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The Immunomodulation Effect of Allogenic Blood Transfusion in Colorectal Cancer – A New Approach

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A B S T R A C T

The total number of 542 patients with colorectal cancer surgery have been analyzed in order to estimate the effect of receiving transfusion local recurrences, and the disease free – survival. It should be examined whether there are changes in general immunity indicators which would be connected with perioperative transfusion. A significant connection has been found between local recurrences and blood transfusion ($p<0.0001$), the most noticeable being in Dukes A ($p=0.045$), localization on rectum ($p=0.036$). The receiving of blood transfusion is linked significantly with disease free – survival reduction ($p=0.0068$; log rank), the most significant being in Dukes A stage ($p=0.0123$; log rank) and with localization on rectum ($p=0.0231$). The analysis of general immunity indicators has shown significant immunocompromitation of patients just before the surgery and this could have effect on immunomodulation caused by transfusion and just as on the treatment prognosis of colorectal carcinoma.

Key words: *colorectal cancer, immunomodulation, allogenic blood transfusion*

Introduction

Intravenous blood transfusion introduces large quantities of foreign antigens, i.e. peripheral blood cells, proteins, lipids, preservatives and anticoagulants. Tests on animal models showed that the intravenous infusion of allogeneic tissue leads to immunosuppression. The mechanism of development of natural immunological tolerance was explained in classic experiments by Medwar, which showed that exposure to foreign bodies during the fetal life leads to lifelong acceptance of skin transplants taken from organism to which the animal was exposed during its fetal life[1].

Those and similar studies have given hope that the tolerance may also be stimulated in patients who were intended for transplantation, and that this would help to reduce the rejection of the transplant. Clinical studies in the later period confirmed the assumption that the use of blood preparations prior to kidney transplantation was a very successful method for improved survival of renal allograft[2].

Previous research on the animal model proved that the infusion of extracts of different tissues from the allogeneic donor before the tumor implantation accelerated growth of the tumor[3].

Gantt's hypothesis[4] that the immunosuppressive influence of transfusion, which was noticed in kidney transplantations, can suppress immunological functions in patients with malignant diseases and in that way contribute to the appearance of local relapse and distant metastases, had been investigated for more than 20 years in over 130 studies, most of them related to colorectal cancer, is still controversial and without final conclusion.

Nor random studies[5–8], nor meta-analyses[9–12] have provided the answer of whether the allogeneic transfusion influences the outcome of treatment of colorectal cancers or not. The transfusion of allogeneic blood causes in the recipient the change in immunological response[13,14], such as the reduction of NK activities and T lymphocytic blastogenesis, and the increase of suppres-

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sive T lymphocytic activity, which can, it is assumed, reduce the resistance to the infection or lead to dissemination of malignant cells.

The studies of the influence of allogeneic blood transfusion on specific changes in immunological functions were carried out. The following was observed: reduced secretion of interleukin 2 (IL-2)[15], reduced activity of natural killer cells (NK cells)[16], reduced proportion of T lymphocyte helper cells (CD 4+ cells) / cytotoxic T lymphocytes (CD 8+ cells) – CD 4/CD 8 proportion[14], reduced macrophagic functions[17], and reduction of postponed type of oversensitivity[18].

Clinical syndromes, whose mechanism is still to be defined, connected with transfusions of allogeneic blood in medical literature are called allogeneic blood transfusion – associated immunomodulation (TRIM)[19].

The goal is to investigate whether there is connection in patients operated on account of colorectal cancer, between the appearance of local relapse and periods of disease free-survival with the transfusion and the quantity of perioperative blood compensation, and to investigate whether there are changes in indicators of general immunity which are related to perioperative blood compensation.

Review of the databases showed that thus far not one analysis of the influence of perioperative allogeneic blood compensation in patients operated on account of colorectal cancer has analyzed the influence of preoperative immunological stage on the possible influence of allogeneic transfusion on the outcome of the treatment.

Patients and Methods

We analyzed the patients who were operated on account of colorectal cancer in the »Sisters of Mercy« Clinical Hospital in Zagreb in the period from 1995 – 1999, who in the moment of operation did not have distant metastases, were not operated on if they were in an inoperable stage or if the palliative operation had been performed. The patients who died during the postoperative period of 30 days, and those who had primary relapsing colorectal cancer were not included in the study. The research also excluded patients who in the period of one month before they were admitted received some kind of blood preparation, who had malignant tumors of other localization, autoimmune diseases, who had corticosteroid therapy, and those whose patohistological analysis determined that their resection margine is not tumor-free.

The data on the acceptance of blood derivatives in perioperative period were recorded – immediately before the surgical intervention, during the operation of colorectal cancer and in the first ten days of the postoperative period. The mentioned period is defined as perioperative period. The transfusion has been defined as reception of any other blood preparation: erythrocyte concentrate, full blood or fresh frozen plasma. The quantity of transfusion was also measured: non-reception or reception of less than 500 ml, more than 500 and less than a thousand milliliters, and more than a thousand milliliters of transfusion. Since most of the patients who received the transfusion also received a combination of several preparations, the analysis of the influence of separate blood preparations was not possible because of mixing of accompanying effects.

The operated patients were postoperatively monitored in days after the surgical intervention. During control examinations, the data about the manifestation of cancer relapse were registered local relapses and distant metastases and/or death, up to 1825 days after the operation.In some randomly chosen patients we measured the indicators of general immunity one day before the operation and on the tenth day after the operation. The scope of subjects was from 42 to 75. Stage of colorectal cancer according to Dukes, as well as the localization in those subjects, were equally represented in patients in all performed analyses.

Also, the same immunological parameters were measured in healthy subjects. The scope of healthy examinees was from 45 to 239. Immunoassays were performed at the Immunology Department at the Clinic for the Oncology and Nuclear Medicine at the »Sisters of Mercy« Clinical Hospital.Blood for the analysis of immunological parameters was taken one day before the operation, and on the tenth day after the operation. In order for the subjects to be accepted for this study, they needed to fulfill the following criteria: not to have received blood preparation transfusion one month before the beginning of the study, not to have had an intestinal obstruction, perforation, intraabdominal abscess, nor the signs of acute inflammatory disease which would influence their immunological status. All patients who were included in this study were operated by surgeons who specialized in colorectal surgery. None of the patients whose immunological indicators were measured on the tenth postoperative day showed signs of sepsis, anastomosis dehiscence, pneumonia, urological infection, or anything that would affect the immunological parameters. All patients whose general immunity indicators were measured on the tenth postoperative day received some transfusion preparation – erythrocyte concentrate, full blood or fresh frozen plasma. Prior to the operation, the values of immunological parameters between the healthy subjects and those who had colorectal cancer were compared. Since all patients received some kind of blood preparation, in the analysis of impact of transfusion on immunological status the patients were divided in respect to the median of received blood preparations. Out of the indicators of general immunity, functional and morphological changes were measured:

- the number of leukocytes, percentage and total number of granulocytes, lymphocytes and monocytes (from standard smears of peripheral blood);
- percentage and total number of T lymphocytes and their subpopulations (CD 4 and CD 8), B lymphocytes (surface immunoglobulins) and natural killer

cells (NK cells, CD 56) by monoclonal antibodies using the method of immunofluorescence;

– functional tests for determining NK activities (cytotoxicity with ^{51}Cr-K562) and phagocytic functions of granulocytes and monocytes (fluorescence with acridine orange and live saccharomyces as target cells).

The instruments and methods used for determining immunological parameters are:

– the light microscope OPTON Axioskop (peripheral blood smears analyzed)

– surface immunocompetent cells (CD 3, CD 4, CD 8, CD 56) were determined with fluorescent microscope with the help of OPTON photomicroscope equipped with epifluorescent condenser III RS;

– the fluorescent method was used to determine phagocytic functions of granulocytes and monocytes, by coloring with acridine orange and with saccharomyces as target cells. The results were examined with OPTON photomicroscope with the use of epifluorescent condenser III RS;

– the radioactive method was used to determine NK – activity with the target cells K-562 marked with chrome ^{51}CR. Radioactivity released in the supernatant of incubation mixture was measured with LKB gamma-counter, model 1272 Clinigamma.

The process of determining fagocytic functions of granulocytes and monocytes. After the incubation of phagocytes with microorganisms, the preparations were colored with acridine orange and microscoped under the ultraviolet light. Dead microorganisms fluoresce red, and the live ones green. The level of microbicidity was determined by comparing the number of ingested dead microorganisms in relation to the total amount of ingested particles.

The process of determining the ingestion and intracellular microbicidity by fluorescence with the aid of saccharomyceses as target cells. With the addition of heparin, peripheral blood sediments, which results with the separation of plasma rich in white blood cells which are then rinsed, counted and their concentration set to a determined value. At the same time, a culture of saccharomyceses is prepared, which are then washed and their concentration set to a determined value. On the slide, with the help of a small plastic ring and vaseline, a chamber is prepared in which a prepared cell suspension is pipetted and incubated in an incubator for cell culture for 30 minutes, because of the sedimentation of cells and adherence to the base. Non-adhered cells are then rinsed, and fungus suspension is added into the chamber, then it is incubated in the incubator for cell culture, free fungi are rinsed, the cells which adhered to the glass are colored with acridine orange and then microscoped under ultraviolet light at the increase of 80x. In that way we have obtained the data on:

a) type of phagocytes (monocyte, granulocyte)

b) number of ingested fungi (ingestion index)

c) percentage of cells which fagocyted (phagocytic activity)

d) number of dead, killed fungi (percentage of microbicidity).

Determining of NK-activity. Mononuclear cells from peripheral blood were separated in Ficoll-Hypaque gradient (Pharmacia, Sweden) and incubated with ^{51}Cr-K562 target cells in RPMI cell culture (Institute for Immunology, Croatia) which contains 10 % of calf serum (Ruđer Bošković Institute, Zagreb) by a proportion of 50:1 over the period of 18 hours. Radioactivity measured with gamma counter was expressed as percentage of cytotoxicity, as explained earlier.

The impact of transfusion and the quantity of received transfusion in particular factors upon the appearance of local relapses were compared with the standard χ^2 test, with measurement of proportion of prospects and limits – the confidence interval at the level of 95%. Kaplan-Meier method was used to present the curve of periods of disease free – survival, and differences in survival were compared by using the log-rank test and Brestow test. In statistical processing the tests of rank sum were used – Wilcoxon test and Mann-Whitney U test.

Results

In the period from 1995 – 1999 on the Surgery Clinic at the Sisters of Mercy Clinical Hospital in Zagreb, 711 patients were operated on account of colorectal cancer. There were 542 (76%) patients who fulfilled the criteria, stated above, for admission into the study, while 169 (24%) of patients did not fulfill the criteria.Dukes A was found in 29.3 % (n=159), Dukes B in 37.8 % (n=205), and Dukes C in 32.8 % (n=178) of patients.

The average reception of erythrocyte concentrate per patient was 699.5 ml (median 580 ml), the reception of full blood was 214 ml (median 0 ml), and fresh frozen plasma 325 ml (median 190 ml).Statistically important connection between the manifestation of local relapse and transfusion was discovered (χ^2=11.774, df=1, p< 0.0001, Odds ratio – 5.139, CI 95% 1.833–14.412), and also that the quantity of received transfusion is statistically significantly connected with the manifestation of local relapses (χ^2=14.09, df=2, p=0.001). As for transfusion, there was significant manifestation of local relapses for rectum and rectosigmoid cancer (χ^2=4.836, df=1, p=0.036, Odds ratio 3.581, CI 95 % 1.072–11.967), and even more significant with regard to the quantity of received transfusions (χ^2=9.766, df=2, p=0.008). There is important connection between the manifestation of local relapses in the transfusion for Dukes A in the data processing with χ^2 test and obtained p value (χ^2=4.282, df=1, p=0.045), but that is not confirmed by proportion of prospects and the reliability interval of 95% (Odds ratio 6.66, CI 95 % 0.856–51.666). Also, transfusions are statistically marginally related to the manifestation of local relapses in Dukes B (χ^2=4.301, df=1, p=0.53, Odds

ratio 6.586, CI 95% 0.862–50.38). In Dukes C there was no statistical connection found between the manifestation of relapse and transfusion (χ^2=2.477, df=1, p=0.171, Odds ratio 3.153, CI 95% 0.704–14.114). In evaluation of statistical significance of local relapses in respect to the quantity of transfusions, there was significance in Dukes A (χ^2=9.551, df=2, p=0.008), while in Dukes B (χ^2=4.156, df=2, p=0.125) and Dukes C (χ^2=4.016, df=2, p=0.134) no significant connection was found.There was significant shortening of disease free – survival period in patients who received transfusions (p=0.0069; log rank, p=0.0139; Brestow signif) (Figure 1).

There was significant shortening of disease free – survival period in the increase of the quantity of received transfusion (p=0.008; log rank, p=0.0139; Brestow signif) (Figure 2).

There was considerably shorter of disease free – survival period in case of transfusion for rectum cancer

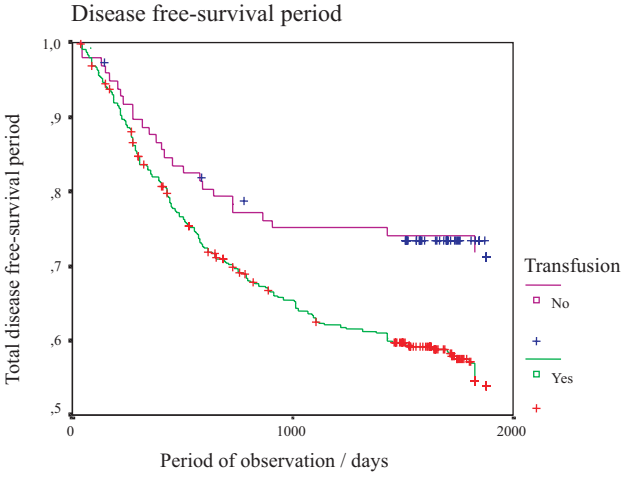

Fig. 1. Curve – disease free – survival period with regard to transfusion.

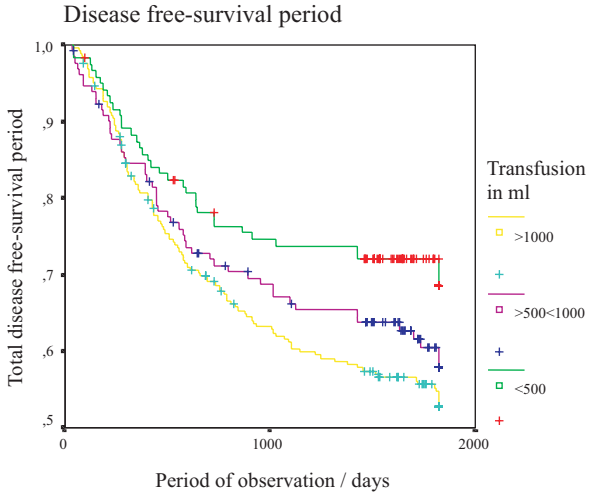

Fig. 2. Curve – disease free – survival period with regard to quantity of received transfusion.

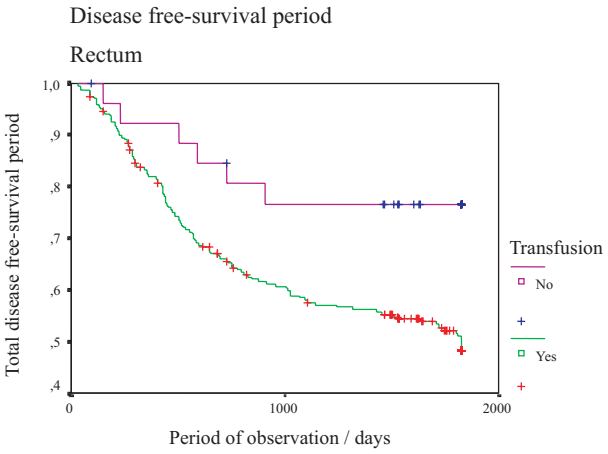

Fig. 3. Curve – disease free – survival period with regard to transfusion for rectum cancer.

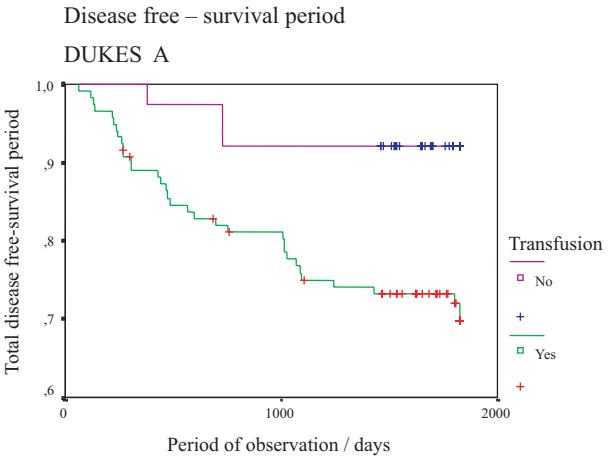

Fig. 4. Curve – disease free – survival period with regard to transfusion for Dukes A.

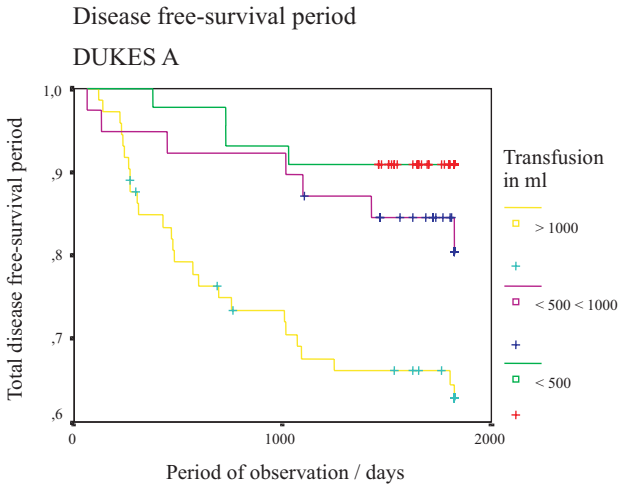

Fig. 5. Curve – period of recurrence free – survival with regard to quantity of received transfusion for Dukes A.

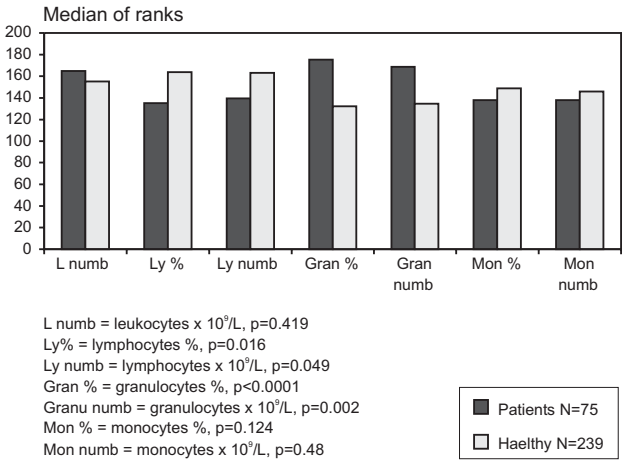

Fig. 6. Number of leukocytes, percentage and number of lymphocytes, granulocytes and monocytes (standard smear of peripheral blood) in healthy subjects and in patients before the operation, i.e. before the transfusion.

(p=0.0231; log rank, p= 0.0316; Brestow signif) (Figure 3), but not in respect to the quantity of received transfusion (p= 0.0654; log rank, p=0.0867; Brestow signif).

There was no important influence to the disease free – survival period in cases of colon cancer with transfusion (p=0.3312; log rank, p=0.3643; Brestow signif), as well as with regard to the quantity of received transfusion (p=0.7853; log rank, p=0.6309; Brestow signif). It was found that transfusion significantly shortens the disease free – survival period in Dukes A (p=0.0123; log rank, p=0.0131; Brestow signif) (Figure 4), as well as the quantity of received transfusion (p=0.0026; log rank, p=0.0021; Brestow signif) (Figure 5).

There was no influence of transfusion to the disease free – survival period for Dukes B (p=0.2098; log rank, p=0.3442; Brestow signif), as well as in respect to the quantity of received transfusion (p=0.6273; log rank, p=0.7151; Brestow signif). There was no influence of transfusion on the disease free – survival period for Dukes C (p=0.8413; log rank, p=0.8501; Brestow signif), as well as in respect to the quantity of received transfusion (p=0.8699; log rank, p=0.8338; Brestow signif).

There was significant decrease of lymphocyte percentage in patients (p=0.016), as well as of the total number of lymphocytes (p=0.049), and the increase of percentage of granulocytes (p<0.0001), as well as of the total number of granulocytes (p=0.002) (Figure 6).

By comparison of patients with healthy subjects, it was noticed that there was significant decrease of percentage of T lymphocytes – CD 3^+ (p<0.0001), of total number of CD 3^+ (p=0.002), percentage of CD 4^+ (p= 0.001), and percentage of CD 8^+ (p= 0.015) (Figure 7).

There was significantly greater percentage of large granulated lymphocytes in patients (p=0.024).

It was noticed that in patients, as compared to healthy subjects, there was significant decrease of percentage of granulocyte function (p=0.006), granulocytic in-

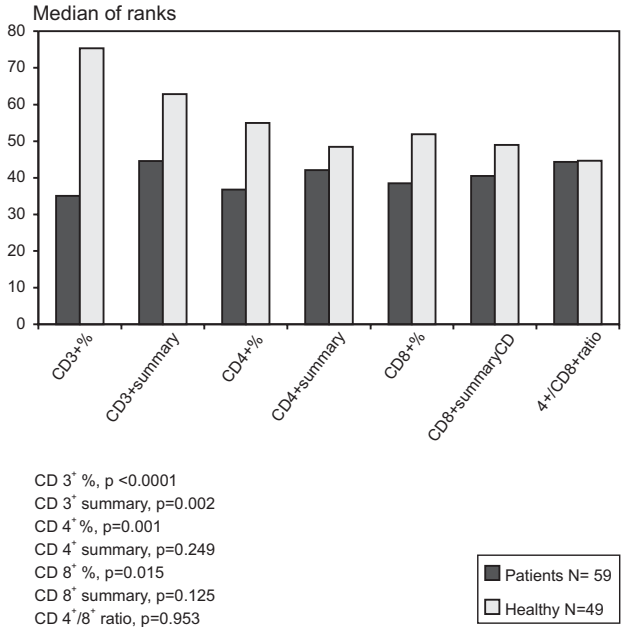

Fig. 7. T lymphocytes (CD 3^+) and their subpopulations (CD 4^+, CD 8^+) in healthy subjects and in patients before the operation, i.e. transfusion.

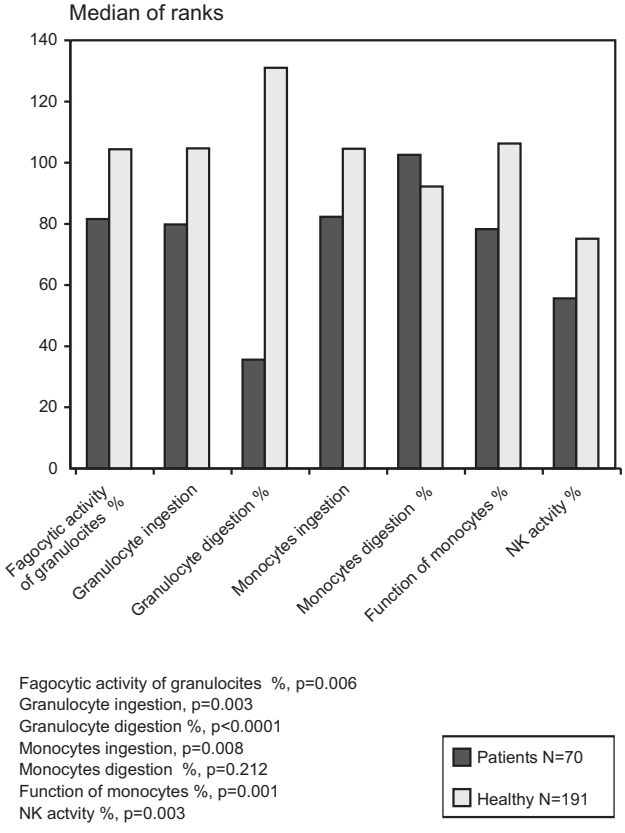

Fig. 8. Tests of phagocytic functions of granulocytes, monocytes and NK activities in healthy subjects and patients before the operation, i.e. transfusion.

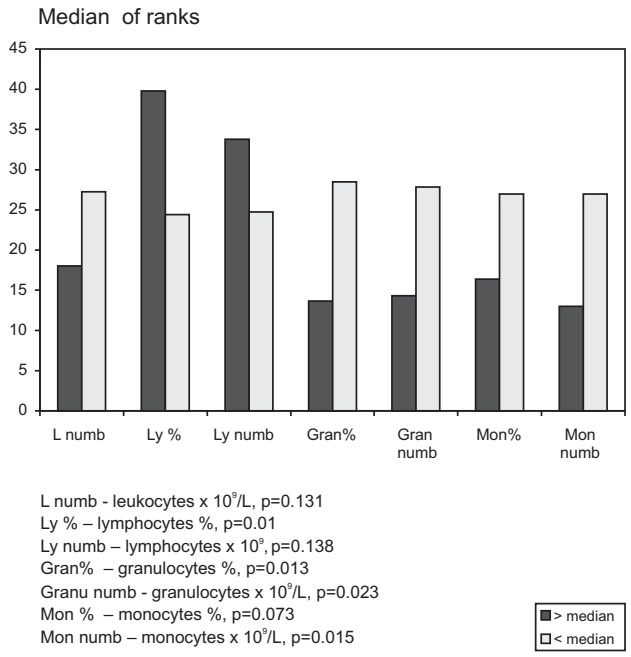

Fig. 9. Number of leukocytes, percentage and number of granulocytes, lymphocytes and monocytes (standard smear of peripheral blood) in patients after the operation, i.e. transfusion, and division in respect to the median of received transfusion in perioperative period.

gestion (p=0.003), percentage of granulocytic digestion (p<0.0001), monocytic ingestion (p=0.008), percentage of monocyte function (p=0.003), and percentage of NK activity (p=0.003) (Figure 8).

In patients who received more than a median of transfusion, there was significant decrease of percentage of lymphocytes (p=0.01), significant increase of percentage of granulocytes (p=0.013), and significant increase of total number of granulocytes (p=0.015), as well as of total number of monocytes (p= 0.015) (Figure 9).

There were no changes in values of T lymphocytes and their subpopulations (CD 4^+, CD 8^+) in patients after the operation, in respect to the median of received transfusion in perioperative period.

There was decrease of value of total number of B lymphocytes in those who received more than a median of transfusion preparations (p=0.022).

There were significantly higher values of percentage of granulocytic digestion in patients after the operation, in patients who received more than a median of blood preparations (p=0.021).

Discussion

The mechanism of immunological changes, which emerge under the influence of transfusion, has not been fully explained. Many authors believe that transfusion is connected with cellular immunosuppression[22–24].

Since the first report on the influence of transfusion on the prognosis for colorectal cancer operation[25], until today, studies often dealt only with the survival in respect to the reception or non-reception of transfusion without including into the studies the other factors which influence both the loss of blood during the operation and the manifestation of relapse of the disease. The later studies started to include the other accompanying factors in the analysis of transfusion influence. But it was the work on randomized studies that provided the clearer image of the possible influence of transfusion to the relapse of colorectal cancer. Three randomized studies compared the frequency of relapse of cancer between buffy-coat reduced allogeneic concentrate of erythrocytes and receiver of autologous full blood[7] or concentrate of erythrocytes[5] or leukocytes reduced, buffy-coat reduced, concentrate of erythrocytes[6], in patients who were operated on account of colorectal cancer. Generally, only one[6] of those three studies reported on relation between allogeneic transfusion and relapse of cancer, and where the control group comprised the patients who received leukocyte reduced concentrates of erythrocytes.Only a few studies had more than one hundred patients with Dukes A[26], and the same was reported by Beynon et al.[27] in his analysis in which he found out that there were not enough patients with Dukes A to make the final conclusion – in his analysis of 519 patients only 77 were those with Dukes A. In that work, the patients who received transfusion had the frequency of relapse 25.5 %, while in the group who did not receive transfusion this frequency was considerably lower and amounted to 6.7 %. Modin et al.[28] in 57 patients with Dukes A who had colon cancer, found the unfavorable influence of transfusion on the period of disease free – survival. According to Modin et al.[28] period of disease free – survival for a six-year period for patients who did not receive transfusion amounted to 69 %, while for patients who received transfusion it was 48 % (p < 0.05). Weiden et al.[29] in his work even found the favorable influence of transfusion in C2 stage on the relapse of disease.

Our study, which included 156 patients in Dukes A, is significant in that respect.Our results suggest that there is connection between transfusion, and particularly the quantity of received transfusion for rectum and rectosigmoid cancer with the manifestation of local relapses, more than the influence on the disease free – survival period. Localization of rectum and rectosigmoid cancer on account of the nature of its localization, and frequent forming of anastomosis deep in pelvis, opens up the question of influence of transfusion on the prognosis of disease. Data on greater connection between transfusion, and particularly the quantity of received transfusion to the manifestation of local relapses, more than to the final prognosis for disease, suggests that there is greater influence of the surgical operation and more aggressive manipulation of tumor in the narrow pelvis area, by which the transfusion is just an indicator of such work, than TRIM influence. The same conclusion was reached by Busch et al.[30] and Brand et al[26].

The immunological parameters in patients with colorectal cancer and in healthy subjects we compared. It was found out that there was significant deviation from normal distributions in differential blood count in patients with colorectal cancer, in comparison with healthy subjects. It can be seen that the number of leukocytes is not significantly different between patients with colorectal cancer and healthy subjects, while there is significant decrease of percentage of lymphocytes (p= 0.016), the total number of lymphocytes (p= 0.049), and increased percentage of granulocytes (AP < 0.0001), as well as the total number of granulocytes (p= 0.001) (Figure 6).The inconsiderable difference in total number of leukocytes, lymphopenia and relative granulocytosis was found by Elsasser-Biele et al.[31] and Ordemann et al[32]. Goto et al.[33] also found the inconsiderable difference in the total number of leukocytes, with significant decrease in the number of lymphocytes, and even more significant decrease of percentage of lymphocytes in patients with several different malignant tumors, as compared to healthy subjects. Also, as well as in our sample, there was no significant change of the total number and percentage of monocytes[33], Ordemann et al.[32] discovered significant increase of percentage of monocytes in patients with colorectal cancer in relation to healthy subjects.

It was found out that the percentage (p<0.0001), as well as the total number (p= 0.002) of T lymphocytes (CD 3^+) were significantly reduced in patients with colorectal cancer, as compared to healthy subjects. The percentage of helper lymohocytes (CD 4^+) is significantly reduced in patients (p= 0.001), but not the total number CD 4^+ (p= 0.249). Also, the percentage of cytotoxic lymphocytes (CD 8^+) is significantly reduced in patients (p= 0.015), but not the total number (p= 0.125). The proportion of CD 4/CD 8 did not change (p= 0.953, Figure 7).

Ordemann et al.[32] discovered significant decrease of CD 4/CD 8 ratio in patients with colorectal cancer in comparison with healthy subjects (p=0.045). They also noticed that with the progress of the disease, stronger immunocompromise would appear. Goto et al.[33] did not find any significant difference between CD 4^+ cells, CD 8^+ cells and the proportion of CD 4/CD 8 between patients with cancer and healthy subjects.

It was observed that there was no significant difference in the percentage and the total amount of B-lymphocytes, between healthy subjects and patients. The percentage of large granulated lymphocytes is significantly increased in patients with colorectal cancer in comparison to healthy subjects (p=0.024), although a significant difference in the total amount (p=0.864) was not found. The percentage of NK cells is higher, but not significantly (p=0.064) in patients with colorectal cancer, while the total amount was not significantly altered (p=0.259).

Mentioned data suggest that there is a significant decrease of cell immunity in patients with colorectal cancer in comparison to healthy subjects.

The most observable changes were found by tests measuring phagocyte activity of granulocytes, monocytes and NK activity in patients in relation to healthy subjects.

The percentage of phagocyte activity in granulocytes is significantly reduced in patients (p=0.006). Likewise, the granulocyte ingestion is significantly reduced in patients (p=0.003) and the percentage of granulocytes digestion even more (AP<0.0001). It has been observed that the function of monocyte ingestion is significantly reduced in patients (p=0.008). The percentage of monocyte digestion is not significantly changed in patients in comparison to healthy subjefcts (p=0212). The percentage of monocyte function is significantly reduced in patients with colorectal cancer in comparison to healthy subjects (p=0.001).

The percentage of NK activity is significantly reduced in patients with colorectal cancer (p=0.003, Figure 8).

Many authors find that the influence of the amount of received transfusions is an even more important factor which influences the relapse of the illness in case of colorectal cancer[28,34–36]. In patients who received less than a median of blood preparations during the perioperative period no changes were observed in the total number of leukocytes in comparison to those who received more than a median of blood preparations (p=0.131). It has been observed that the percentage of lymphocytes was significantly reduced in patients who received more than a median of blood preparations (p=0.01), but the total amount of lymphocytes did not significantly change (p=0.138), while the percentage of granulocyte significantly increased (p=0.013), as well as the total amount of granulocyte in patients who received more than a median of blood preparations (p=0.023). The number of monocytes was significantly increased in patients who received more than a median of blood preparations (p=0.015, Figure 9).

No important difference was observed in subpopulations of T lymphocytes (CD 4^+, CD 8^+, ratio CD 4 / 8) in patients who received less, compared to those who received more than a median of blood preparations.A significant decrease was observed in the total amount of B lymphoytes in patients who received more than a median of blood preparations (p=0.022), while the percentage of B lymphocytes was marginally reduced (p=0.059). The total number of large granulated lymphocytes, percentage of NK cells (CD 56) and total amount NK cells (CD 56) were not significantly changed. Ziv and al.[37] also found out a significant decrease in the number of B lymphocytes in patients who received transfusion, and were operated on because of colorectal cancer in Dukes C. Also they did not find any important differences in percentage and total amount of leukocytes, lymphocytes, T lymphocytes, helper T lymphocytes, cytotoxic T lymphocytes and NK cells, in the first and the fifth week after the operation.

In our patients, who were divided in a different way, a lymphopenia with reactive granulocytosis and monocytosis was observed and, likewise, a significant decrease in the total number of B lymphocytes which appeared with the increase of the amount of transfusion.

Intake of antigens changes the flow of lymphatic cells in lymph nodes and other lymphatic organs. During 24 hours after the intake of antigens, the lymphatic reactive

clone completely disappears from the blood circulation and can be found in the area of lymph nodes. In fact, clone members accidentally enter the lymph node, but then the reaction to the antigen retains them there. Only after a couple of days, lymphocytes come out from the lymph node, mostly in the shape of lymphoblasts, and spread through the body. Mentioned facts are a possible cause for the decrease in the number of B lymphocytes, which this study observed in patients who received more than a median of blood preparations.

In the test analysis of phagocyte functions of granulocytes, monocytes and NK activity, a significant rise in the percentage of granulocyte digestion was observed in patients who have taken more than a median amount of blood preparations ($p=0.021$), while no significant results were noticed in other tests. Kaplan et al.[38] found a decrease in the proportion of CD 4/8 in patients who received an allogeneic transfusion, whereas Waymackas et al.[39] found a decrease in macrophages functions.On the whole, neither of the previous analyses has taken into consideration that patients were already immunocompromised before receiving transfusions, i.e. prior to the operation, which should be taken into account in the future studies.

It is also possible that a different level of immunocompromise before the operation, which is linked to the cancer stage, could be connected to the influence of transfusion on the outcome of the treatment. In patients with Dukes A, which are less immunocompromised before the very operation, immunocompromisation caused by allogeneic transfusion could be strongly manifested, while in patients with dissemination malignant illnesses (Dukes C) this influence would not be manifested.

In the further research an analysis of the immunological status should be carried out prior to the operation, which would be connected with the stage of disease, then patients should be divided according to the type of received transfusion – autologous / leucoreduced vs. allogeneic, and a postoperational analysis of the immunological status needs to be carried out. Furthermore, the development of the local relapses and distant metastases should be monitored, taking into account all prognostic factors which influence the course of colorectal cancer treatment. All of this would give a clearer picture of the possible immunomodulation activity of allogeneic transfusion.

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IMUNOMODULACIJSKI UČINAK PRIMANJA TRANSFUZIJE KOD KOLOREKTALNOG KARCINOMA

S A Ž E T A K

Analizirano je 542 bolesnika operiranih zbog kolorektalnog karcinoma radi procijene utjecaja primanja transfuzije na pojavu lokalnih recidiva i na period bez bolesti – preživljavanje. Istražene su promjene u pokazateljima opće imunosti i njihovu moguću povezanost sa perioperacijskom transfuzijom. Nađena je značajna povezanost pojave lokalnih recidiva i primanja transfuzije ($p<0,0001$), najizraženije kod Dukesa A ($p=0,045$), lokalizacije na rektumu i rektosigmoidu ($p=0,036$). Količina transfuzije najviše je povezana sa pojavom lokalnih recidiva kod Dukesa A ($p=0,008$), lokalizacije na rektumu ($p=0,008$). Primanje transfuzije ($p=0,0123$; log rank) značajno je povezano sa skraćenjem perioda bez bolesti – preživljavanje, najznačajnije kod Dukes A stadija ($p=0,0123$; log rank), te pri lokalizaciji na rektumu ($p=0,0231$; long rank). Analizom pokazatelja opće imunosti uočena je značajna imunokompromitacija bolesnika prije same operacije, što bi moglo djelovati na stupanj imunomodulacije uzrokovane transfuzijom, a tim i na prognozu liječenja kolorektalnog karcinoma.