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Features of the State of Cellular and Humoral Markers of the Immune System in Breast Cancer among Women in Aktobe Region

Azhar Zhexenova[1], Aiman Kaldybaeva[2], Ainur Amanzholkyzy[2], Gulaim Taskozhina[3]*, Saulesh Kurmangalieva[3], Altyn Almagambetova[3], Gulnara Gubasheva[3], Gulbanu Mukyshova[1], Dina Yegisbaeva[1]

[1]Department of Pathological Physiology, Non-Commercial Joint Stock Company "West Kazakhstan Marat Ospanov Medical University", Aktobe, Kazakhstan; [2]Department of Normal Physiology, Non-Commercial Joint Stock Company "West Kazakhstan Marat Ospanov Medical University", Aktobe, Kazakhstan; [3]Departament of Clinical Laboratory and Visual Diagnostics, Non-Commercial Joint Stock Company "West Kazakhstan Marat Ospanov Medical University", Aktobe, Kazakhstan

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***Correspondence:** Gulaim Taskozhina, Department of Clinical Laboratory and Visual Diagnostics, Non-Profit Joint-Stock Company "West Kazakhstan Marat Ospanov Medical University", Maresyev str.68, Aktobe 030000, Kazakhstan. E-mail: tge77@mail.ru
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Abstract

BACKGROUND: Globally, breast cancer (BC) is considered one of the most common cancers among women and the second most common cancer worldwide. BC is also more common in developed countries, and its prevalence is increasing globally by 2% each year.

AIM: The aim of this study is to determine the features of the state of cellular and humoral markers of the immune system, as well as the relationship of specific and non-specific immunity in women in the compared groups in BC in Aktobe region.

METHODS: Statistical processing of this analysis was performed using licensed Statistica 10.0 and SPSS 25 software. Methods of descriptive statistics with calculation of central tendencies and their range for quantitative variables were used. Results were expressed as median and upper and lower quartiles. To compare the studied groups by quantitative variables, we used non-parametric Spearman's criterion for independent samples and to assess the relationship between ordinal and quantitative signs. Differences were considered statistically significant at $p < 0.05$. The study was conducted on 251 patients at Medical Center of West Kazakhstan Marat Ospanov Medical University with the established diagnosis of BC. The study period was 3 years (2018–2020). The study is funded by the Ministry of Education and Science of the Republic of Kazakhstan project IRN 0118RK01065. Two groups of patients were formed, among them in the control group – BC before chemotherapy and in the main group – BC after chemotherapy. To address the objectives, in addition to the general clinical study, all patients were conducted immune system study. Clusters of differentiation for determining cellular immunity are (CD)3+CD19-, CD3-CD19+, CD4+CD8-, CD4-CD8+, IRI, CD3+HLA-DR+, NK (CD16+56+), CD3+/CD16+56+; to determine humoral immunity, the content of JgM, JgG, and JgA was determined.

RESULTS: Correlation analysis revealed a strong positive relationship between IRI and T helper (CD4+CD8-) ($r = 0.79$; $p \leq 0.05$) and a strong negative correlation between T cytotoxic lymphocyte (CD4-CD8+) and IRI ($r = -0.8$; $p \leq 0.05$) in both patient groups. The ratio of the two T- cell subpopulations CD4+/T cell CD8+ is at the regulatory T-cell level (IRI). A direct median correlation between T lymphocyte (CD3+CD19-) and T killer (CD3+/CD16+56+) $r = 0.35$; ($p \leq 0.05$) was found, which indicates an integral regulatory role of natural killer cells in immune system function. The following correlation analysis showed a weak negative association between cellular and humoral immunity in the control group of women between T cytotoxic lymphocytes (CD4-CD8+) and IgG ($r = -0.2$; $p \leq 0.05$). Further, the relationship revealed between cellular and humoral immunity in the main group between IGM and NK cells ($r = -0.2$; $p \leq 0.05$).

CONCLUSION: It can be assumed that identifying the possibility of altering the antitumor immune response before and after chemotherapy in patients with a primary breast tumor may set the stage for its early detection and application of targeted chemoprophylaxis.

Introduction

Worldwide, breast cancer (BC) is considered one of the most common types of cancer among women and the second most common cancer worldwide. BC is also more common in developed countries, with an annual increase of 2% worldwide [1], [2]. In recent years, BC has been regarded as a group of diseases containing at least 21 distinct histological subtypes and four major molecular subtypes that consistently correlate with distinctive clinical manifestations and/or outcomes [3]. BC is the most common malignant neoplasm in women and is defined as a heterogeneous disease at the molecular level. In recent years, it has become obvious that BC is an immunogenic tumor [4], [5].

Recent studies have shown that the growth of most malignant tumors is accompanied by significant disturbances in various parts of the body's immune response [6]. The immune system is active in BC, playing a dual role in tumor progression and in immune surveillance. Immune cell infiltration is both a prognostic factor and an indicator of response to standard BC treatments [7]. BC is a complex and heterogeneous disease in which the body's immune response plays a significant role. The measurement of T-infiltrating lymphocytes has been proposed as a powerful new

tool for predicting the early evolution of BC, especially in HER2-positive and triple-negative subtypes [8], [9].

In connection with the active introduction of immunological approaches into clinical practice in the treatment of cancer patients, it is relevant to search for immunological disorders in the structure of immunocompetent cells, including the linear structure of lymphocytes and their T-cell link [10], [11].

It is believed that the immune system recognizes the tumor process, forms specific antibodies and a whole pool of specific cytotoxic immunocompetent cells, which is an important condition for the activation and implementation of antitumor immunity [12], [13]. Innate and adaptive immunity cells (NK, NKT, and T cells) participate in the immune response to a tumor. These populations are heterogeneous and contain both cells with antitumor activity and regulatory (suppressor) cells that suppress the immune response and promote tumor progression [14], [15], [16]. The interaction between a malignant tumor and the immune system of a tumor carrier is described in modern literature by the term “immune editing” [17]. A fundamental aspect of the pathogenesis of cancer is recognized as a change in the immune status. Presumably, the tumor is able to induce immunosuppression, which can lead to the ineffectiveness of antitumor immunotherapy [18]. The close relationship between the mechanisms of non-specific resistance of the body and the activity of the immune system is currently beyond doubt [19]. Thanks to the widespread use of modern methods of treatment, there is an improvement in survival rates among women with this diagnosis. Despite significant advances in the treatment of patients, early diagnosis of this disease remains a serious problem [20].

The purpose of this study is to determine the characteristics of the state of cellular and humoral markers of the immune system, as well as the relationship of specific and non-specific immunity in women in the compared groups for BC in the Aktobe region.

Materials and Methods

Study design

This was a cross-sectional study.

Inclusion criteria

I–III stages of BC (T1-3, N0-2, and M0).

18–65 years old.

Exclusion criteria

- Women with BC of all ages with Stage IV
- Under 18 years old and over 65 years old
- A study of 251 patients at the Marat Ospanov Medical Center of WKMU with a diagnosis of BC was carried out. The study period was 3 years (2018–2020).

Authors declare the absence of conflict of interest. The study is funded by the Ministry of Education and Science of the Republic of Kazakhstan project IRN 0118RK01065. Two groups of patients were formed, of which in the control group - patients with BC before chemotherapy and in the main group - patients with BC after chemotherapy. The study was carried out in the scientific molecular genetic laboratory on the basis of the Scientific and Practical Center of the WKMU named after Marat Ospanov. The project was approved by the local ethical committee of the university (approval number: No. 20 dated 09/11/2017). Written informed consent was obtained from all study subjects. Immunological research methods included the determination of lymphocyte subpopulations by the level of expression of membrane antigens of lymphocytes using a set of monoclonal antibodies specific to the differentiation antigens CD3+CD19-, CD3-CD19+, CD4+CD8-,CD4-CD8+, IRI, CD3+HLA-DR+, NK (CD16+56+), and CD3+/CD16+56+. Samples were analyzed on a FacsCalibur flow cytometer (Becton Dickenson, USA) using the CellQuest software. Software The CellQuest FacsCalibur flow cytometer analyzes up to 50,000 cells in one sample simultaneously by several parameters: Forward scatter, side scatter, and multicolor fluorescence - to conduct a multiparameter analysis of cell populations and also to determine humoral immunity was evaluated by the content of IgM, IgG, and IgA.

Statistical analysis

Statistical processing of this analysis was carried out using the licensed program Statistica 10.0. Descriptive statistics methods were used with the calculation of central trends and their range for quantitative variables. Results were expressed as median and upper and lower quartiles. To compare the studied groups by quantitative variables, non-parametric criteria were used using the Mann–Whitney U-test for independent samples, and Spearman’s rank correlation method was used to assess the relationship between ordinal and quantitative characteristics. Differences were considered statistically significant at $p < 0.05$.

Results

According to the results of the study, the average age of all patients was 58 years, while this age was different in the control group of patients. The following are the average values of all parameters in the general specified group, as well as in separate ranked groups, as: The first group is the control group of patients before

Table 1: Comparative analysis of immunological parameters in breast cancer patients before and after chemotherapy

S. No.	Parameters	Mean value		p-value
		Control group, n = 90	Main group, n = 161	p < 0.05
Age women (years)				
1	Age	60.0 (53.0; 67.0)	56.0 (47; 64)*	0.01
Humoral immunity (g/l)				
2	IgM	1.1 (0.8; 1.5)	1.2 (0.9; 1.7)	0.1
3	Ig G	13.2 (11.9; 15.3)	13.4 (11.5; 15.1)	0.7
4	Ig A	2.3 (1.7; 2.9)	2.3 (1.7; 3.2)	0.5
Cellular immunity (%)				
5	CD3+CD19-	71.7 (65.5; 7.67)	71.3 (66.1; 76.7)	0.8
6	CD3-CD19+	10.4 (7.8; 13.0)	7.9 (3.7; 12.2)*	0.001
7	CD4+CD8-	40.0 (33.2; 44.1)	40.9 (32.7; 45.0)	0.6
8	CD4-CD8+	28.6 (24.5; 36.5)	31.6 (25.9; 36.7)	0.1
9	IRI	1.3 (1.0; 1.7)	1.3 (0.9; 1.6)	0.6
10	CD3+HLA-DR+	5.9 (2.0; 12.7)	8.6 (4.5; 14.5)*	0.02
11	NK (CD16+56+)	11.3(7.4; 18.1)	11.7 (8.2; 17.0)	0.9
12	CD3+/CD16+56+	6.3 (4.3; 9.6)	5.9 (3.3; 9.1)	0.1

*- Significant differences between groups p ≤ 0.05 Me – median, Q1 – 25% lower quartile, Q3 – 75% upper quartile.

chemotherapy and the second group is the main group of patients, the components after chemotherapy.

According to the data, the mean age was 58.0 years; whereas in the control group, there were older women (60.0) than in the main group of women with BC (56.0), a significant difference was found (p = 0.01).

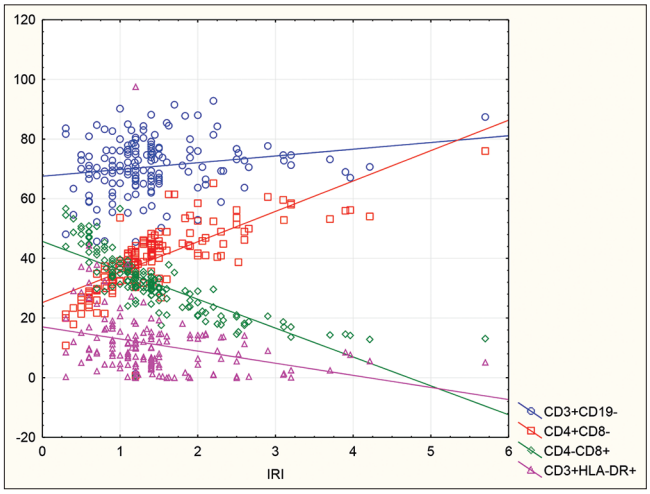

Figure 1: Correlation between IRI and subpopulations of T lymphocytes in the main group of patients

Studies of peripheral blood parameters, which were carried out before and after chemotherapy, revealed an imbalance in immunopoiesis.

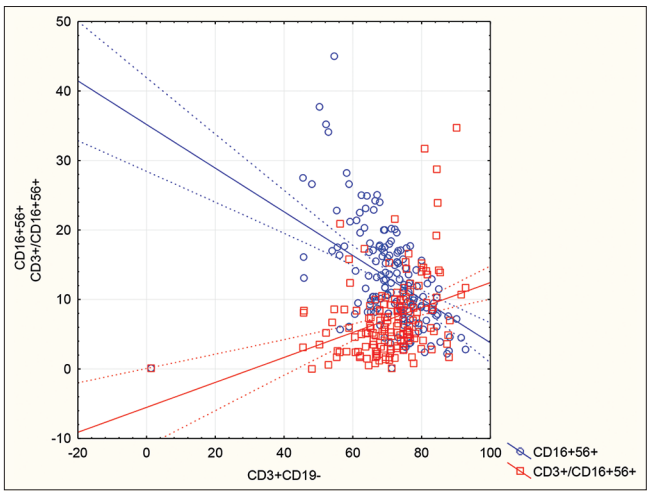

Figure 2: Correlation between T lymphocyte and killer cells in the main group of patients

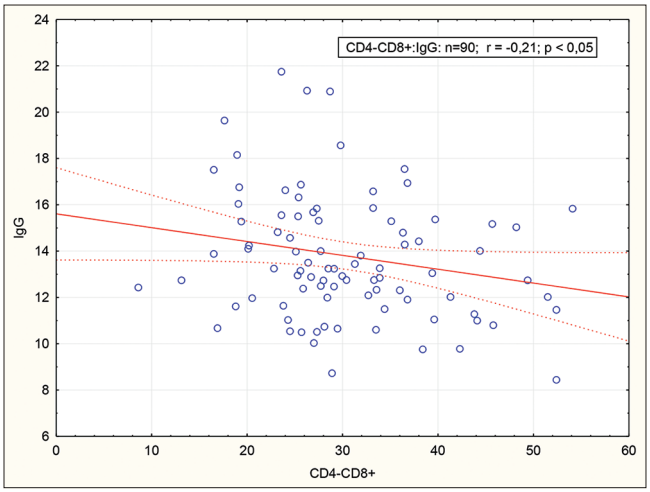

Figure 3: Correlation between T cytotoxic lymphocyte and IgG in the control group of patients

Among all examined women with BC, the results showed that the parameters of cellular immunity such as B lymphocytes clusters of differentiation (CD3-CD19+) were lower than the reference values and T cytotoxic lymphocytes (CD4-CD8+), on the contrary, there was an increase in their content. All other populations of T lymphocytes and humoral immunity were within the normal range.

As can be shown in Table 1, a comparative analysis of the parameters of humoral and cellular immunity between the control and main groups revealed significant differences in the following markers: B lymphocyte (CD3-CD19+) p = 0.001 and activated T lymphocytes (CD3+HLA+DR+) p = 0.02.

The results of our own study indicate that immunoglobulins, being at normal values, play an important role as mediators in the cascade development of the immune response and may partially determine the effectiveness of the final effector reactions of cellular immunity in inactivation and elimination of mutant cells. It is known that circulating antibodies are one of the

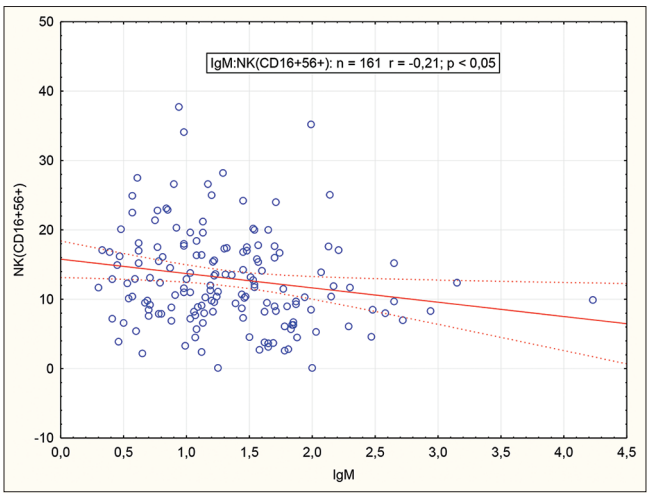

Figure 4: Correlation between T cytotoxic lymphocyte and IgM in the main group of patients

effector factors of immunity providing antigen-specific protection [21], [22].

The study of the concentration of serum immunoglobulins revealed a trend toward normal fluctuations in all groups of patients with BC, when compared, no significant differences were found. Thus, the features of changes in the humoral immunity in patients with BC were a reduced expression of B lymphocytes (CD3-CD19+) and a significant tendency to increase the T marker of cytotoxic lymphocytes (CD4-CD8+) in venous blood.

BC in women over 40 occurs much more often than in young women, from this point of view, with increasing age, the frequency of this disease increases. It is known that older patients have a less aggressive course of the tumor process than younger patients. According to many authors, the influence of age on the development of tumors largely depends on the age-related characteristics of the immune system, one of the most important factors controlling tumor growth and influencing the clinical efficacy of various types of anticancer therapy [23], [24].

In our study, there is a tendency for an age range of more than 60 years without aggressive changes in the pre-chemotherapy group. Whereas in the main group who received chemotherapy at the age of 56, there is a 2-fold change in the cellular immune response, which reacts in contrast to the control group.

T lymphocytes are those cells that are able to activate CD4-CD8+ independent mechanisms of innate immunity with the help of natural killers and macrophages. In patients with BC before chemotherapy, cytotoxic T cells (CD4-CD8+) showed themselves in the active form, and T helpers (CD4+CD8-) did not show significant changes in the immune response activity formula. Furthermore, after chemotherapy, these same patients showed activation of the anti-inflammatory response of a significant part of T lymphocytes (CD4-CD8+), which really appeared only during chemotherapy, but before the start of specific antitumor therapy (chemotherapy), these same cells were in a semi-active state.

The humoral link of immunity was studied by the expression of the CD3-CD19+ marker on B-lymphocytes and the serum concentration of the main classes of immunoglobulins – IgA, IgG, and IgM. Expression of CD3-CD19+ on B-lymphocytes was significantly higher in the main group of patients with BC compared to the control group. Thus, the relative number of B lymphocytes (CD3-CD19+) in the group of patients with BC was 1.1 times higher than in the control group p = 0.001, which is shown in the table.

In our study, in the main group of patients, strong and weak positive correlations were found between IRI and T helpers (CD4+CD8-) r = 0.8 (p = 0.0000), also T lymphocytes r = 0.2 (p = 0.03), and between IRI and T cytotoxic lymphocyte (CD4-CD8+) strong r= −0.8 (p = 0.0000) and medium negative correlation with activated T lymphocytes r= −0.3 (p = 0.0002). In this regard, it can be seen and assumed that IRI, the ratio of two subpopulations of CD4+T cells/CD8+T cells, is at the regulatory level of T cells and is the main predictor of immune surveillance and tolerance, including subpopulations that are highly correlated with each other (Figure 1).

In the scatterplot representing the relationship in the main group of patients after chemotherapy, a direct average correlation was found between T lymphocyte (CD3+CD19-) and T killer (CD3+/CD16+56+) r= 0.3; (p = 0.00001), as well as the inverse average correlation between natural killers (CD16+56+) r= −0.5; (p = 0.0000), which indicates an integral regulatory role in the functioning of the immune system by natural killers, since they combine the functions of cells involved in adaptive and innate immunity by increasing the absolute number of mature NKT-lymphocytes (CD3+CD56+CD16+), the tendency of bilateral auxiliary the response of the cells themselves as T killers and T lymphocytes (Figure 2).

A weak negative relationship was found between cellular and humoral immunity in the control group of women between T cytotoxic lymphocyte (CD4-CD8+) and IgG (r = −0.2; p ≤ 0.05), and in the main group between Ig M and NK cells (r= −0.2; p ≤ 0.05) (Figures 3 and 4).

Discussion

Among immune cells, NK cells are part of a large and diverse cluster of innate lymphoid cells. It is also known that they are able to control the growth of some tumors and can regulate the function of other immunocompetent cells: Macrophages, dendritic and endothelial cells, and B and T lymphocytes [25], [26].

In this study, a strong negative correlation was found between the number of T cells (CD3+CD19-) and the number of NK cells (CD16+56+) (r = −0.82; p ≤ 0.05) before chemotherapy, and the average correlation (r = −0.56; p ≤ 0.05) was detected after chemotherapy. The revealed negative relationship suggests that a comparative increase in the activity of T lymphocytes leads to a decrease in antitumor immunity.

Along with this, an important response of NK cells, natural killers in the mode of an adequate response to subsequent activating stimuli, is in the process of the effector response of innate immunity, that they are able to control the growth of some tumors, and can also regulate the function of other immunocompetent cells: Macrophages, dendritic and endothelial cells, and B and T lymphocytes. Accordingly, this makes it possible to identify activated NK cells (cytotoxic and

cytokine producing), which allows us to reconsider their role in the antitumor immunity system. In addition, lymphocytes are considered the main effector cells in the formation of an effective immune response. An adaptive immune response to a tumor antigen requires both T-cell cytotoxicity (CD4-CD8+) and functional T-helper activity (CD4+CD8-). A special subpopulation of lymphocytes with a high immunoregulatory potential of innate and adaptive immunity are NK cells that are stimulated without prior antigenic stimulation [27].

In addition, in oncoimmunology studies, the expansion of NKT cells is an urgent task, due to the possibility of using these cells to achieve a cytotoxic antitumor effect [28], [29].

In addition, Savchenko *et al*. (2017) noted an increase in the level of NKT cells and observed an inverse relationship between a decrease in T cytotoxic cells (CD4-CD8+) and an increase in the relative content of T regulatory cells [30] in peripheral blood in patients with kidney cancer.

According to some authors reporting that despite the decrease in the number of B cells and NK cells, the response to chemotherapy may be negative in neoadjuvant treatment of BC, as there is a decrease in the number of Treg and T cells (CD4+CD8-), but non-T cells (CD4-CD8+); in addition, the CD8/Treg ratio increases, especially in Her2+, and this, accordingly, may open up a new tool for monitoring the immune response in BC receiving chemotherapy in neoadjuvant therapy [31].

In our study, correlations were revealed – a strong positive correlation between IRI and T helper (CD4+CD8-) ($r = 0.79$; $p \leq 0.05$), and a strong negative correlation between T cytotoxic lymphocyte (CD4-CD8+) and IRI ($r = -0.8$; $p \leq 0.05$) in both groups of patients. In this regard, it can be seen and assumed that IRI the ratio of two subpopulations of CD4+CD8- T-cells/ CD4- CD8+ T-cells is at the regulatory level of T-cells and is the main predictor of immune surveillance and tolerance, including strongly correlate with each other. Basically, CD4-CD8+ T cells lyse and eliminate cancer cells directly, but after they are primed by CD4+CD8- T cells, the interaction of these T cells is part of the cancer immune cycle, which is supported by our assumptions based on the data obtained.

A weak negative relationship was found between cellular and humoral immunity in the control group of women between T cytotoxic lymphocyte (CD4-CD8+) and IgG ($r = -0.2$; $p \leq 0.05$), and in the main group between IgM and NK cells ($r = -0.2$; $p \leq 0.05$).

In addition, studies of recent years have shown that the growth of most malignant tumors is accompanied by significant disturbances in various parts of the body's immune response. The role of the immune system in the pathogenesis of oncological diseases is considered in terms of its interaction with the tumor, aimed at rejecting a malignant neoplasm, or contributing to its growth and progression. The main role in antitumor immunity is played by the cellular immune response, the key participants of which are T-lymphocytes. T-lymphocytes express differentiation antigens, which are combined into CD. Subpopulations of CD cells differ in their functions, stages of development, and activation of T-lymphocytes and can serve as immunological markers of oncogenesis, including BC [32].

In our study, in the group of patients after chemotherapy, a direct average correlation was found between T lymphocyte (CD3+CD19-) and T killer (CD3+/CD16+56+) $r = 0.35$; ($p \leq 0.05$), which indicates an integral regulatory role in the functioning of the immune system by natural killers, since they combine the functions of cells involved in adaptive and innate immunity by increasing the absolute number of mature NKT-lymphocytes (CD3+CD56+CD16+) by the tendency of bilateral auxiliary response reactions of the cells themselves as T killers and T lymphocytes.

Conclusion

With the development of the pathology of BC in the course of treatment, there are problems of the toxic effect of the chemotherapy drugs used, which entails a decrease in their dosage or even the complete abolition of chemotherapy. Ultimately, there is a failure of the chosen treatment tactics and, accordingly, undesirable results of the disease with its progression.

Monitoring of the state of the immune status suggests that cytotoxic cells, B-lymphocytes (CD3-CD19), T-lymphocytes (CD3-CD19), and serum NK levels can be used as a predictor to determine the response to chemotherapy in BC.

It can be assumed that the identification of the possibility of changing the antitumor immune response before and after chemotherapy in patients who have been diagnosed with a primary breast tumor can create prerequisites for its early detection and the use of targeted chemoprophylaxis, which can be recommended for prevention and treatment for doctors of a large circle at the level PHC, oncological hospitals, and employees of educational and scientific organizations.

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin. 2022;72(1):7-33. https://doi.org/10.3322/caac.21708.
   PMid:35020204
2. Simonian M, Ghaffari MH, Negahdari B. Immunotherapy for

breast cancer treatment. Iran Biomed J. 2021;25(3):140-56. https://doi.org/10.29252/ibj.25.3.140
PMid: 33724757

3. Jaafar M. Diversity of breast carcinoma: Histological subtypes and clinical relevance. Clin Med Insights Pathol. 2015;8:23-31. https://doi.org/10.4137/CPath.S31563
PMid:26740749
4. Zabotina TN, Chertkova AI, Borunova AA, Zakharova EN, Shoua EK, Artamonova EV, *et al*. Relationship of lymphocyte subpopulations in breast cancer patients with treatment results. Russian J Biother. 2021;20:25-33. https://doi.org/10.17650/1726-9784-2021-20-3-25-33
5. Harbeck N, Penault-Llorca F, Cortes J, Gnant M, Houssami N, Poortmans P, *et al*. Breast cancer. Nat Rev Dis Primers. 2019;5(1):66. https://doi.org/10.1038/s41572-019-0111-2
PMid:31548545
6. Oshi M, Asaoka M, Tokumaru Y, Angarita FA, Yan L, Matsuyama R, *et al*. Abundance of regulatory T cell (Treg) as a predictive biomarker for neoadjuvant chemotherapy in triple-negative breast cancer. Cancers (Basel). 2020;12(10):3038. https://doi.org/10.3390/cancers12103038
PMid:33086518
7. Cimino-Mathews A, Foote JB, Emens LA. Immune targeting in breast cancer. Oncology (Williston Park). 2015;9(5):375-85.
PMid:25979549
8. Cruz-Merino L, Chiesa M, Caballero R, Rojo F, Palazón N, Carrasco FH, *et al*. Breast cancer immunology and immunotherapy: Current status and future perspectives. Int Rev Cell Mol Biol. 2017;331:1-53. https://doi.org/10.1016/bs.ircmb.2016.09.008
PMid:28325210
9. Cruz-Merino L, Palazón-Carrión N, Henao-Carrasco F, Nogales-Fernández E, Álamo-de la Gala M, Vallejo-Benítez A, *et al*. New horizons in breast cancer: The promise of immunotherapy. Clin Transl Oncol. 2019;21(2):117-25. https://doi:10.1007/s12094-018-1907-3.
PMid:29916188
10. Farhood B, Najafi M, Mortezaee K. CD8+ cytotoxic T lymphocytes in cancer immunotherapy. J Cell Physiol. 2019;234(6):8509-21. https://doi.org/10.1002/jcp.27782
PMid:30520029
11. Speiser DE, Ping-Chih Ho PC, Verdeil G. Regulatory circuits of T cell function in cancer. Nat Rev Immunol. 2016;16(10):599-611. https://doi.org/10.1038/nri.2016.80
PMid:27526640
12. O'Donnell JS, Teng MW, Smyth MJ. Cancer immunoediting and resistance to T cell- based immunotherapy. Nat Rev Clin Oncol. 2019;16(3):151-67. https://doi.org/10.1038/s41571-018-0142-8
PMid:30523282
13. de Aquino MT, Malhotra A, Mishra MK, Shanker A. Challenges and future perspectives of T cell immunotherapy in cancer. Immunol Lett. 2015;166(2):117-33. https://doi.org/10.1016/j.imlet.2015.05.018
PMid:26096822
14. Zhang L, Liu M, Yang S, Wang J, Feng X, Han Z. Natural killer cells: Of-the-shelf cytotherapy for cancer immunosurveillance. Am J Cancer Res. 2021;11(4):1770-91.
PMid:33948388
15. Huang Y, Ma C, Zhang Q, Ye J, Wang F, Zhang Y, *et al*. $CD4^+$ and $CD8^+$ T cells have opposing roles in breast cancer progression and outcome. Oncotarget. 2015;6(19):17462-78. https://doi.org/10.18632/oncotarget.3958
PMid:25968569
16. Liu M, Meng Y, Zhang L, Han Z, Feng X. High-efficient generation of natural killer cells from peripheral blood with preferable cell vitality and enhanced cytotoxicity by combination of IL-2, IL-15 and IL-18. Biochem Biophys Res Commun. 2021;534:149-56. https://doi.org/10.1016/j.bbrc.2020.12.012
PMid:33309274
17. Mittal D, Gubin MM, Schreiber RD, Smyth MJ. New insights into cancer immunoediting and its three component phases--elimination, equilibrium and escape. Curr Opin Immunol. 2014;27:16-25. https://doi.org/10.1016/j.coi.2014.01.004
PMid:24531241
18. Siska PJ, Rathmell JC. T cell metabolic fitness in antitumor immunity. Trends Immunol. 2015;36(4):257-64. https://doi.org/10.1016/j.it.2015.02.007
PMid:25773310
19. Valachis A, Nearchou AD, Lind P. Surgical management of breast cancer in BRCA- mutation carriers: A systematic review and meta-analysis. Breast Cancer Res Treat. 2014;144(3):443-55. https://doi.org/10.1007/s10549-014-2890-1
PMid:24567198
20. Siu AL, U.S. Preventive Services Task Force. Screening for breast cancer: US preventive services task force recommendation statement. Ann Intern Med. 2016;164(4):279-96. https://doi.org10.7326/M15-2886
PMid:26757170
21. Carmi Y, Spitzer MH, Linde IL, Burt BM, Prestwood TR, Perlman N, *et al*. Allogeneic IgG combined with dendritic cell stimuli induces anti-tumor T cell immunity. Nature. 2015;521(7550):99-104. https://doi.org/10.1038/nature14424
PMid:25924063
22. Garaud S, Zayakin P, Buisseret L, Rulle U, Silina K, de Wind A, *et al*. Antigen specificity and clinical significance of IgG and IgA autoantibodies produced *in situ* by tumor-infiltrating B cells in breast cancer. Front Immunol. 2018;9:2660. https://doi.org/10.3389/fimmu.2018.02660
PMid:30515157
23. Liu X, Zheng D, Wu Y, Luo CH, Fan Y, Zhong X, *et al*. Treatment patterns and outcomes in older women with early breast cancer: A population-based cohort study in China. BMC Cancer. 2021;21(1):226. https://doi.org/10.1186/s12885-021-07947-w
PMid:33673816
24. Chertkova AI, Zabotina TN, Tsiklauri VT, Zakharova EN, Tabakov DV, Borunova AA, *et al*. Age related features of systemic antitumor immune response in patients with primary operable breast cancer and cancer of the oral mucosa. Russ J Biother. 2020;19:81-8. https://doi.org/10.17650/1726-9784-2019-19-1-81-88
25. Wu S, Fu T, Jiang Y, Shao Z. Natural killer cells in cancer biology and therapy. Mol Cancer. 2020;19(1):120. https://doi.org/10.1186/s12943-020-01238-x
PMid:32762681
26. Wu F, Xie M, Hun M, She Z, Li C, Luo S, *et al*. Natural killer cell-derived extracellular vesicles: Novel players in cancer immunotherapy. Front Immunol. 2021;12:658698. https://doi.org/10.3389/fimmu.2021.658698
PMid:34093547
27. Saraiva DP, Jacinto A, Borralho P, Braga S, Cabral MG. HLA-DR in cytotoxic T lymphocytes predicts breast cancer patients' response to neoadjuvant chemotherapy. Front Immunol. 2018;9:2605. https://doi.org/10.3389/fimmu.2018.02605
PMid:30555458
28. Khan M, Arooj S, Wang H. NK cell-based immune checkpoint inhibition. Front Immunol. 2020;11:167. https://doi.org/10.3389/fimmu.2020.00167
PMid:32117298

29. Chiossone L, Dumas PY, Vienne M, Vivier E. Natural killer cells and other innate lymphoid cells in cancer. Nat Rev Immunol. 2018;18(11):671-88. https://doi.org/10.1038/s41577-018-0061-z
PMid:30209347
30. Savchenko A, Modestov AA, Moshev AV, Tonacheva OG, Borisov AG. Flow cytometry of NK and NKT cells in patient with renal cell carcinoma. Russian J Immunol. 2014;8(17):1012-18.
31. Sánchez-Margalet V, Barco-Sánchez A, Vilariño-García T, Jiménez-Cortegana C, Pérez- Pérez A, Henao-Carrasco F, *et al*. Circulating regulatory T cells from breast cancer patients in response to neoadjuvant chemotherapy. Transl Cancer Res. 2019;8(1):59-65. https://doi.org/10.21037/tcr.2018.12.30
PMid:35116734
32. Ohue Y, Nishikawa H. Regulatory T (Treg) cells in cancer: Can Treg cells be a new therapeutic target? Cancer Sci. 2019;110(7):2080-9. https://doi.org/10.1111/cas.14069
PMid:31102428