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The Function of Systemic Inflammatory Response Indicators in the Development of Thrombotic Problems in Malignancy

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Abstract

The work aimed to analyze the intensity of H3-type citrus in the blood of women with genital and breast cancer. Group 1 included 90 women with cancerous tumors of the uterus (26), ovaries (30), malignant neoplasms of the cervix (10), and mammary glands (24). Group 2 included 66 women who had benign tumors in the same locations. Heston levels were determined by the immunoassay method. Group 1 showed an elevated concentration of histone (from 1.4 to 2.1 ng per 1 ml), and Group 2 was normal (0.3-0.4 ng per 1 ml). Depending on the diagnosis, the concentration of citrules in patients with breast cancer was 0.3-0.9 ng per 1 ml, in those with ovarian cancer 1.3-2.1 ng per 1 ml, and in those with uterine cancer 2.2-3.0 ng per 1 ml. The parameters of C-reactive protein and D-dimer concentration were also increased at higher values of histone concentration. Increased content of neutrophils, and platelets. The presence of increased ketosis activity was found in women in group 1, with a diagnosis of uterine and ovarian cancer.

Keywords: Cancer, Thrombosis, Histone, Neutrophils, Platelets, Inflammation

Introduction

Among the cells involved in innate immunity, neutrophils are among the most important. The role of neutrophils comes down to minimizing the influence of pathogens, including bacteria, viruses, and antibodies. Neutrophils also reduce the influence of interleukin-8, as well as the factor which causes tumor necrosis processes (Lazzaretto & Fadeel, 2019). If a specific process responsible for neutrophil death is triggered, chromatin decondensation occurs. As a result, network structures are formed, which are also called neutrophil traps. These structures were discovered in 2004 (Nirmala & Lopus, 2020). Their composition includes low-concentration DNA, as well as components represented by proteins and enzymes. The compounds included in the traps include myeloperoxidase, tissue factor, and elastase (Libby *et al*., 2019). Myeloperoxidase cofactor heme is involved in the formation of HOCl, with H2O2, and Cl-, during oxidative reactions occurring in neutrophil cells. The role of elastase is limited to protein hydrolysis; this process takes place in the lysosomes that make up the neutrophils. These lysosomes are called azurophilic membranes. They are involved in the degradation of outer membrane compounds such as protein A and also neutralize virulence factors of microbes (Pertiwi *et al*., 2019). In addition, elastase can degrade the matrix of extracellular origin included in the body, including collagen and elastin. The formation of neutrophil traps is associated with the formation of citrus histone by peptidyl arginine deaminase, a type 4 enzyme (Bryk *et al*., 2019). According to recent data, histone type H3 can be regarded as the main component of neutrophil traps, which determines the intensity of the necroptosis process. Moreover, this still applies to blood and body tissues (Middleton *et al*., 2020). Citrula formation is one of the main steps in the formation of neutrophil traps, and histone type 3 is a marker determining the intensity of this process. Neutrophil trap networks affect pathogens by oxidation, including that involving HOCl. When the intensity of neutrophil trapping is disturbed, acute inflammatory processes begin, the consequence of which can be tissue perforation, the beginning of hypercoagulation, thrombosis, increased tumor formation, fibrosis, and the formation of new metastases (Yang & Liu, 2021).

Theoretical Framework or Literature Review

Local processes of thrombosis can start when the neutrophil trap network appears. These processes are pathogenic at the initial stage since they are directed against pathogens. The process of deployment of neutrophil trap networks is directly related to fibrosis, including for lungs (Döring *et al*., 2017; Tatarina, 2022). Among the causes of cancer mortality, thrombotic processes are the most common. Cancer is known to be 4-7 times more likely to cause venous thromboembolism. Bleeding after anticoagulants is 2 times more likely, as is mortality from thromboembolism of arterial origin. These figures are given in comparison with the group of patients without tumors (Streiff *et al*., 2020; Bodnar, 2022). Thrombosis at diagnosis of cancer can be related to a group of factors, such as ethnicity, age, and the presence of other pathologies. Factors include the type of tumor, the extent of the

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tumor, the location in the body, and the period since the tumor was detected. In addition, the type of therapy, the time of hospitalization, and the medications used are also influential. One of the most important factors is the level of such biomarkers as the number of blood cells (leukocytes, platelets), D-dimer concentration, and hemoglobin level (Zhou *et al*., 2021). It has been established that neutrophil traps can support procoagulants when they associate with red blood cells as well as platelets. This leads to thrombosis both in clinical cases and in laboratory experiments (Efrimescu *et al*., 2021). Traps contain proteins that are activators of the coagulation process, resulting in thrombus formation (Chen *et al*., 2021). It follows that neutrophil traps are an important component of clot formation, including in patients with cancer. Neutrophil traps can induce such processes as nontoxic. This occurs when hypoxic conditions are formed during oxidative stress. The triggering of ketosis is related to the formation of cytokines, and proteases, in tumor cells. In combination, hypoxia, and oxidative stress are the main factors of tumor induction in surrounding tissues during its development and metastasis formation (Hong *et al*., 2018). Neutrophil traps can stimulate endothelial and mesenchymal transition, which enhances formation, tumor growth, and triggers metastasis processes. In this case, there is an accumulation of neutrophil traps in the tissues adjacent to the tumor (Hedrick & Malanchi, 2021). Thus, neutrophil traps can perform migration of tumor cells, carrying out their protection. In addition, the attack on tumor cells by lymphocytes is suppressed. Enzymes included in neutrophil traps, such as elastase, metalloproteinase 9, and cathepsin are capable of destroying the membrane, the connections between cells. In this regard, the permeability of endothelial cells is increased. The formation of metastases is also promoted by the activation of endothelial cells, resulting in the recruitment of tumor cells circulating in blood and tissues (Chen *et al*., 2021; Efrimescu *et al*., 2021). One study showed that tumor-associated neutrophil traps were found in patients with a negative prognosis of the cancer course. These traps were also found in colorectal metastases and lymph nodes. The concentration of neutrophil traps was found to decrease from the center to the edge of the metastasis. At the same time, concentration indices in lymphoma and plasma had a direct correlation. With these parameters, there was a direct connection and poor survival rate of patients (Martins-Cardoso *et al*., 2020). It is known that neutrophil trap concentration is higher in patients with liver cancer than in healthy patients or patients with benign tumors of this organ (Yang & Liu, 2021). It remains unclear what role the level of H3-type citrulline in plasma, which is a marker of the nontoxic process, can play. This predetermined the relevance of this study. The work aimed to analyze the level of citrus histone type H3 concentration in the blood of patients diagnosed with uterine, ovarian, and breast cancer (Ilina-Stohniienko & Malets, 2022). The objectives of the study were: a) to establish quantitative parameters of blood form elements (erythrocytes, leukocytes, thrombocytes); b) to establish parameters of D-dimer and C reactive protein in blood plasma in women from different groups. The theoretical framework will establish the theories on which the study is based. The literature review will establish the most relevant investigations of the object of study. El marco teórico establecerá las teorías sobre las que se fundamenta el estudio.La revisión de literatura establecerá las investigaciones más relevantes del objeto de estudio./ O referencial teórico estabelecerá as teorias em que se baseia o estudo e a revisão da literatura estabelecerá as investigações mais relevantes sobre o objeto de estudo.

Materials and Methods

Study Design

The study was conducted between 2019 and 2022. The study included 156 women whose ages ranged from 31 to 73 years. All women were on routine hospitalization in an inpatient setting and awaiting surgery. The study is a controlled prospective study. The study is non-randomized.

Sampling

Group 1 consisted of 90 women diagnosed with cancer at stages 1-3. The localization of cancer was ovarian (a subgroup of 26 people), cervical and corpus uteri (30 and 10 people, respectively), and breast cancer (24 patients). Group 2 was a control group of 66 women with benign tumors of similar localization.

Criteria for Participation and Non-participation in the Study

Group 1 included women diagnosed with cancer in the indicated localizations. The diagnosis in each case was confirmed by the results of instrumental and clinical analysis. In addition, a signed written consent agreement was necessary to participate in the study. Group 2 included women with a benign tumor in the same localization who had no history of cancer. In addition, all of these women had no thrombosis or thromboembolism. No inflammatory processes leading to pathology were recorded in group 2 women. All women in this group also signed a consent agreement to participate in the study. Group 2 did not include women with an active stage of inflammation or infection or allergy to contrast agents. It did not include women who had undergone chemotherapy or had the thrombotic syndrome.

Study Methods

Blood sampling was performed in all patients, in all cases on an empty stomach 24 hours before the proposed operation. Blood sampling was performed using a dry sterile needle. The ulnar vein was chosen as the sampling site. Blood samples were placed in a test tube containing an anticoagulant (Na citrate solution, concentration 3.8%). The ratio was 9 to 1. Histone citrus type 3 levels were determined using an enzyme immunoassay. The ELISA Kit manufactured by Cayman Chemical, country of origin USA, was used for this purpose. The analysis was performed according to the manufacturer's instructions. The D-dimer level was determined using the Dimertest system (manufacturer Agen, country of origin Australia). The essence of the method is that antibodies of a specific type interact with D-dimer. D-dimer concentration can reflect the level of thrombosis development, as well as determine the intensity of fibrin polymerization. In addition, we performed a general blood test and C-reactive protein level measurement according to the standard methods.

Ethics of Research

The study was conducted following the provisions of the WHO Declaration of Helsinki. In a written contract, all women were acquainted with the essence of the study. The study adhered to the principles of ethics and morality, the patients were guaranteed anonymity and confidentiality of the data obtained.

Statistical Analysis

Statistical calculations were performed using Statistics (version 10). Since the data were normally distributed, nonparametric statistical methods were used. Mann-Whitney and Kruskal-Wallis criteria were calculated. The significance level was assigned as $p \leq 0.05$. Spearman correlation was used to calculate possible relationships between the parameters. Coefficient values greater than 0.7 are considered high correlation.

Results and Discussion

We found no statistically significant differences between age and body mass index between women from both groups **(Table 1)**.

Table 1. Indicators of women in groups 1 and 2 based on clinical examinations and medical history

Indicator name	Group 1 (90 women)	Group 2 (66 women)
Age, average, minimum, maximum	48.4 (32-70)	46.8 (33-69)
Indicators of body mass index, kg per 1 m^2	24.9 (17-37)	24.1 (16-36)
Concomitant pathologies, number of patients		
Hypertension	28	22
Diabetes mellitus	2	0
Asthma (bronchial type)	2	0
Heart irregularities, irregular rhythm	8	6
Chronic liver disease	12	0
Chronic kidney disease	8	4
Obesity	18	10
Anemia	20	0

There were many obese women in both groups. An especially high number of such women were in groups 1 and 2 among endometrial and mammary gland tumors. The maximum number of patients with anemia was recorded among the women from group 1, including endometrial and ovarian tumors. The laboratory parameters of the patients from the two groups are presented in **Table 2**.

Table 2. Results of laboratory blood tests for women from two groups

Parameter name, average, maximum, minimum	Group 1	Group 2
D-dimer level, μg per 1 ml	1.64 (0.5-3.1) *	0.38 (0.2-0.7)
Number of neutrophils, units 10^9 per 1 liter	5.0 (3.3-8.8)	3.4 (1.7-5.5)
Platelet count, units of 10^9 per 1 liter	255.8 (173-414) *	191.4 (116-287)
Histone type H3 level, μg per 1 ml	1.75 (0.2-3.6) *	0.33 (0.1-0.7)
The ratio of neutrophils to lymphocytes	6.2 (1.7-15.6) *	1.9 (1.3-3.1)
C-reactive protein level, μg per 1 dL	3.6 (0-9) **	0.21 (0-0.3)

Note * - $p \leq 0.05$; ** - $p \leq 0.01$.

It was found that there were significant differences ($p \leq 0.05$) between the two groups in D-dimer and histone H3 concentrations. In group 1, the concentration of D-dimer was 1.64 μg per 1 mL, whereas in group 2 it was 0.38 μg per 1 L ($p \leq 0.05$). For histone, these values were 1.75 ng per 1 mL in group 1 and 0.33 ng per 1 mL in group 2 ($p \leq 0.05$). The ratio of neutrophils to lymphocytes in group 1 was 6.2, whereas in group 2 it was 1.9 ($p \leq 0.05$) the number of platelets was also significantly different, $255.8*10^9$ per 1 L in group 1 and 1 in group 1 and 1 in group 2 ($p \leq 0.05$). In addition, C-reactive protein levels were also significantly different between the groups: 3.6 mg per 1 dL in group 1 and 0.21 mg per 1 dL in group 2 ($p \leq 0.01$).

Histone-type H3 concentrations were significantly different between groups, as shown by the Mann-Whitney test (p=0.001). Whereas 95% of the women in group 1 had histone H3 concentrations of 1.4 to 2.0 ng per 1 ml, a similar number of women in group 2 had histone H3 concentrations of 0.3 to 0.4 ng per 1 ml **(Figure 1)**.

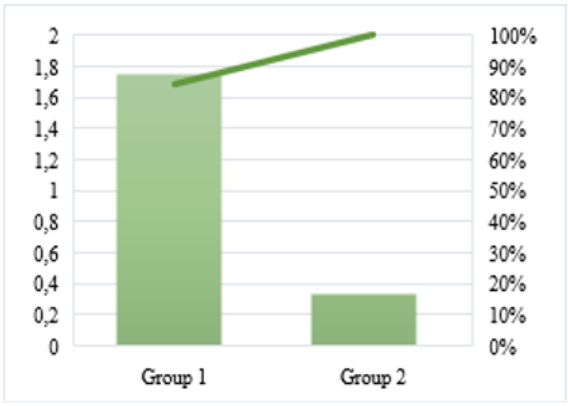

Figure 1. Histone H3-type concentrations in the blood of women from the two study groups

There are also differences between the subgroups of Group 1. While women with uterine oncology had histone H3 concentration values ranging from 2.2 to 3.0 ng per 1 ml, patients with ovarian oncology had values of 1.3-2.1 ng per 1 ml. Patients with breast cancer had the lowest values (0.3-0.8 ng per 1 mL, $p \leq 0.05$ with other subgroups, $p \geq 0.05$ with Group 2), **(Figure 2, Table 3)**.

Figure 2. Histone H3 blood levels in various localizations of the neoplasm

Table 3. Histone type H3 plasma levels in women in group 2 and subgroups of group 1

Group and subgroup number	Histone-type H3 concentration	95% confidence interval
2	0.33 ± 0.03	0.29-0.38
1.1 (uterine cancer)	2.62 ± 0.14*,**	2.1-3.0
1.2 (ovarian cancer)	1.69 ± 0.18*,**	1.4-2.0
1.3 (breast cancer)	0.60 ± 0.11	0.4-0.8

Notes. * - p≤0.05 between groups 1 and 2, ** - p≤0.05 between subgroups 1.2, 1.1 та 1.3.

It was found that there were significant Spearman correlation values (p=0.001) between the Histone H3 level and all other parameters (C-reactive protein, neutrophil, and platelet counts and their ratio, D-dimer level). High correlation values were established between C-reactive protein level and Histone type H3 (0.77), whereas for other parameters the correlation value was 0.3-0.69.

When comparing the number of platelets for women from both groups, we found that there were significant differences (Mann-Whitney test, p=0.001). Whereas in group 2 the platelet count was 177.8-203.1*109, in group 1 it was from 235.7 to 274.3*109.

Thus, the level of histone H3 type is interconnected with many parameters triggering pathological processes in the body.

In addition to protecting the body against bacteria and other agents, neutrophils play an important role in processes of neoplastic origin. In particular, neutrophils can promote the growth of cancerous tumors, as well as contribute to the formation of blood clots (Snoderly *et al*., 2019).

To date, there are no therapies that could effectively neutralize this negative aspect of neutrophils' influence on neoplastic growth and the risk of thrombosis. At the same time, when analyzing the links between the activity of neutrophils and the processes triggering cancer and thrombus formation, one can come to a better understanding of the algorithm of a new therapy based on target cells. Neutrophil traps can be linked to proliferative processes in tumors, metastases, and clots. Neutrophil traps are found in elevated concentrations in various pathologies, such as colorectal and hepatocellular cancers, as well as in vascular vasculitis of autoimmune origin (Faget *et al*., 2017; Nie *et al*., 2018; van der Windt *et al*., 2018).

Our study showed that nontoxic can significantly contribute to the development of uterine cancer, ovarian cancer, and breast cancer. At the same time, it was found that the level of H3-type histone was significantly higher in uterine and ovarian cancers compared to the control group with benign tumors. Based on the data obtained, neutrophils and neutrophil traps may influence the progression of malignant uterine, ovarian tumors. Patients diagnosed with cancer usually have leukocytosis (Lee *et al*., 2018; Wolach *et al*., 2018; Mammadova-Bach *et al*., 2019).

In tumor progression, there is a presence of neutrophils at the mature stage of the patient's blood (Scharf, 2018). It is related to the fact that neoplasm cells produce so-called granulocytic colony-stimulating factors. The latter increases the number of neutrophils and also stimulates the effect of their activity (Abdel-Razeq *et al*., 2017). Besides, high neutrophil counts can be associated with a high probability of cancer death (Lee *et al*., 2018). Experiments on healthy laboratory animals showed that neutrophil counts increased in intensity after increasing histone concentration. In the case of animals already having tumors, an increase in the number of neutrophil traps, microscopic clot formation in lungs and kidneys were noted (Reddel *et al*., 2019). Neutrophil traps are thus capable of activating platelets, which can lead to thrombus formation. The data we obtained allowed us to establish that histones of H3 type have a direct correlation with the numerical indices of neutrophils, as well as their ratio with lymphocytes. From this, we can conclude that neutrophils are the main suppliers of neutrophil traps and that ketosis plays an essential role in the progression of neoplasms.

When inflammatory processes occur at the systemic level, the concentration of C-reactive protein increases (Lee *et al*., 2020). It is known that in the preoperative period, C-protein is a prognostic factor that does not have any dependence on the stage of oncology (Suzuki-Inoue, 2019).

Cells involved in the formation of the inflammatory response can be used in the prognosis of patients diagnosed with cancer. In addition to neutrophils, these include lymphocytes, platelets, and monocytes (Anene *et al*., 2018; Bodnar *et al*., 2021). The ratio of lymphocytes and neutrophils considered in our work has already been used previously in the prognosis of the survival rate of cancer patients diagnosed with metastasis. This is due to the fact that histone H3 concentration is directly related to C-reactive protein levels. Thrombosis is the second leading cause of death in the course of oncology. Neutrophil traps can act as clot-forming factors in cancer, as they are the building material in clot formation. In breast cancer, the number of neutrophils increases, which is related to the progression of the tumor. Thrombosis begins to manifest itself when histone type H3 is already detected in blood plasma analysis (Reddel *et al*., 2019). Moreover, thrombocytosis can act as a factor determining the possibility of the formation of distant metastases, as is observed in colorectal cancer (Suzuki-Inoue, 2019). Our study showed that the number of platelets was increased in the subgroup of women with ovarian, uterine, and breast cancer compared to the control group. We also found an

association between the platelet count and the level of histone type H3 in group 1 patients. From this, we can assume that there is an association between the inflammatory process and thrombus formation. In addition, an association between high levels of D-dimer and levels of type H3 histone has been established. The D-dimer level is a factor indicating an increased likelihood of clot formation. Therefore, the presence of high concentrations of neutrophil traps may be a determinant of clot formation. In addition, neutrophil traps can be considered targets in the search for new types of anti-clotting therapy.

Conclusion

The study demonstrated that there is a difference in the level of nontoxic among women with different cancer diagnoses, such as uterine cancer, ovarian cancer, and breast cancer. Group 1 women were found to have elevated Heston H3-type concentrations, with levels ranging from 1.4-2.1 ng per 1 ml, which is higher than those in group 2 (0.3-0.4 ng per 1 ml). There were also differences in Heston levels within Group 1: patients with uterine cancer had 2.2-3.0 ng per 1 ml, those with ovarian cancer had 1.3-2.1 ng per 1 ml, while women with breast cancer had 0.3 to 0.9 ng per 1 ml. The values of the latter subgroup did not differ statistically significantly from those of Group 2 women. Increased H3-type histone concentration indices were reliably associated with increased concentration of C-reactive protein, D-dimer, as well as with numerous neutrophil, platelet, and neutrophil ratios. Group 1 was found to have more platelets compared to Group 2 (237-274*109 per 1 L and 178-203*109 per 1 L). The present study has illuminated in full the links between clot formation and tumor growth, the process itself is characterized by a large number of links. Therefore, it is necessary to conduct further studies, as their results can improve the effectiveness of cancer and thrombosis therapy.

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Ethics statement: Authors state that the research was performed using an online anonymous survey and exempt from ethical approval requirements. Informed consent was received from each participant who volunteered. Data was anonymous, and confidentiality was maintained throughout the research process. The authors further state that they followed the standards of their institutional ethical committee and the WMA Helsinki Declaration (with its latest amendments) throughout the study.

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