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LYMPHOCYTE COUNT IN PERIPHERAL BLOOD IS A SENSITIVE TOOL IN PRETREATMENT ASSESSMENT OF PATIENTS WITH UROLOGICAL CANCER

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Cancer, inflammation and immune surveillance recruit lymphocytes as common key cellular players. *The aim* of the study was to assess a utility of the absolute and relative lymphocyte counts (ALC and RLC) in peripheral blood of patients with urological cancer as sensitive tool in pretreatment assessment of patient, which correlates with postoperative outcome of the disease, and outlines the overall reactivity of the patient. *Materials and Methods*: We retrospectively studied correlation between lymphocyte count in peripheral blood of the patients with urological cancer (n = 789) and number of clinical parameters: cancer localization, stage of the disease, treatment outcome, complications. Mann — Whitney two-tailed test and logistic regression models were used. *Results*: Lymphocyte counts (both absolute and relative) correlate with the cancer stage, and status of the disease, allowing differentiate patients with urological cancer, from healthy individuals, and from the cancer patients after radical surgery. In patients with kidney and bladder cancer, lymphocyte count allowed differentiate the stages of the disease. Lower rate of the reactivity of the patient to the cancer treatment is accurately predicted by the ALC and RLC: those in highest quartile for lymphocytes count have shorter postoperative recovery. Patients in lowest quartile demonstrated worst postoperative performance, including cases of early postoperative mortality due to weak somatic status. *Conclusion:* The study presents evidence that pretreatment lymphocyte count in the peripheral blood of patients with urological cancer is a sensitive marker of cancer stage, and the reactivity of the patient to the cancer treatment, which can be used in the pretreatment assessment of the patient.

***Key Words*: urological cancer, lymphocytes, postoperative morbidity, reactivity, immune status assessment, treatment outcome.**

Cancer progression, chronic inflammation and strength of immune response are the phenomena tightly connected and interrelated in a diseased person [1, 2]. Each of entities either predisposes, maintains, or facilitates the progression of each of those conditions, in particular, chronic inflammation serves as basis for cancer initiation, cancer progression causes suppression of the immune system, including induction of apoptosis of T-lymphocytes [3–5], immunosuppression facilitates carcinogenesis [3]. The cellular (white blood cells) and molecular (cytokines, chemokines) mediators are common for all those three processes, which make them universal indicators of the state of the disease and reactivity of the patient.

The assessment of the patients' reactivity is an important step in managing urological malignancy, as it relates to the ability to recover after the planned treatment, have less postoperative complications and shorter stay after surgery [6]. It is well known that any modality in cancer treatment bears the immunosuppressive potential. Thus, knowledge of the basic reactivity of the patient and scope of its ability to cope with the stress caused by cancer itself and cancer treatment can be additional study in pretreatment clinical evaluation of the patient.

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Abbreviations used: ALC – absolute lymphocyte count; N/L ratio – neutrophil-to-lymphocyte ratio; RLC – relative lymphocyte count; TNM – clinical classification of tumors.

The aim of the study was to validate the absolute and relative count of lymphocytes in peripheral blood in patients prior to surgical treatment as sensitive and informative tool in pretreatment assessment of the patient, which correlates with postoperative outcome of the disease, and outlines the overall reactivity of the patient.

MATERIALS AND METHODS

We retrospectively studied the medical records of 789 patients who during 2014–2016 received surgical treatment at the Department of Urology for urological cancer. The study objects included: 1) blood analysis before treatment, in particular, absolute lymphocyte count (ALC) and relative lymphocyte count (RLC) — percentage of lymphocytes in relation to all white blood cells in peripheral blood; 2) final diagnosis at the discharge; 3) stage of cancer; 4) treatment outcome, 5) length of postoperative stay. Blood analyses were performed at the same automatic blood analyzer, the results entered into a database, and analyzed with MS Office Excel software.

The study protocol was reviewed and endorsed by the Ethics Committee.

All patients were distributed into three groups: group I — 722 patients with primary urological cancer scheduled to receive cancer treatment; group II — 67 cancer patients who had received radical cancer treatment more than 3 months ago and at the moment of this study had no signs of recurrence or progression, and were considered "recovered"; group III — 62 healthy individuals without cancer, who came to the

clinic for a regular check-up. The results of their blood analysis were considered as control group for the purpose of this study.

We used the data from these three groups to conduct mathematical analysis of correlation between the status of the disease (primary urological cancer, recovered cancer patients, and cancer-free patients) and lymphocyte counts in peripheral blood. We applied Mann — Whitney two-tailed test with a significance level of 0.05 to assess if difference exists between study groups based on the investigated countable parameters (ALC and RLC).

At the second part of our study we focused only on the patients from the group I — those with most frequent urological cancer (urinary bladder, prostate, kidney) — to test if ALC and RLC may differentiate the stage of the cancer. Table 1 presents the distribution of patients among the cancer types. Table 2 presents the distribution of patients with most frequent cancer per stage based on TNM classification.

Table 1. Localization of urological tumors in the study group

Tumors	Number of patients
Urinary bladder cancer	269
Kidney cancer	140
Prostate cancer	138
Testicular cancer	87
Penile cancer	21
Renal pelvis cancer	20
Adrenal gland cancer	7
Sarcoma	6
Ureteral cancer	4
Multiple tumors of urological organs	30
Total	722

Table 2. Distribution of patients by tumor localization and TNM stage

Localization, stage	Number of patients
Urinary bladder	
Stage I	170
Stage II	43
Stage III	32
Stage IV	24
Subtotal	269
Kidney	
Stage I	59
Stage II	35
Stage III	26
Stage IV	20
Subtotal	140
Prostate	
Stage I	1
Stage II	55
Stage III	43
Stage IV	39
Subtotal	138
TOTAL	547

We applied Mann — Whitney two-tailed test to each pair of stages (I and II, I and III, etc.) in every cancer type subgroup to assess if difference exists between study population of particular stage in each cancer type subgroup based on the ALC and RLC.

To assess the correlation between preoperative lymphocyte count and length of postoperative stay we used the data on 35 patients with kidney cancer of stage II who underwent radical nephrectomy. The model of binary logistic regression was used for this purpose.

RESULTS

Mean ALC and RLC in study groups (I, II, III) are presented in Tables 3, 4, and points at evident differences in these indexes between study groups. In particular, the lowest ALC and RLC values were observed in the group of cancer patients, and the highest — in cancer patients after radical treatment. This fact highlights the suppressive effect of the tumor on lymphopoetic branch, and stimulatory effect on myeloid lineage of hemopoesis, which maintains the inflammatory state. Surgical removal of the tumor, even in the setting of the stage IV patients with cytoreductive purpose, leads to correction of the lymphocytes count in the blood analysis, which even exceeds such of the healthy control patients (group III). Based on these data we can acknowledge that surgical removal of the tumorous tissue reduces the immunosuppressive effect of the cancer, and on other hand, alleviates the inflammatory effect of the tumor on the body.

Table 3. Mean ALC in peripheral blood in groups I–III, $\cdot 10^3$/ml

	Group	Number of patients	ALC			Standard deviation
			Minimum	Maximum	Mean	
I	Primary cancer before treatment	722	0.3	4.1	1.720	0.548
II	Cancer after definitive treatment	67	1.0	4.5	2.034	0.755
III	Healthy controls	62	1.0	3.6	1.974	0.582

Table 4. Mean RLC in peripheral blood of total white blood cell count in peripheral blood in groups I–III, %

	Group	Number of patients	RLC			Standard deviation
			Minimum	Maximum	Mean	
I	Primary cancer before treatment	722	5.8	59.7	27.890	8.647
II	Cancer after definitive treatment	67	16.7	60.0	32.888	8.874
III	Control	62	13.6	48.8	32.294	7.504

To evaluate if patients' study groups (I, II, and III) differ by mean absolute and relative level of lymphocytes in peripheral blood, we ran Mann — Whitney two-tailed test for ALC and RLC for each pair of comparison. The results are presented in Tables 5, 6.

Table 5. Results of Mann – Whitney two-tailed test for mean ALC in peripheral blood in paired study groups

Criteria	Pairs of patients' groups for comparison		
	I–II	I–III	II–III
U	10,893.500	9280.000	724.000
Expected value	14,340.000	12,547.500	700.000
Variance (U)	1,804,682.55	1,568,879.1	8818.613
p-value (two-tailed)	0.010	0.009	0.802
Alpha	0.05	0.05	0.05

Table 6. Results of Mann – Whitney two-tailed test for RLC in peripheral blood in paired study groups

Criteria	Pairs of patients' groups for comparison		
	I–II	I–III	II–III
U	9777.000	8518.000	727.000
Expected value	14,340.000	12,547.500	700.000
Variance (U)	1,811,568.207	1,574,665.593	8864.901
p-value (two-tailed)	0.001	0.001	0.778
Alpha	0.05	0.05	0.05

Table 5 data indicate that there is statistically significant difference in mean ALC count in peripheral blood between cancer patients and healthy controls ($1.72 \cdot 10^3$/ml *vs* $1.97 \cdot 10^3$/ml, $p = 0.009$), and cancer patients and those who received radical cancer treatment ($1.72 \cdot 10^3$/ml *vs* $2.03 \cdot 10^3$/ml, $p = 0.01$).

Cancer patients after radical treatment do not statistically differ in their ALC from the healthy controls ($2.03 \cdot 10^3$/ml *vs* $1.97 \cdot 10^3$/ml, $p = 0.8$).

The data from Table 6 demonstrate statistically significant difference in RLC between cancer patients and healthy controls (27.9% *vs* 32.3%, $p = 0.001$), and between cancer patients and cancer patients after radical treatment (27.9% *vs* 32.9%, $p = 0.001$). As with ALC, the RLC in cancer patients after radical treatment does not differ from such in healthy controls (32.9% *vs* 32.3%, $p = 0.78$).

In order to answer the question if ALC and RLC can differentiate clinical stages (I, II, III or IV) in each type of cancer (urinary bladder, prostate and kidney) we determined mean for each parameter, and then performed Mann — Whitney two-tailed test for each pair of clinical stages. The results are presented in Table 7.

Table 7. ALC and RLC in peripheral blood in most frequent urological cancer types per TMN stage

Cancer and stage	Patients, n	Minimum ALC, $\cdot 10^3$/ml	Maximum ALC, $\cdot 10^3$/ml	Mean ALC, $\cdot 10^3$/ml	Minimum RLC	Maximum RLC	Mean RLC, %
Urinary bladder cancer							
Stage I	170	0.300	3.900	1.777	7.500	53.000	29.529
Stage II	43	0.500	2.600	1.702	6.900	45.600	25.391
Stage III	32	0.700	3.500	1.816	11.400	39.600	25.803
Stage IV	24	0.500	3.100	1.596	8.700	43.600	23.271
Subtotal	269						
Kidney cancer							
Stage I	59	0.800	2.900	1.861	16.200	59.700	31.880
Stage II	35	0.900	2.600	1.654	15.400	45.000	27.786
Stage III	26	0.900	3.300	1.762	14.300	43.600	27.986
Stage IV	20	0.400	2.900	1.555	7.400	44.400	22.690
Subtotal	140						
Prostate cancer							
Stage I	1	–	–	1.6	–	–	26.7
Stage II	55	0.600	3.000	1.669	9.200	54.200	28.782
Stage III	43	0.700	4.100	1.799	14.100	53.400	29.395
Stage IV	39	0.700	3.400	1.544	12.700	42.500	26.372
Subtotal	138						
TOTAL	547						

The data from Table 7 indicate that in all urological malignancies with progressing clinical stage one can observe the drop in absolute and relative number of lymphocytes in peripheral blood, favoring rise in neutrophil granulocytes. These data support the notion that progression of cancer occurs against progressing immunosuppression, reflected in declining level of lymphocytes.

The results of Mann — Whitney two-tailed test assessing the significance of difference in ALC and RLC between stages I through IV in each type of cancer are presented in Tables 8–10 in binary fashion ("Yes" — there is difference, "No" — there is no difference).

Table 8. Difference in ALC and RLC between clinical stages of urinary bladder cancer cases

Cancer stage	I		II		III		IV	
	ALC	RLC	ALC	RLC	ALC	RLC	ALC	RLC
I	–	–	No	Yes	No	Yes	No	Yes
II	–	–	–	–	No	No	No	No
III	–	–	–	–	–	–	No	No
IV	–	–	–	–	–	–	–	–

Note: RLC proved to be a valid criterion allowing differentiate patients with stage I bladder cancer from all others. To date, patients with bladder cancer of stage II–IV had RLC lower than in stage I.

Table 9. Difference in ALC and RLC between clinical stages of prostate cancer cases

Cancer stage	I		II		III		IV	
	ALC	RLC	ALC	RLC	ALC	RLC	ALC	RLC
I	–	–	–	–	–	–	–	–
II	–	–	–	–	No	No	No	No
III	–	–	–	–	–	–	No	No
IV	–	–	–	–	–	–	–	–

Table 10. Difference in ALC and RLC between clinical stages of kidney cancer cases

Cancer stage	I		II		III		IV	
	ALC	RLC	ALC	RLC	ALC	RLC	ALC	RLC
I	–	–	Yes	Yes	No	Yes	Yes	Yes
II	–	–	–	–	No	No	No	Yes
III	–	–	–	–	–	–	No	No
IV	–	–	–	–	–	–	–	–

Note: ALC and RLC in patients.

From the data presented in Table 8, we can conclude that for patients with bladder cancer the mean ALC may not differentiate stages of the cancer. Contrary, there is statistically significant difference for RLC between stage I and others stages. This means that the patients with bladder cancer of stage I have the highest RLC. Starting with stage II and up the RLC difference loses significance.

Table 9 represents the data on prostate cancer cases. Neither ALC nor RLC in peripheral blood of patients with prostate cancer play a role of valid differentiation tool between stages. As there was only one patient with stage I prostate cancer, his data could not be included in calculation.

Table 10 demonstrates that in patients with kidney cancer ALC and RLC are of higher differentiating value, in particular, ALC allows differentiate stages I and II, and I and IV stage, while RLC allows differentiate stages I and all others (II, III and IV), and stages II and IV.

DISCUSSION

In routine clinical practice we use different qualitative and quantitative criteria and parameters which describe the somatic condition of the patient. We need to know how fit the patient is for the treatment. We also need to know how reactive the patient is, meaning how much resources patient has for recovering from the trauma caused by cancer treatment, how probable the complications are and what would be the anticipated length of postoperative stay.

It is commonly accepted and validated in the literature that ALC and neutrophil-to-lymphocyte (N/L) ratio are valid prognostic factors for survival in cancer patients [7–12]. The cut-off value for ALC is above $1.3 \cdot 10^3$/ml, and for N/L ratio is below 2.4. The normal range for RLC in peripheral blood is above 19%. Falling ALC or rising N/L ratio (dropping RLC) are considered as predictors of poor prognosis, cancer progression, recurrence, or unfavorable treatment outcome. This statement reflects the notion that development of cancer occurs against lymphocytopenia and neutrophilia. Neutrophils are the cells producing pro-angiogenic, anti-apoptotic and diverse growth factors, whereas lymphocytes are cells yielding innate and adoptive immunity against cancer cells.

Another research has long time ago postulated and validated [13] that absolute and relative counts of lymphocytes in peripheral blood are reliable indicators of reactivity of the organism and strength of immune system response required for combatting the disease. It was demonstrated, that "stress" (reaction of the physiological systems to the extreme forces affecting the body) causes extreme lymphopenia below the cut-off threshold of 1.3 • 10^3/ml for ALC, or 19% for RLC. Whereas factors less extreme and less stressful might lead to milder reaction of the immune system, which range from soft immunosuppression (falling ALC and RLC to above 1.3 • 10^3/ml, or 19%) to even stimulation of immune reactivity (ALC and RLC rise to the upper limits of the normal range). The countable criterion, which reflects this phenomenon is relative count of lymphocytes in peripheral blood, thus making RLC a valuable diagnostic tool readily available in clinical blood test.

Study of urinary bladder carcinoma yielded valuable understanding of how cancer and immunity interact. Bladder carcinomas have developed a mechanism to avoid immune-induced apoptosis [5]. Under normal conditions the Fas/Fas-ligand system mediates programmed cell death in cancerous tissues. Fas ligand is found primarily on T-lymphocytes and natural killer (NK) cells [14, 15]. Fas activation via binding of Fas ligand results in apoptosis of the cell bearing the receptor. Bladder carcinomas have developed a mechanism to avoid this immune response by removing Fas, effectively evading apoptosis [5]. Additionally, it has been shown that high-grade bladder cancers have developed resistance to Fas-ligand-induced apoptotic events downstream of Fas. It has been suggested that the production and secretion of soluble Fas (sFas), produced by all bladder cancer cell lines may be able to block the action of T-lymphocytes and even induce apoptosis in immune cells [3, 5], reducing level of T cells in the peripheral blood.

Assessment of the immune status is not yet a routine practice in cancer clinic. The data presented above highlight that suppression of immune surveillance promotes the development and progression of cancer, which makes the immunological study a vital diagnostic tool in the arsenal of oncologist.

The assessment of the immune status can be done at different levels of complexity: 1) basic blood analysis (which was used for this study), 2) analysis of cellular fractions (lymphocytes), functional cellular activity and humoral aspects of immune system (immunogram), 3) HLA-phenotyping of lymphocytes to assess the defects in antigen-presentation machinery [16, 17]. All of those methods deliver particular diagnostic value to the assessment of immune system at rising cost and with rising level of accuracy. It is important to combine all available diagnostic tools. Still we need to acknowledge that the simplest and always readily available test — blood analysis — delivers accurate and valid data about the strength of immune surveillance and reactivity of the patient, which may guide the clinical judgment. To illustrate this, out of our dataset, four patients with cancer of stage IV and lowest lymphocyte counts — ALC = 0.3–0.5 • 10^3/ml, and RLC% = 7.5–9.0 died in 3–4 weeks after surgery. At the same time, most of the patients with highest preoperative ALC and RLC had shortest postoperative stay compared to other patients after similar surgical procedures, but with worse results of ALC and RLC.

Based on this observation and on the results of our study, we can conclude that ALC and RLC in peripheral blood of patients with urological cancer are efficient and valid criteria, which allow to differentiate patients with urological cancer from those who were radically treated from cancer, and from those who are healthy. For the purpose of differentiating stages of the cancer, ALC and RLC are valid tools in some cases, such as: RLC differentiates stage I bladder cancer from the rest of stages; in kidney cancer ALC differentiates stage I and stage II, and I and IV, RLC — stage I from all others, and stage II from IV.

The reactivity of the patient's immune system converts into outcome of the cancer treatment. The patients with highest preoperative ALC and RLC performed best and had shortest postoperative stay, and those with lowest ALC and RLC demonstrated poorest results of postoperative stay and recovery, including four postoperative deaths during month after surgery for urological cancer.

CONCLUSION

ALR and RLC in peripheral blood are valid and sensitive tests easily available in everyday clinical practice, correlating with the cancer stage and reflecting the progress, or status of the disease. The results of these tests allow to differentiate patients with urological cancer from healthy individuals, and from the cancer patients after radical surgery. Lymphocyte counts (both absolute and relative) are higher in patients after radical cancer treatment than in healthy controls, which signify immune-stimulative effect of radical surgical excision of the tumor and reduction of its immunosuppressive effect. In patients with kidney and bladder cancer, lymphocyte count allows differentiate the stages of the disease. The reactivity of the patient to the cancer treatment is accurately predicted by the ALR and RLC: those in highest quartile for ALC and RLC (above 2 • 10^3/ml, and above 32%, respectively) have shorter postoperative recovery and no postoperative complications. Patients with lowest ALC and RLC (below 1.3 • 10^3/ml, and below 19%, respectively) demonstrated worst postoperative performance, including cases of early postoperative mortality due to weak somatic status.

REFERENCES

1. Grivennikov S, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell 2010; **140**: 883–99.

2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; **144**: 646–74.

3. Thompson DB, Siref LE, Feloney MP, ***et al.*** Immunological basis in the pathogenesis and treatment of bladder cancer. Expert Rev Clin Immunol 2015; **11**: 265–79.

4. Mizutani Y, Yoshida O, Miki T. Adriamycin-mediated potentiation of cytotoxicity against freshly isolated bladder cancer cells by autologous non-activated peripheral blood lymphocytes and tumor infiltrating lymphocytes. J Urol 1999; **162**: 2170–5.

5. Perabo FG, Kamp S, Schmidt D, ***et al.*** Bladder cancer cells acquire competent mechanisms to escape Fas-mediated apoptosis and immune surveillance in the course of malignant transformation. Br J Cancer 2001; **84**: 1330–8.

6. Clavien PA, Barkun J, de Oliveira ML, ***et al.*** The Clavien-Dindo classification of surgical complications: five-year experience. Ann Surg 2009; **250**: 187–96.

7. Kim HS, Ku JH. Systemic inflammatory response based on neutrophil-to-lymphocyte ratio as a prognostic marker in bladder cancer. Dis Markers 2016; Article ID 8345286: 1–12.

8. Mano R, Baniel J, Shoshany O. Neutrophil-to-lymphocyte ratio predicts progression and recurrence of non-muscle-invasive bladder cancer. Urol Oncol 2015; **33**: e1–7.

9. Morgan TM. Editorial Comment to: Saroha S, Uzzo RG, Plimack ER, *et al*. Lymphopenia is an independent predictor of inferior outcome in clear cell renal carcinoma. J Urol 2013; **189**: 460.

10. Motzer RJ, Bacik J, Schwartz LH, ***et al.*** Prognostic factors for survival in previously treated patients with metastatic renal cell carcinoma. J Clin Oncol 2004; **22**: 454–63.

11. Ohno Y, Nakashima J, Ohori M, ***et al.*** Pretreatment neutrophil-to-lymphocyte ratio as an independent predictor of recurrence in patients with nonmetastatic renal cell carcinoma. J Urol 2010; **184**: 873–8.

12. Ozyalvacli ME, Ozyalvacli G, Kocaaslan R, ***et al.*** Neutrophil-lymphocyte ratio as a predictor of recurrence and progression in patients with high-grade pT1 bladder cancer. Can Urol Assoc J 2015; **9**: 126–31.

13. Garkavi LK, Kvakina EB, Kuzmenko TS. Antistress reactions of activation therapy. Reaction of activation as a way towards the health through the process of self-organization. M: Imedis, 1998. 656 p. (in Russian).

14. Gruss HJ. Molecular, structural, and biological characteristics of the tumor necrosis factor ligand superfamily. Int J Clin Lab Res 1996; **26**: 143–59.

15. Chicheportiche Y, Bourdon PR, Xu H, ***et al.*** TWEAK, a new secreted ligand in the tumor necrosis factor family that weakly induces apoptosis. J Biol Chem 1997; **272**: 32401–10.

16. Aptsiauri N, Garcia-Lora AM, Cabrera T. MHC Class I antigens in malignant cells: immune escape and response to immunotherapy. Briefs in Cancer Research 6. New York: Springer, 2013. 59 p.

17. Drannik GN, Drannik AG. Introduction in clinical immunology. Handbook for Students. K: Yuston, 2015. 200 p. (in Russian).