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TGF-β1 AND TNF-α AFTER RED BLOOD CELL TRANSFUSION IN COLORECTAL CANCER PATIENTS

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Operations in patients with cancer are associated with blood transfusion to restore normal physiology. Blood transfusion can cause the immunomodulatory effect. There is lack of the literature data about influence of blood transfusion on the blood serum levels of cytokines TGF-β and TNF-α, though these cytokines are important in neoplasia development. *The aim* of our study was to estimate changes in the concentration of cytokines TGF-β1 and TNF-α in colorectal cancer patients' peripheral blood after surgery and allogenic red blood cell transfusion. *Methods*: Venous blood of 64 patients with colorectal adenocarcinoma in stage III was tested before and after the surgery. Concentration of cytokines TGF-β1 and TNF-α was quantitatively measured by ELISA. *Results*: TGF-β1 and TNF-α concentration significantly increased in the group of transfused colorectal cancer patients (before operation: TGF-β 10.1 ± 1.3 ng/ml, TNF-α 20.9 ± 1.7 pg/ml and after operation and RBC transfusion: TGF-β 15.9 ± 1.7 ng/ml; TNF-α 27.0 ± 2.1 pg/ml). Statistical analysis has shown that serum levels of cytokines didn't change significantly after surgery in non-transfused patients group. *Conclusion*: These results indicate that levels of multipotent cytokines TGF-β1 and TNF-α were elevated after red blood cell (RBC) transfusion in colorectal cancer patients.

***Key Words*: transforming growth factor TGF-β, tumor necrosis factor-α, red blood cell transfusion, colorectal cancer.**

Surgery is the oldest and most frequent method of primary treatment for colorectal cancer. Operations of cancer are rather complicated and usually associated with blood transfusion to restore normal physiology. There is evidence that blood transfusion associated with an increased rate of tumor recurrence and that decreased the survival of cancer patients [1–3]. However, other authors haven't found any increase in cancer recurrence after perioperative blood transfusion [4, 5].

Perioperative blood transfusions may have a deleterious effect on the survival of cancer patients, possibly because of its immunosuppressive effect [6]. Whether or not blood transfusion exerts an immunosuppressive effect on the recipient remains an area of controversy. The mechanisms of this effect are insufficiently known and not fully understood. Dzik et al. has presupposed that blood transfusion can stimulate the synthesis of the immunosuppressive cytokine TGF-β. However, we have not found the information about the changes of TGF-β after blood transfusion. Miki C et al. [1] have shown that blood transfusion increased levels of proinflammatory cytokine IL-6 in blood of colorectal cancer patients. However, we have not found literature date about the changes of another important proinflammatory cytokine TNF-α after blood transfusion.

Recently paced red blood cells (RBC) are more often used in transfusion. There are scarce scientific literary data about the influence of allogenic RBC transfusion on changes of immunosupressive TGF-β and proinflammatory TNF-α cytokines in colorectal cancer patients although these cytokines are important in neoplasia process [8–11].

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Abbreviations used: RBC – red blood cell; TGF-β – transforming growth factor-β, TNF-α – tumor necrosis factor-α.

The aim of our study was to estimate changes in the concentration of cytokines TGF-β1 and TNF-α in colorectal cancer patients' peripheral blood after surgery and allogenic RBC transfusion.

PATIENTS AND METHODS

Forty eight patients with diagnosed primary colorectal cancer in the Department of Abdominal Surgery, Institute of Oncology, Vilnius University, Lithuania were involved in the study. The study was approved by the local research ethics committee, and written informed consent was obtained from all patients.

Colorectal cancer patients' eligibility criteria are summarized in Table 1.

Table 1. Colorectal cancer patients' eligibility criteria

Inclusion criteria:	Exclusion criteria:
Age 45–65 years	Patients with active infections
Patients with histological confirmed colorectal adenocarcinoma in stage III	Patients with chronic pulmonary, chronic active hepatitis and ischemic cardiac disease
Without previous blood transfusion	Patients taking immunosuppressive drugs or on chronic anticoagulation
Parameters before surgery: Haemoglobin concentration > 100 g/L; Haematocrit > 36%; Platelet count >150 × 10^9/L	
Weight > 60 kg	

We studied patients with histologically documented colorectal adenocarcinoma in stage III ($T_{1-4}N_+M_0$) with ECOG performance status 0–2 and reasonable hematology, liver and renal functions. All patients underwent curative operation.

The median age of patients was 58 years (range of years 45–65). Among them 38 patients were men and 26 were women. All patients were divided in two groups: transfused patients group consisted of 30 persons (average age 56.5 ± 3.8 years; 18 men and 12 women) and non-transfused patients group consisted of 34 persons (average age 59.8 ± 3.0 years; 20 men and 14 women).

Transfused patients group was considered that received 1–2 packet allogenic RBC intraoperation (blood

lost > 500 ml) and that received 1–2 packet allogenic RBC within 1–5 days after the surgery. Allogenic red blood transfusions were administered after operation if haemoglobin dropped to < 100 g/L and haematocrit values decreased to < 25%.

Venous blood of cancer patients was tested before surgery (1st analysis) as well as 7 and 14 days after surgery (2nd and 3rd analysis, respectively).

RBC packets have been prepared from whole blood by removing most of the plasma and red blood cells suspended in saline-adenine-glucose-mannitol (SAGM) in Public Institution National Blood Center of Lithuania. RBC packets have been prepared according to the recommendations of the Council of Europe and leukocytes count was < $1.2 \cdot 10^9$/L.

Cytokine measurement by ELISA. Peripheral venous blood was obtained from 64 individuals and serum was separated. The samples were frozen and stored at 70 °C until assayed. Cytokines TGF-β1 and TNF-α concentrations were quantitatively measured using ELISA kits obtained from Biosource (Niveles, Belgium) according to the manufacturer's instructions. According to the manufacturer, the sensitivity of these assays is 15.6 pg/ml for TGF-β1 and 3 pg/ml for TNF-α. The optical density at 450 nm was measured with an ELISA reader (Multiskan EX, Labsystem Oy).

Hematological parameters. The following haematological indices were determined: total erythrocytes number, haemoglobin concentration, total leukocytes number, granulocyte, lymphocyte and platelet count was determined by the Abbott Cell Dyn 1700 hematology analyzer. Percent values of lymphocytes, granulocyte were determined by microscopic examination of smears in peripheral blood stained by Pappenheim method (May — Grunwald — Giemsa).

Statistical analysis. Statistical analysis was performed using the statistical software SAS version 8.2. Paired Student's t-test was used to compare the difference in cytokines and haematological parameters in peripheral blood of patients' before and after operation and transfusion. The differences of comparative values were estimated as reliable at $p \leqslant 0.05$.

RESULTS

Allogenic RBC transfusion can be related to the variations of cytokines and cellular parameters in the colorectal cancer patient's blood. Consequently, cellular parameters in peripheral blood of patients after RBC transfusion were analyzed in addition to the analysis of cytokines TGF-β1 and TNF-α concentration.

We investigated those indices after 7th (2nd analysis) and 14th (3rd analysis) days in post-operational period to estimate the parameters' changes in dynamics during stay in hospital. All parameters investigated in post-operational period were compared to the appropriate parameters detected before the treatment (1st analysis).

Statistical analysis has shown that serum levels of cytokines TGF-β1 and TNF-α didn't change significantly in 2nd and 3rd analysis after surgery in non-transfused patients group (Table 2).

Table 2. Serum levels of TGF-β1 and TNF-α in non-transfused patients' group

Concentration of cytokines	n	Analysis		
		1st	2nd	3rd
TGF-β1, ng/mL	34	11.2 ± 1.0	12.0 ± 1.2	13.2 ± 1.2
TNF-α, pg/mL	34	25.1 ± 2.9	30.0 ± 3.0	26.0 ± 2.4

Note: 1st analysis – before operation; 2nd analysis – 7 days after operation; 3rd analysis – 14 days after operation.

Leukocyte and granulocyte count also didn't change in peripheral blood of that group. Percent and absolute count of lymphocytes decreased in the 2nd analysis but returned to baseline level in the 3rd analysis (Table 3).

Table 3. Haematological parameters of peripheral blood in non-transfused patients' group

Variables	n	Analysis		
		1st	2nd	3rd
RBC 10^{12}/L	34	4.7 ± 0.11*°	4.0 ± 0.13°	4.1 ± 0.18*
HB g/L	34	143 ± 3.49*°	121 ± 3.44°	123 ± 4.45*
Platelets 10^9/L	34	270 ± 16*°	352 ± 30.4°	454 ± 44.9*
WBC 10^9/L	34	7.5 ± 0.59	7.6 ± 0.44	8.0 ± 0.78
Granulocytes %	34	63.9 ± 2.69°	72.8 ± 2.13°	71.5 ± 2.90
Granulocytes 10^9/L	34	5.3 ± 0.68	5.5 ± 0.43	6.3 ± 0.80
Lymphocytes %	34	27.4 ± 2.45°	19.7 ± 1.96°	21.7 ± 1.90
Lymphocytes 10^9/L	34	1.8 ± 0.11°	1.4 ± 0.14°	1.6 ± 0.15

°$p \leqslant 0.05$ (2nd analysis was compared to 1st analysis); *$p \leqslant 0.05$ (3rd analysis was compared to 1st analysis); 1st analysis – before operation; 2nd analysis – 7 days after operation; 3rd analysis – 14 days after operation.

TGF-β1 concentration significantly increased in the 3rd analysis, and TNF-α increased in the 2nd and 3rd analysis transfused patients group (Table 4).

Table 4. Serum levels of cytokines TGF-β1 and TNF-α in transfused patients' group

Groups	Concentration of cytokines	n	Analysis		
			1st	2nd	3rd
Common	TGF-β1 (ng/mL)	30	10.1 ± 1.3*	11.8 ± 1.5	15.9 ± 1.7*
	TNF-α (pg/mL)	30	20.9 ± 1.7*°	32.5 ± 3.1°	27.0 ± 2.1*
Patients with complication	TGF-β1 (ng/mL)	12	9.3 ± 1.0*	15.0 ± 1.7	16.9 ± 2.0*
	TNF-α (pg/mL)	12	21.5 ± 2.1°	33.0 ± 3.1°	24.5 ± 2.5
Without complication	TGF-β1 (ng/mL)	18	10.7 ± 1.3*	10.7 ± 1.3	15.0 ± 1.6*
	TNF-α (pg/mL)	18	20.5 ± 2.1*°	31.9 ± 3.2°	28.7 ± 2.5*

°$p \leqslant 0.05$ (2nd analysis was compared to 1st analysis); *$p \leqslant 0.05$ (3rd analysis was compared to 1st analysis); 1st analysis – before operation; 2nd analysis – 7 days after operation; 3rd analysis – 14 days after operation.

Leukocyte and granulocyte count significantly increased in transfused patients group however lymphocyte count decreased in that group (Table 5).

Table 5. Haematological parameters of peripheral blood in transfused patients' group

Variables	n	Analysis		
		1st	2nd	3rd
RBC 10^{12}/L	30	4.3 ± 0.08*°	3.8 ± 0.10°	3.9 ± 0.11*
HB g/L	30	122 ± 3.8*°	106 ± 2.9°	109 ± 2.7*
Platelets 10^9/L	30	323 ± 41.0*°	291 ± 25.0°	452 ± 39.4*
WBC 10^9/L	30	6.7 ± 0.55*°	9.6 ± 0.69°	9.8 ± 0.74*
Granulocytes %	30	64.1 ± 3.76*	76.0 ± 6.12	75.3 ± 2.04*
Granulocytes 10^9/L	30	4.4 ± 0.60*°	6.9 ± 0.86°	6.9 ± 0.73*
Lymphocytes %	30	28.1 ± 2.46*°	13.2 ± 1.33°	13.4 ± 1.49*
Lymphocytes 10^9/L	30	1.7 ± 0.19*°	1.3 ± 0.20°	1.4 ± 0.15*

°$p \leqslant 0.05$ (2nd analysis was compared to 1st analysis); *$p \leqslant 0.05$ (3rd analysis was compared to 1st analysis); 1st analysis – before operation; 2nd analysis – 7 days after operation; 3rd analysis – 14 days after operation.

Similar tendencies were observed in the other haematological parameters changes both in non-transfused and transfused patients group. The erythrocyte count and haemoglobin level decreased and platelet count increased significantly in the 2nd and 3rd analysis (Table 3, 5).

Postoperative complications (perineum abscess, surgery seam suppuration, perineum fistula) were observed in 15 from 64 patients: for 12 transfused patients and for only 3 non-transfused patients. The

statistical analysis of data was performed only for the transfused patients group with postoperative complications because the lack of patients with complication in non-transfused group.

The statistical analysis has shown that the concentration of cytokine TNF-α in serum of patients with complications significantly increased in the 2nd analysis and the concentration of the cytokine returned to baseline level in the 3rd analysis. The level of cytokine TGF-β1 significantly increased in the 3rd analysis (Table 4).

The concentration of cytokine TNF-α significantly increased in the 2nd and 3rd analysis in patients without complications. TGF-β1 concentration increased only in the 3rd analysis in the case (Table 4).

The similar tendency of haematological parameters changes was observed in patients both with and without complications.

DISCUSSION

The results of our study have indicated that the changes of cytokines TGF-β1 and TNF-α concentration may be determined by blood transfusion because levels of both cytokines increased markedly in the sera of patients after colorectal cancer surgery in the transfused group and changed only insignificantly in the non-transfused group.

TGF-β1 is a multipotent cytokine and a potent suppressor of immune cells. The cytokine plays an important role in late-stage carcinogenesis [10] by stimulating the invasive behavior of cancer cells, promoting neo-angiogenesis and helping cancer cells to escape surveillance by the immune system [11, 12]. Narai et al. [13] have also shown that TGF-β1 concentration was significantly higher in patients with colorectal cancer.

TNF-α, another multipotent cytokine, performs several immunologic functions and it is involved in maintaining the homeostasis of the immune system. It is known, that TNF-α, like IL-1 and IL-6, suppresses erythropoesis by direct inhibitory effects on bone marrow RBC production [14]. TNF-α (cachectin) that probably causes cachexia and anemia is increased in cancer patients and may enhance tumor growth and invasion [15], whereas after surgery cytokine TNF-α level not elevate in patients with rectal cancer [16].

Thus, cytokines TGF-β1 and TNF-α are important in cancer and their increase after allogenic RBC transfusion can influence on negative current of the disease course.

Leukocyte count increased and lymphocyte count decreased in the 2nd analysis of the transfused group patients and similar tendency remained in the 3rd analysis. Leukocytes increased at the expense of granulocytes because the total lymphocyte count was lowered. It seems reasonably to suggest that white blood cells have changed as a consequence of allogenic RBC transfusion because no significant differences between pre- and postoperative leukocyte and lymphocyte count were found in non-transfused patients. Fenwick [17] and Izbicki [18] have shown that blood transfusion is the cause of leukocytosis in critically ill patients.

Blood transfusion is associated with the restoration of haemoglobin level and erythrocyte count. Allogenic RBC transfusion was administered if after operation haemoglobin level decreased below 100 g/L. After transfusion the haemoglobin was restored to a lower than normal level and remained in the same level till 14 days after operation. Haemoglobin level, erythrocyte and platelet count showed a similar tendency of changes in the analysis of the transfused and non-transfused patients groups. It may be determined by operation stress and operation trauma.

Thus, the quantitative changes of TGF-β1 and TNF-α, leukocytes and lymphocytes in the peripheral blood of colorectal cancer patients after RBC transfusion imply that transfusion may have a negative influence on the immune system of patients.

Interestingly, we observed postoperative complications (perineal abscess, surgery seam suppuration, perineal fistula) in 12 patients from the transfused group and only in 3 patients from the non-transfused group. Postoperative complications in those patients often developed after 4–6 days. The cytokine TNF-α level increased at 7 days after surgery but it returned close the baseline level after 14 days, possibly under the effects of systemic and local anti-inflammatory treatment. The level of cytokine TGF-β1 significantly increased 14 days after operation. All the parameters studied in non-complicated patients changed similarly as in the blood transfusion patients' group. The reason might be that these post-operative complications are local processes and are not reflected in systemic changes.

The influence of RBC transfusion is complicated. It remains unclear what physiological conditions or stimuli induce postoperative complications in the transfused patients.

Thus, blood transfusion poses a difficult problem and there are still many controversial aspects related to its benefits.

Considerably, more detailed examination is needed to evaluate the influence of blood transfusion on the organism of colorectal cancer patients. Moreover, complex investigations of the other organism systems parameters of colorectal cancer patients are necessary in perspective to provide a more exhaustive answer to the problem. It may be that allogenic RBC administration is not a solution for colorectal cancer patients.

In conclusion, in our study we have found that the level of multipotent cytokines TGF-β1 and TNF-α significantly increased after RBC transfusion. The data of our study corroborate findings of other investigators who showed that allogenic RBC transfusion might exert immunomodulatory effects.

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ИЗМЕНЕНИЯ КОНЦЕНТРАЦИИ ТРАНСФОРМИРУЮЩЕГО ФАКТОРА РОСТА (ТФР-β1) И ФАКТОРА НЕКРОЗА ОПУХОЛИ (ТФР-α) ПОСЛЕ ПЕРЕЛИВАНИЯ АЛЛОГЕННОЙ ЭРИТРОЦИТАРНОЙ МАССЫ БОЛЬНЫМ КОЛОРЕКТАЛЬНЫМ РАКОМ

Операции больных онкологического профиля нередко сложные и сопровождаются переливанием крови для восстановления кровопотери. Данные литературы свидетельствуют о том, что переливание крови оказывает иммуномодулирующий эффект на организм пациентов. Однако существует недостаточно данных относительно влияния переливания крови на изменения концентраций цитокинов ТФР-β1 и ФНО-α, играющих важную роль в развитии опухолевой болезни. ***Цель*****: определение влияния переливания аллогенной эритроцитарной массы на изменения концентрации цитокинов ТФР-β1 и ФНО-α в периферической крови больных колоректальным раком после операции.** ***Методы*****: концентрацию цитокинов ТФР-β1 и ФНО-α определяли методом ELISA у 64 больных раком толстой кишки III стадии до и после операции.** ***Результаты*****: концентрация цитокинов ТФР-β1 и ФНО-α достоверно повышалась в периферической крови больных, которым после операции переливали аллогенную эритроцитарную массу (перед операцией: ТФР-β1 10,1 ± 1,3 ng/ml, ФНО-α 20,9 ± 1,7 pg/ml, после операции и переливания массы аллогенных эритроцитов: ТФР-β1 15,9 ± 1,7 ng/ml; ФНО-α 27,0 ± 2,1 pg/ml).** ***Выводы:*** **полученные результаты показали, что концентрация мультипотентных цитокинов ТФР-β1 и ФНО-α повысилась у больных колоректальным раком III стадии после переливания аллогенной эритроцитарной массы.**

Ключевые слова**: трансформирующий фактор роста-β1, фактор некроза опухоли-α, переливание аллогенной эритроцитарной массы.**