)	Plt (g/L)
Hb (g/L)	Plt (g/L)	Hb (g/L)	Plt (g/L)	Hb (g/L)	Plt (g/L)
131	323	154	264	18	-18
120	242	136	202	13	-17
118	322	123	186	4	-42
123	128	118	200	-4	56
161	203	173	204	7	0
151	183	163	259	8	42
80	338	109	265	36	-22
133	224	156	184	17	-18
134	268	135	112	1	-58
131	268	176	339	34	26
151	313	133	247	-12	-21
140	136	123	93	-12	-32
164	236	141	196	-14	-17
116	203	116	142	0	-30
133	203	151	181	14	-11
138	290	134	207	-3	-29
117	197	119	217	2	10
118	289	114	232	-3	-20
147	193	158	145	7	-25
Excluded from the study					
SEM*				5.947368	-11.9847
p-value**				0.11	0.022

SEM* - Standard error of the mean

p-value** - Statistical significance

initial and final visits. Differences in the platelets and haemoglobin during the initial visit prior to bevacizumab administration and during the final visit after the completion of treatment are shown in Table 1.

Analysis of the whole group of 19 patients revealed a statistically significant decrease in the average platelet counts from the initial to the final measurements (p=0.022). Similar results were obtained for the 14 patients who continued the study (p=0.025). There were no statistically significant differences in the haemoglobin concentrations before or after initiation of bevacizumab, either in the overall population (p=0.11) or in the 14 patients who continued the study (p=0.179).

Analysis of platelet counts before and after bevacizumab administration by gender

In terms of gender, the test group was divided into two subgroups, and differences in platelet counts were assessed during the initial and final visit by gender. This analysis was performed in all 19 patients who entered the study and in the 14 patients who continued the study. Statistical analyses of the average platelet counts are presented in Table 2.

There were no significant differences in platelet counts between the initial and final visits in male patients in either tested subgroup. However, in the female patients in both subgroups, there was a very statistically significant reduction in platelet counts after compared with before bevacizumab treatment.

Analysis of platelet counts before and after treatment by age

The patients were divided into two subgroups by age: patients ≥ 60 and < 60 years old. The platelet counts were determined during the initial and final visits for the subgroup of all patients and for those who continued the study. Statistical analysis of the average values for the platelet counts are presented in Table 3.

From Table 3, we can conclude that there were no statistically significant differences in platelet counts from the initial to the final visit in patients ≥ 60 years of age; however, in the patients < 60 years old, a statistically significant reduction in the platelet counts was identified in the group of 19 patients. The haemoglobin concentration was also assessed before and after treatment by age.

Table 4 shows that there were no significant differences in average haemoglobin concentrations between the initial and the final visit in male patients, either in the test group

or in the group that continued treatment. In both female subgroups, there was a statistically significant increase in haemoglobin concentrations after compared with before the treatment.

Analysis of haemoglobin concentrations before and after treatment by age

Table 5 shows the statistical analysis of the average haemoglobin concentrations by subgroups.

Table 5 shows that there were no statistically significant differences in the haemoglobin concentrations between the first and final visits in patients ≥ 60 years old; however, a significant difference was identified in those < 60 years old. In the present study, we examined the correlation between platelet counts and haemoglobin concentrations by gender for the overall population. The correlations were performed to evaluate the association of platelets and haemoglobin before and after initiation of bevacizumab treatment by gender and for comparison with the results of clinical studies demonstrating an association of these parameters. These correlations were performed using the Pearson coefficient (r) and statistical value p .

Based on these parameters ($r=0.590$; $p=0.095$), there was a significant positive correlation between the platelet counts and haemoglobin concentrations after bevacizumab treatment.

Table 2. Comparison of statistical parameters determined for platelet counts by gender

Gender Parameters	Male gender (entered the study)	Male gender (continued the study)	Female gender (entered the study)	Female gender (continued the study)
$\bar{x}$	-7	0.8	-21.9	-19.66667
p-value	0.3444	0.4657	0.0036	0.0081

Table 3. Comparison of statistical parameters for platelet counts by age

Age Parameters	≥ 60 years old (entered the study)	≥ 60 years old (continued the study)	< 60 years old (entered the study)	< 60 years old (continued the study)
$\bar{x}$	-7.44444	-12.16667	-15.9	-12.5
p-value	0.1103	0.1058	0.0270	0.0859

Table 4. Comparison of statistical parameters for haemoglobin levels by gender

Gender Parameters	Male gender (entered the study)	Male gender (continued the study)	Female gender (entered the study)	Female gender (continued the study)
$\bar{x}$	4.666667	2.6	7.1	7.444444
p-value	0.2403	0.3983	0.0286	0.0385

Table 5. Comparison of statistical parameters for haemoglobin levels by age

Age Parameters	≥ 60 years old (entered the study)	≥ 60 years old (continued the study)	< 60 years old (entered the study)	< 60 years old (continued the study)
$\bar{x}$	3.555556	-1.83333	8.1	11.375
p-value	0.1575	0.7080	0.1181	0.0553

Figure 1.

zumab treatment in males in the overall population (Figure 1). The platelet counts and haemoglobin concentrations before bevacizumab treatment in females in the overall population showed a tendency toward towards a negative correlation, which may indicate an important difference in larger samples ($r=-0.517$; $p=0.126$) (Figure 2).

DISCUSSION

To date, there are no published data regarding the incidence of the type and grade of proteinuria in patients in Montenegro treated with bevacizumab, nor are there data related to the success of therapy. The purpose of this study was to determine the percentage of proteinuria in patients with metastatic colorectal cancer who were treated with bevacizumab in Montenegro. By evaluating platelet counts and haemoglobin levels by age and gender, we were able to predict which target population has a greater risk of developing proteinuria. In the present study, proteinuria occurred in one of the 14 patients (7%). This percentage is consistent with the available literature and the results of clinical trials showing proteinuria in proportions of patients ranging from 0.7 % to 38 % (12).

It is known that chemotherapy destroys the cells in the body that are rapidly dividing, as well as those in the bone marrow. In patients who had already received a combination of chemotherapeutic agents as first-line chemotherapy, it was necessary to control basic laboratory blood parameters prior to the introduction of second-line chemotherapy. In the present study, emphasis was placed on examining the platelet counts and haemoglobin concentrations because platelets are the main source of VEGF in the blood, and the production of VEGF is greatest during episodes of hypoxia or low levels of haemoglobin. Application of the anti-VEGF neutralizing antibody bevacizumab resulted in changes in the blood concentrations of these two elements. Studies have indicated that bevacizumab administered as mono or combination therapy leads to in-

Figure 2.

creased haemoglobin levels due to the inhibition of VEGF (16) as well as the reduced numbers of platelets (17).

In the present study, after the five bevacizumab treatments, increased haemoglobin concentrations were noted in 57% of the patients (ranging from 2% to 36%). There were no statistically significant differences in the haemoglobin levels before compared with after the bevacizumab treatment in the total population.

A comparison between the platelet counts during the initial visit before bevacizumab treatment and the final visit revealed a significant decrease in 10 out of 14 patients (71 %), ranging from 11 % to 58 %. The largest increase in platelet counts (56%) was recorded in a patient who had platelet counts that were lower than the reference value at the beginning of treatment. This patient developed proteinuria. The present study results revealed a statistically significant difference in the platelet counts measured before treatment compared with after the injection of bevacizumab in the overall population of patients who began the study, as well as in the population that completed the study ($p = 0.025$). Our results confirm previous research and mechanisms to date showing an association among VEGF, platelets and bevacizumab.

The decreased risk of colorectal cancer in postmenopausal women on hormone replacement therapy and increased incidence in patients with breast cancer suggest that sex hormones play a role in the pathogenesis and development of colorectal cancer and, therefore, may have prognostic value (18). Oestrogen stimulates the production of anti-angiogenic factors through a mechanism that involves the activation of PAF-mediated NF- κ B factor (19). Studies have shown that oestrogen plays a major role in the epithelisation of the colon. The isolation of colorectal cancer cells has revealed that a significant number of cells are positive for beta oestrogenic receptors (20). Hendifar and colleagues showed that among post-surgical patients with colorectal cancer, women younger than 55 years of age have a better

survival rate than young males. In contrast, women older than 55 years of age demonstrated a worse overall survival compared with older men (21). All of the above findings suggest that oestrogen has a prominent role in preventing the development of colorectal cancer. Moreover, antibodies have a longer half-life in women, resulting in an improved response to treatment with monoclonal antibody (22). Wakelee and associates examined the effects of age and gender on the survival of patients with NSCLC with and without bevacizumab. The administration of bevacizumab resulted in a significantly different average survival time in women under 60 years of age (the longest survival time) without affecting males (23). The administration of bevacizumab in combination with chemotherapy in patients with colorectal cancer improved overall survival and the period without disease progression similarly in patients under 65 years of age, patients from 65 to 70 years old and patients older than 70 years of age (24).

The present results for gender and age provided interesting information. Differences in the number of platelets in the male population were not statistically significant, whereas in women, statistically significant differences were obtained, in terms of reduced platelet counts ($p = 0.0036$, $p = 0.0081$) and increased haemoglobin concentrations after bevacizumab treatment ($p = 0.0286$, $p = 0.0385$).

Regarding age, there was a significant reduction in platelet counts ($p = 0.0270$) and an increase in haemoglobin concentrations ($p = 0.0553$) after treatment with bevacizumab in the population under the age of 60 years, while the results for those over 60 years of age were not statistically significant. It must be noted that the average age of the patients assessed herein was 58 years; 58 % of the patients were under 65 years of age, 32 % were between 65 and 70 years of age and only 10 % were older than 70 years. Considering the total number of female patients, 30 % of the women older than 60 years of age and previous research on the importance of oestrogen, we can conclude that our results confirm previous study findings and mechanisms associated with oestrogen, colorectal cancer and bevacizumab.

The present study has some limitations, among which the small study population was prominent. The significant difference in platelet counts in the tested population suggests the potential for even more meaningful results with a larger number of specimens. Furthermore, there was a significant difference in haemoglobin concentrations in females, which would probably be more evident in a larger patient cohort. Another limitation of this study was the exclusion of patients after the identification of grade 2 proteinuria; thus, the application of a larger dose of bevacizumab was not tested to determine whether it would lead to an increased degree of proteinuria.

CONCLUSION

The present results demonstrate that proteinuria developed in one patient, thus representing 7% of the tested

population. These data are consistent with the published literature, although a smaller percentage was obtained herein due to the small number of patients in the tested population.

In the total population, there was a statistically significant difference between the platelet counts before and after the initiation of bevacizumab. In females, but not in males, a highly significant difference in platelet counts was obtained significant difference. Furthermore, significant differences were even noted in patients under 60 years of age. The haemoglobin concentrations before and after bevacizumab treatment were not significantly different in the overall population. However, analyses by gender and age revealed statistically significant differences in female patients and in patients <60 years old. A significant positive correlation was evident between the platelet counts and haemoglobin concentrations in male patients after bevacizumab treatment. Based on the present results, proteinuria occurred in only one patient, who presented an apparent deviation in laboratory parameters from the expected values in terms of a significant increase in platelet counts and a significant decrease in haemoglobin levels.

Because the study was performed in a small group of patients, it will be necessary to perform detailed analyses in a larger cohort to confirm the hypothesis that platelet counts and haemoglobin concentrations may serve as a prognostic biomarker for the development proteinuria, and furthermore, that based on the initial values determined for these biological parameters, a target group of patients with respect to gender and age may be predicted to obtain optimal benefits from bevacizumab therapy.

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REFERENCES

1. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nature Medicine* 1995;1:27-31.
2. Jain RK, "Molecular Regulation of Vessel Maturation," *Nature Medicine* 2003; 9:685-693.
3. Ferrara N. Vascular endothelial growth factor and the regulation of angiogenesis. *Department of Molecular Oncology*. 2000; 55:15-35.
4. Dunst, J., Pigorsch, S., Hansgen, G., Hintner, I., Lautenschlager, C., Becker, A. Low hemoglobin is associated with increased levels of vascular endothelial growth factor (VEGF) in cancer patients. Does anemia stimulate angiogenesis? *Strahlenther Onkologie*. 1999; 175: 93- 96.
5. Gerber H, Condorelli F, Park J, Ferrara N. Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia. *Journal of Biological Chemistry* 1997; 272: 23659-67.

6. Katoh O, Tauchi H, Kawaishi K, Kimura A, Satow Y. Expression of the vascular endothelial growth factor (VEGF) receptor gene, KDR, in hematopoietic cells and inhibitory effect of VEGF on apoptotic cell death caused by ionizing radiation. *Cancer Research* 1995; 55(23):5687-92.
7. Battinelli EM, Markens BA, Italiano JE. Release of angiogenesis regulatory proteins from platelet alpha granules: modulation of physiological and pathological angiogenesis. *Blood*. 2011;118(5):1359-69.
8. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature*. 2000; 407(6801): 249-57.
9. Poon RT-P, Fan S-T, Wong J. Clinical implications of circulating angiogenic factors in cancer patients. *Journal of Clinical Oncology* 2001; 19(4): 1207-25.
10. Jain RK. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. *Nature Medicine* 2001; 7(9): 987-9.
11. FDA Approval for Bevacizumab. National Cancer Institute. (Accessed in May 2013 at <http://www.cancer.gov/cancertopics/druginfo/fda-bevacizumab>)
12. SPC Avastin (Accessed in Sep 2012 at <http://www.medicines.org.uk/emc/medicine/15748>)
13. Abbate M, Zoja C, Remuzzi G. How Does Proteinuria Cause Progressive Renal Damage? *Journal of the American Society of Nephrology* 2006; 17(11): 2974-84.
14. Loon NR. Diabetic Kidney Disease: Preventing Dialysis and Transplantation. *Clinical Diabetes* 2003; 21(2): 55-62.
15. Clinical Research Protocols. National Institutes of Health. (Accessed in June 2012 at <http://www.niehs.nih.gov/about/od/ethics/protocols/>)
16. Harshman LC, Kuo CJ, Wong BY, Vogelzang NJ, Srinivas S. Increased hemoglobin associated with VEGF inhibitors in advanced renal cell carcinoma. *Cancer Investigation*. 2009 Oct; 27(8):851-6.
17. Mutlu H, Berk V, Karaca H, Erden A, Aslan T, Akca Z. Treatment Regimen With Bevacizumab Decreases Mean Platelet Volume in Patients With Metastatic Colon Cancer. *Clinical and Applied Thrombosis/Hemostasis* 2012; 18 (5): 546-548.
18. DiLeo A, Messa C, Russo F, et al. Prognostic value of cytosolic estrogen receptors in human colorectal carcinoma and surrounding mucosa: preliminary results. *Dig Dis Sci*. 1994;39:2038-2042.
19. Heon Seo K, Lee HS, Jung B, et al. Estrogen Enhances Angiogenesis through a Pathway Involving Platelet-Activating Factor-Mediated Nuclear Factor-KB Activation. *Cancer Research* 64, 2004; 6482-6488.
20. Witte D, Chirala M, Younes A, Li Y and Younes M: Estrogen receptor beta is expressed in human colorectal adenocarcinoma. *Human Pathology* 2001;32: 940-944.
21. Hendifar A, et al. Gender Disparities in Metastatic Colorectal Cancer Survival. *Clinical Cancer Research* 2009; (15): 6391.
22. Schmetzer O, Flörcken A. Sex differences in the drug therapy for oncologic diseases. *Handbook of Experimental Pharmacology*. 2012;(214):411-42.
23. Wakelee HA, Dahlberg SE, Brahmer JR, et al.: Differential effect of age on survival in advanced NSCLC in women versus men: analysis of recent Eastern Cooperative Oncology Group (ECOG) studies, with and without bevacizumab. *Lung Cancer* 76, 2012; (3): 410-5.
24. Cassidy J, Saltz LB, Giantonio BJ, Kabbinavar FF, Hurwitz HI, Rohr UP. Effect of bevacizumab in older patients with metastatic colorectal cancer: pooled analysis of four randomized studies. *Journal of Cancer Research and Clinical Oncology* 2010; 136(5): 737-743.