

# Assessment of the Diagnostic Value of IL-10 and Lactoferrin by BIRADS Categories in Breast Neoplasms

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## **Absrtact**

Breast cancer is the most common cancer among women around the world. The real way to improve the results of treatment of breast tumours is an early, and in some cases, preclinical diagnosis. This problem can only be solved if complex diagnostic methods are used. The aim of our work is to determine the change in the level of IL-10 and lactoferrin in the blood serum of patients with breast neoplasms corresponding to the BIRADS categories. The sensitivity, specificity, diagnostic values of these biochemical parameters and the correlation between them were also studied. According to the results of our studies, increasing levels of BIRADS would lead to a raise in the levels of IL-10 and lactoferrin in blood serum in patients with breast tumours. The results showed that lactoferrin had a high diagnostic value compared to IL-10.

**Keywords:** Breast tumors, IL-10, lactoferrin, BIRADS

## **Introduction**

Breast cancer (BC) is a leader in the structure of oncological diseases and mortality rate among female population. Survival after treatment of breast cancer, taking into account the quality of life and the total cost of treatment, Taking the life quality and treatment high cost into account, the survival of breast cancer treatment directly depends on the stage of the oncological process. Over the course of decades, X-ray mammography has been considered almost the main both screening and refinement method in the diagnosis of breast diseases (Amiraslanov &

Gaziyev, 2010). Mammography well differentiates pathological processes in the mammary glands with a large amount of adipose tissue on the background of involution. The age-related features of the architectonics of the mammary gland make the results of mammography in young women with a glandular structure of the mammary glands less convincing. To date, the possibilities of ultrasound diagnostics in mammology are no longer disputed.

An ultrasound examination (ultrasound) of the mammary glands is a safe, affordable and highly informative method of breast examination, which is used both for preventive examination and for detection of various diseases of the mammary glands in the presence of relevant complaints (Rumack *et al.*, 2011; Zabolotskaya & Zabolotsky, 1997). To standardize the assessment of the results of mammography, magnetic resonance imaging (MRI) and ultrasound according to the degree of risk of malignant tumours of the mammary gland, the classification or BIRADS scale was proposed by the American Society of Radiology (American College of Radiology - ACR) in the late of nineties of the last century. The BIRADS classification is primarily aimed at facilitating interpretation of complex diagnostic cases in detection of neoplasms and differential diagnosis with breast cancer (American College of Radiology, 2003).

With the use of modern instrumental diagnostic methods in clinical practice, the potential of medicine in early detection of tumours has been improved and means of laboratory diagnostics have significantly increased in recent years.

It is known that the pathogenetic mechanisms of the onset and progression of tumour growth are the application of protein mediators - cytokines, chemokines and growth factors. Cytokines are secreted by both lymphoid and tumour cells, affecting many different target cells, and the cytokine network is the most important regulatory mechanism of intercellular interactions (Muftuoglu, 1993). On the one hand, cytokines manifest themselves as factors of tumour progression through activating the angiogenesis and migration of tumour cells. They change function of target cells and are involved in the mechanism of evading tumour cells from the immune surveillance system. On the other hand, cytokines can be the main mediators of antitumor immunity. The concentration and balance of the levels of cytokines and their antagonists contribute to the enhancement or inhibition of the growth of breast cancer. Changes in the relative concentration of some cytokines can directly and indirectly stimulate breast cancer (Kazakov *et al.*, 2015)

Currently, in science and medicine, much attention is paid to antimicrobial peptides (AMP). The data of numerous publications devoted to the study of AMP as molecular factors of the innate immunity system indicate that these substances

have significant therapeutic potential and can be considered as candidates for the role of not only antimicrobial drugs, but also antitumor drugs of a new type. AMP are characterized by a variety of mechanisms of cytotoxic action, which can lead to both necrosis and apoptosis of target cells. The basis of these effects is selective interaction with the membranes of tumour cells, similar in a number of structural and physiological characteristics to the membranes of microorganisms. It has also been found that AMP can inhibit tumour growth through disrupting formation of its vascular network. Like antimicrobial activity, the antitumor effect of AMP can be enhanced by modulating the body's protective functions. The described properties of AMP give hope for development of new drugs based on which they/we can overcome the resistance of tumour cells (Balandin *et al.*, 2016).

The aim of our work was to assess the level of cytokine IL-10 and lactoferrin (LF) by BIRADS categories, to evaluate sensitivity, specificity and diagnostic value of these biochemical parameters, as well as the correlation between them.

## Materials and Methods

In this work, we presented the results of a study of 92 patients undergoing examination for breast cancer at the Oncology Clinic of the Azerbaijan Medical University for the period from 2014 to 2017. The age of patients ranged from 18 to 79 years. All patients underwent an ultrasound examination with a combination of dopplerography and mammography. An ultrasound examination has been performed by using the MINDRAY D70 device (China) and a mammography was conducted by using the SIEMENS MAMMOMAT INSPIRATION device (Germany). Out of all examined patients, 48 women were malignant and 28-benign breast tumours. 16 apparently healthy women have been included into the control group. All women have been divided into 4 groups according to the BIRADS category: BIRADS1, BIRADS2, BIRADS4, BIRADS5.

In the blood of all women included in the study, serum levels of IL-10 and LF have been studied. The concentration of the studied parameters has been determined by enzyme-linked immunosorbent assay on a STAT Fax 303 Plus apparatus (USA) using a reagent kit of the VEKTOR – BEST firm (Russia) to define cytokines and Cloud-clone (China) for LF.

The ELISA method is a highly sensitive and highly specific immunodiagnostic method, with the help of which a qualitative and quantitative determination of various substances with the properties of antigen, hapten (defective antigen) or antibody is carried out. The ELISA method is widely used to diagnose infectious

and non-infectious diseases in humans and animals and can also be used to confirm the quality of biological drugs. The principle of ELISA consists in the specific interaction of an antigen and an antibody, creation of an Ag/Ab complex and a conjugate, and determination of a resulting complex according to the degree of colour by using a substrate mixture.

To calculate the sensitivity, specificity and diagnostic value of tumour markers and cytokines a “cut of point” has been determined in SOCS-20 by using ROC analysis in advance and statistical parameters calculated in binary classification with respect to this point in MS EXCEL-2013.

## Results and discussion

IL-10 is an anti-inflammatory cytokine. During infection, it inhibits the activity of Th1 cells, NK cells, and macrophages, all of which are required for optimal pathogen clearance but also contribute to tissue damage. In consequence, IL-10 can both impede pathogen clearance and ameliorate immunopathology (Couper *et al.*, 2008). As a result of our studies, it was revealed that in women included in the BIRADS 1 category, the level of IL-10 ranged from 2.8-9.5 ng/ml, averaging 6.13 ng/ml (Table 1). In the BIRADS 2 group, the level of this indicator was 3.8-16.2 ng/ml, an average of 8.77 ng/ml ( $p < 0.05$ ). In patients included in the BIRADS 4 category, the level of IL-10 averaged 6.13 ng/ml, min and max levels were respectively 5.4-19.1 ng/ml ( $p < 0.001$ ). In the BIRADS 5 category, the level of IL-10 ranged between 7.5-23.2 ng/ml, averaging 14.78 ng/ml ( $p < 0.001$ ).

Table 1. The level of IL-10 in breast tumours according to BIRADS classification

						95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Min.	Max.
IL-10	B1	16	6,13	2,20	0,55	4,96	7,30	2,8	9,5
	B2	19	8,77	3,56	0,82	7,05	10,48	3,8	16,2
	B4	26	10,47	4,34	0,85	8,72	12,22	5,4	19,1
	B5	31	14,78	4,73	0,85	13,04	16,51	7,5	23,2
	Total	92	10,82	5,09	0,53	9,76	11,87	2,8	23,2

According to our data, in women included in the BIRADS 2 group, in comparison with the control group, the level of IL-10 increased 1.43 times, and in the BIRADS

4 and BIRADS 5 groups the level of this cytokine was even higher and increased 1.7 and 2.4 times respectively.

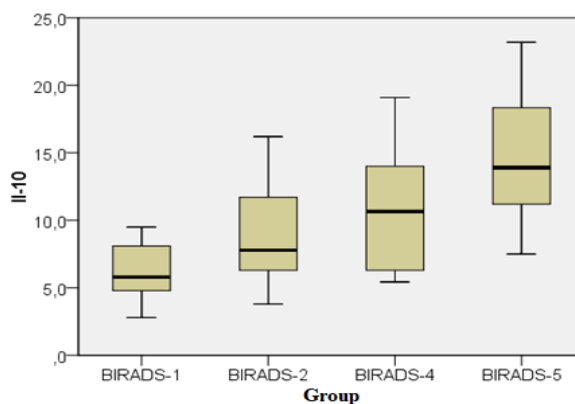


Figure 1. The level of IL-10 in neoplasms of the breast according to the classifications BIRADS

Interleukin-10, which has an important coordinated role in breast carcinogenesis (Sheikhpour & Mohiti, 2014), is an anti-inflammatory cytokine that regulates the immune response and inhibits the pro-inflammatory functions of antigen-presenting cells (APCs) through expression of antagonizing costimulatory molecules. Its low expression is associated with poor survival outcome. The role of IL-10 in breast cancer is controversial. Interleukin -10 has both pro- and anti-tumor effects. IL-10 mRNA expression is seen in more than 50% of tumor samples. Also, greater IL-10 protein concentrations are seen in serum of breast cancer patients than in that of healthy individuals and this is associated with poor clinical outcomes. Interleukin-10 promoted proliferation and metastasis of tumor cells and inhibited T-cell proliferation and function (Li *et al.*, 2014; Fernandez, 2006; Sheikhpour *et al.*, 2018).

We also determined the level of LF in the blood serum of patients with breast neoplasms according to the BIRADS categories (Table 1). In the BIRADS group 1, the LF level ranged from 0.9-5.8 ng/ml, averaging 3.26 ng/ml. In women included in the BIRADS 2 category, the average level of this indicator was 5.81 ng/ml, the interval of variation was 1.9-9.2 ng / ml. In the BIRADS 4 min and max categories, the indicators were 5.1 and 18.8 ng / ml, respectively, the average level was 10.24 ng/ml ( $p < 0.001$ ). In patients included in the BIRADS 5 group, the LF value ranged between 8.2-42.7 ng/ml, the average level was 30.84 ng/ml (Figure 2).

As compared with the control group, in women included in the BIRADS 2 group, the LF value increased 1.78 times, and in the BIRADS4 and BIRADS 5 groups the level of this indicator increased 3.14 and 9.5 times, respectively.

Table 2. LF level in case of breast neoplasms according to BIRADS classification.

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
LF	B1	16	3,26	1,37	0,34	2,53	3,99	0,9	5,8
	B2	19	5,81	2,41	0,55	4,65	6,97	1,9	9,2
	B4	26	10,24	3,66	0,72	8,76	11,72	5,1	18,8
	B5	31	30,84	9,41	1,69	27,39	34,29	8,2	42,7
	Total	92	15,05	12,97	1,35	12,37	17,74	0,9	42,7

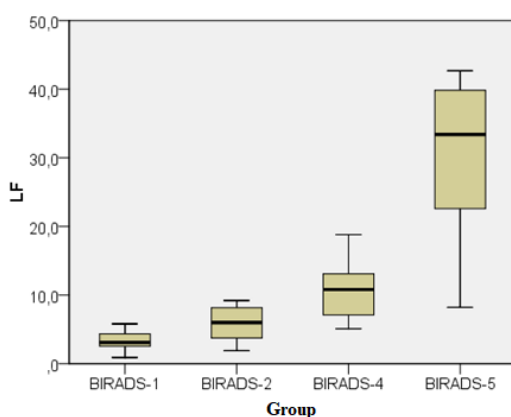


Figure 2. LF level in case of breast neoplasms according to BIRADS classification

Lactoferrin (LF) has a direct effect on cell growth, due to which it can act as a regulatory element in protecting the body from the development of tumours and metastases. The protein has a direct effect on the growth of tumour cells, which is confirmed by the slowdown or lack of splicing of its mRNA in cancer cells. LF blocks transition from G1 to S stage of the cell cycle (Borzenkova *et al.*, 2010). It adversely affects cell proliferation, causing changes in the expression and/or activity of important regulatory cell cycle proteins. By activating the signaling pathway of fatty acid synthase in cancer cells, the protein induced apoptosis. In addition, LF stimulates the production and/or activation of various immune cells

including lymphocytes and normal killer cells, and also increases the sensitivity of target cells to lysis by killer cells (Balandin, 2016; Naidu, 2010).

In our work, we also evaluated the sensitivity, specificity, and diagnostic value of IL-10 and LF. According to our data, the sensitivity of IL-10 in assessing the malignancy of a breast tumour was  $60.4 \pm 7.1\%$ , and specificity was  $78.6 \pm 7.8\%$ . The predictive usefulness of a positive result was  $82.9 \pm 6.4\%$ , the predictive usefulness of a negative result was  $53.7 \pm 7.8\%$ . The likelihood ratio of a positive result was 2.82 and was assessed as mediocre, and a negative result of 0.50 and assessed as unsuitable. The total diagnostic weight of the test was  $67.1 \pm 5.4\%$ .

In the course of studies, it was found that the sensitivity of the LF in assessing the malignancy of a breast tumour was  $83.3 \pm 5.4\%$ , and the specificity was  $85.7 \pm 6.6\%$ . The predictive usefulness of a positive result was  $90.9 \pm 4.3\%$ , the predictive usefulness of a negative results was  $75.0 \pm 7.7\%$ . The likelihood ratio of a positive result was 5.83 and a negative result of 0.19 and both parameters were rated as good. The total diagnostic weight of the test was  $84.2 \pm 4.2\%$ .

Table 2. The sensitivity and specificity of the cytokines IL-10 and LF in breast tumors

	Se, %	Sp, %	pPV, %	nPV, %	LR+	LR-
<b>IL-10</b>	60,4±7,1	78,6±7,8	82,9±6,4	53,7±7,8	2,82	0,50
<b>LF</b>	83,3±5,4	85,7±6,6	90,9±4,3	75,0±7,7	5,83	0,19

It should also be noted that in the course of our studies, a statistically significant positive correlation was observed between IL-10 and lactoferrin ( $\rho=0.645$ ,  $p<0.001$ ).

Taking into account the results of our study we can conclude that these data may serve in the diagnosis and monitoring of breast cancer treatment.

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