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LACTOFERRIN EXPRESSION IN BREAST CANCER IN RELATION TO BIOLOGIC PROPERTIES OF TUMORS AND CLINICAL FEATURES OF DISEASE

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***Aim*: To determine the patterns of lactoferrin (LF) expression in breast cancer (BC) in relation to biologic properties of the neoplasms and clinical features of the disease course. *Materials and Methods*: Clinical specimens of 266 BC patients (115 patients with BC of stages I–II — retrospective study, and 151 BC patients — prospective study) were analyzed. Morphological, immunohistochemical and statistical methods were used. *Results*: The number of LF-positive tumors in retrospective and prospective groups was similar (52.1 and 52.8%, respectively). Among common clinical criteria for prognosis of the disease outcome in BC patients (patient's age; stage of the disease; histological type, differentiation grade, receptor status; presence of metastases), a strong correlation was found only between expression indexes of LF and estrogen receptors (ER). In ER-positive tumors expression of LF was significantly higher than in ER-negative tumors (35 *vs* 18%). 5-Year survival rate of BC patients was higher in LF-positive group (70 *vs* 52% in LF-negative group). The presence of regional metastasis tended to correlate with an increased number of LF-positive tumors. In the patients with invasive ductal carcinoma, expression level of LF moderately correlated with occurrence of luminal A subtype ($r = 0.43$), while in the patients with invasive lobular carcinoma this index strongly correlated with occurrence of luminal B subtype ($r = 0.71$). LF expression correlated positively with low and moderate differentiation grade of luminal B or basal tumors, and negatively with luminal B or basal tumors of high differentiation grade ($r = -0.57$ and -0.63, respectively). *Conclusion*: It has been shown that LF expression in breast tumors correlated with life expectancy of BC patients and important physiologic and clinical features of the disease, while the character of such relation strongly depended on molecular phenotype of tumor, i.e. luminal A, luminal B or basal.**
***Key Words*: lactoferrin, breast cancer, molecular phenotype, estrogen receptors, prognosis of the disease course.**

Breast cancer (BC) is the most prevalent oncologic pathology in women. It is believed that an effectiveness of therapy of this disease could be improved by the development of new means for biologic correction of iron homeostasis as an important factor of stable functioning of all body systems. New knowledge about the role of iron in the development and progression of tumors has been gained due to the studies of the intracellular content and tissue distribution of iron-containing proteins and their receptors.

It is known that at cellular and molecular levels the regulation of iron metabolism is exerted through the balanced action of iron-containing proteins, including transporter proteins. Transport of iron in a protein-bound form minimizes its capability for participation in the reactions of free-radical oxidation, and therefore decreases possible oxidative damage of cells and tissues of an organism [1].

While the metabolic patterns of such iron-containing proteins as ferritin, transferrin, ferroportin in BC patients are studied in detail [2], the role of lactoferrin (LF) in pathogenesis, clinical course and prognosis of BC is still poorly understood. LF is an iron-binding glycoprotein of transferrin family of iron transporter proteins, with a molecular weight of 76.8 kD composed of a single polypeptide chain. LF contains 703 aminoacids and forms two homological domains, N- and C-parts of which contain an iron-binding center and have a special function [3].

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Abbreviations used: BC – breast cancer; ER – estrogen receptor; LF – lactoferrin; PR – progesteron receptor.

Different LF isoforms have been identified. LF-α is capable to bind iron ions but has no ribonuclease activity while isoforms LF-β and -γ exert ribonuclease activity but are incapable to bind metal ions [4]. Apart from this, the protein could exist in iron-saturated form (hololactoferrin) or iron-unsaturated form (apolactoferrin). One molecule of the protein could be bound with two ions of iron, copper, zinc or other metals.

Also it has been shown that some functional features of LF depend on its oligomeric state. As a monomer, LF is capable to bind with DNA and regulate granulopoiesis, while tetrameric form of LF lacks such features [5]. It has been established that the process of conformational rearrangement of LF with formation of mono-, di-, tri-, or tetrameric forms requires adenosine triphosphate [6].

In adult human body, LF is stored in neutrophils, specific (secondary) granules of which contain large amounts of LF. LF content may vary depending on gender and age, however, the results of different studies are contradictory. After degranulation of neutrophils, LF is released into the blood and is quickly bound to and absorbed by parenchymatous cells of liver and spleen. Under normal conditions, blood plasma LF concentration is 0.4–2.0 mg/l. There are some data on significant elevation of LF content in biological fluids (up to 200 mg/l) upon some pathologies, including cancer, and especially upon inflammatory states, therefore LF content could be used as a biochemical marker of inflammation [7].

An important feature of LF is its capability to bind with nucleic acids, especially with double-stranded DNA. LF is internalized with participation of LF receptor along with iron ions bound with its molecule. Also,

LF regulates the concentration of iron ions in blood and secretory fluids, exerts antimicrobial and antiviral action and is considered as an important immune factor of milk. It directly participates in defense reactions of a body and mediates the development of cellular immunity. LF interacts with polyamines and heparin. Apart from this, LF exerts antioxidant, immunomodulating and anticancer activities [8]. LF is capable to enter cell nuclei and activate transcription of specific genes, but such target genes have not been yet identified.

Therefore, LF is a protein with a wide spectrum of biologic functions. The role of LF in the clinical course of BC remains unclear. There are a few studies of LF in BC cells *in vitro*, in particular, LF increases migration and invasion of triple receptor-positive and receptor-negative BC cells [9]. Also it has been shown that LF isolated from cow milk is capable to decrease the viability of human breast carcinosarcoma HS578T cells and human ductal breast epithelial tumor T47D cells by 47 and 54%, respectively, and to induce 2-fold increased apoptosis in these cells [10]. The results of our studies have shown that exogenous LF can modify the molecular profile and invasive properties of cultured BC cells with different potential of malignancy, including drug-resistant MCF-7/Cp and MCF-7/Dox cell lines [11]. Other researchers have demonstrated that recombinant human LF variants affect the properties of tumor cells *in vitro* in a way that could be considered antitumorigenic [12, 13].

Presently there are scarce or no data on the role of LF in the pathogenesis of BC, relation between LF content and clinical, morphological and molecular-biologic characteristics of BC at different stages of the disease, as well as LF validity as prognostic criterion for clinical course of BC. Therefore, the aim of the study was to analyze the patterns of LF expression in tumor cells of BC patients taking into account their biological properties and clinical features of the disease.

MATERIALS AND METHODS

The clinical specimens of 266 BC patients was analyzed [14]. The assessment of prognostic value of LF for the disease outcome was based on a retrospective analysis of the data of 115 BC patients stages I–II treated in Kyiv Municipal Clinical Oncologic Center in 2005–2007. A prospective study of 151 BC patients treated in the same clinics in 2013–2014 was aimed at an assessment of the relation between LF expression in tumors of different molecular phenotype and clinical characteristics of BC affecting the course of the disease. All patients provide an informed written consent on the use of individual clinical data for scientific purposes.

All patients received surgical treatment (quadrant- or lumpectomy with regional lymph node dissection, radical mastectomy by Madden). The patients with BC of stages I–II were not treated with neoadjuvant therapy. Adjuvant polychemotherapy was performed: CMF (cyclophosphamide, methotrexate, fluorouracyl), CAF (cyclophosphamide, doxorubicine, fluorouracyl), 4–6 courses; radiotherapy on postoperative cicatrix and the zone of regional metastases at a total dose of 42–44 Gy. The patients with positive expression of hormone receptors in removed tumor tissue were treated with prolonged hormonal therapy by standard scheme (tamoxiphene, aromatase inhibitors) depending on the individual clinical data.

General clinico-pathological characteristics of BC patients are presented in Table 1. As one may see, the patients from both groups were of similar age, the majority of them being at menopause. By histological structure, the most common BC type was invasive ductal cancer of moderate differentiation grade. By the data of molecular-biologic study, luminal A subtype was diagnosed in the majority of BC patients from both groups.

Table 1. Clinico-pathologic characteristics of BC patients

Indexes	Retrospective group		Prospective group	
	Number of patients			
	n	%	n	%
Total number of patients	115	100	151	100
Average age, years	54.2 ± 3.1		56.5 ± 9.6	
Age range, years	23–75		28–89	
Active menstrual cycle	39	33.9	29	19.2
Menopause	76	66.1	122	80.8
Stage I (T1N0M0)	27	23.5	47	31.1
Stage II (T2N1–2M0)	88	76.5	104	68.9
T2a (T1N0M0)	53	46.0	81	53.6
T2b (T2N1–2M0)	35	30.5	23	15.3
Invasive ductal carcinoma	86	74.8	101	66.9
Invasive lobular carcinoma	29	25.2	50	33.1
Differentiation grade G1 (high)	32	27.8	42	27.8
Differentiation grade G2 (moderate)	59	51.3	78	51.7
Differentiation grade G3 (low)	24	20.9	31	20.5
Molecular subtype				
Luminal A	53	46.0	81	53.6
Luminal B	31	27.0	35	23.2
Basal	31	27.0	35	23.2

All patients were examined with the use of common clinical and laboratory methods in accordance with the standards for diagnostics and therapy of cancer patients approved by the orders of Ministry of Health No.140 of 27.07.1998 and No.554 of 17.09.2007. The stage of cancer was estimated according to International TNM classification (2008).

For morphologic research, surgically removed tumor specimens was fixed in 10% neutral formaline solution and further treated by standard histological method. The preparations were prepared from paraffine blocks, stained with hematoxylin and eosin, and examined using light microscopy.

Immunohistochemical determination of LF expression in the tumors was performed with the use of standard streptavidine-biotine-peroxydase method on histological slides prepared from paraffine blocks after their fixation in 10% solution of neutral formaline. The 4–5 mm histologic sections were placed on Super Frost Plus slides (Germany). Further procedures were performed by routine technique. Anti-LF MoAbs (Abcam, USA) were used as primary antibodies. Visualization was done using EnVision system (DakoLSAB2 system, Denmark). After detection of peroxydase activity, the slides were stained with Mayer's hematoxylin. Assessment of LF expression was performed by semiquantitative method. In each histological preparation, LF expression was analyzed per 1000 tumor cells, the number of immunopositive and immunonegative cells was expressed in percents ac-

counting for the degree of the marker expression (high, moderate, strong). If the number of immunopositive cells was higher than 10%, LF expression was considered as a strong positive one.

For immunohistochemical study of other markers we have used antibodies against estrogen receptors (ER) (clone 1D5), progesterone receptors (PR) (clone PgR636), epidermal growth factor receptor HER2/neu (clone c-erbB-2), proliferation marker Ki-67 (clone MIB-1) from DakoCytomation, Denmark. Assessment of expression of mentioned proteins in breast tumors was performed by counting immunopositive cells using H-Score method [15]. Expression from 0 to 100 scores was considered low, 101–200 — moderate, and > 200 — high. In the study light microscope Primo Star (Zeiss, Germany), magnification ×100–400, was used.

Statistical analysis of the data was done with the use of STATISTICA 6.0 program. The relation between the indexes was assessed using Pearson's coefficient, its significance analyzed with the use of χ^2 test.

RESULTS AND DISCUSSION

Immunohistochemical detection of LF in BC samples revealed a positive LF-specific reaction in cytoplasm of tumor cell in both groups of the patients. The number of LF-positive (LF+) tumors in retrospective and prospective groups did not differ and at average was equal to 52.1 and 52.8%, respectively.

In BC patients from group 1, LF expression indexes were analyzed in relation to a number of clinical characteristics each of which affecting the clinical course of the disease, in particular, age and menstrual function of the patients, the stage of the disease, histological type of the tumor, its differentiation grade and degree of malignancy, metastasis in regional lymph nodes, receptor status of tumor cells.

The results of correlation analysis evidenced on an absence of the difference in the numbers of LF+ tumors dependent on the age of patients with preserved reproductive function (24 (43.6%)) and patients at menopause (40 (45.4%)). No correlation was found between LF expression in tumor, BC stage and histological type of BC. There was observed a tendency between an increased number of LF+ tumors in the patients with high differentiated BC (19 (47.5%)) compared to these with moderately differentiated tumors (17 (42.5%)) and low differentiated BC (17 (40.4%)).

The number of LF+ tumors (26%) tended to increase if regional lymph node metastasis N1 was present.

As far as receptor status of BC is considered as the most important prognostic marker and an objective criterion for hormonal therapy [16], we have studied a relation between an expression of ER in BC cells and LF expression in tumor cells of these patients. Interestingly, in ER-positive (ER+) tumors an expression level of LF was found to be significantly, nearly twice higher (35%) than in ER-negative (ER–) tumors (18%) (Fig. 1).

According to the data of literature, regulation of LF synthesis depends on histological type of the cells producing this protein, while amount of LF synthesized in mammary gland is controlled by prolactin, and its synthesis in reproductive tissue is regulated by estrogens [17]. Also, it has been reported that LF is a ligand for specific receptors on cell surface, which are selective for each histological type [18]. This observation could possibly explain a high expression level of LF in RE+ BC.

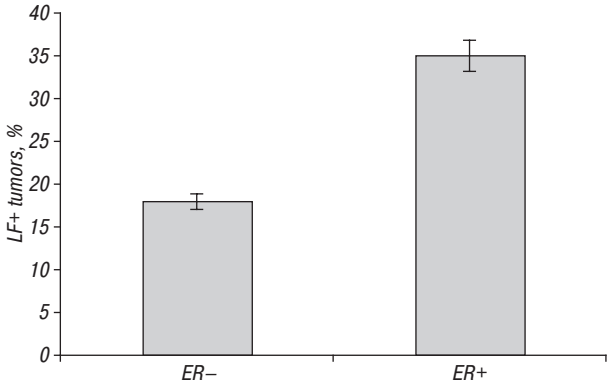

Fig. 1. LF expression in ER+ and ER– breast tumors

Also, we have studied whether LF expression in BC tissue could be related to survival time of BC patients, the most valid clinical criterion [19]. An analysis of Kaplan — Meyer's curves for the groups of BC patients with LF+ or LF-negative (LF–) tumors (Fig. 2) has shown that 5-year survival was higher in LF+ group. We suppose that higher LF levels could increase functional activity of immune system, as it has been detected in the case of inflammation when increased LF levels are related to antiinflammatory cytokines [20]. It could be considered reasonable as far as some researchers claim common features of inflammation and cancer [21]. A relation between LF and antiinflammatory cytokines has been found in the studies of lymphoproliferative diseases [22] and malignant melanoma [23]. Further studies of LF expression in BC tissue and its relation to survival of BC patients are required.

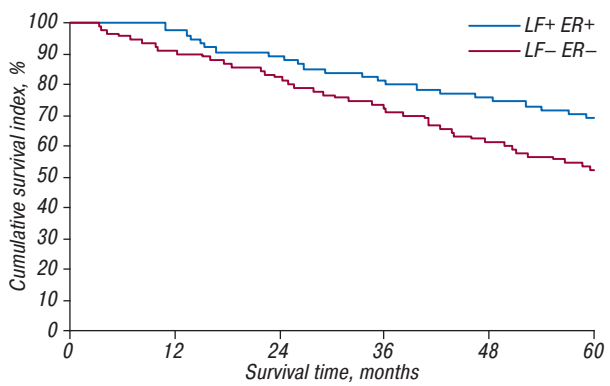

Fig. 2. Overall survival of BC patients depending on LF and ER expression in tumor cells

Presently BC is considered as a complex pathology with various biologic subtypes which differ in the causes of development, clinical and molecular features, have different prognosis and require special therapeutic strategies [24, 25]. In recent years a classification of molecular portraits of BC proposed by Perou et al. [26] became popular, because different molecular BC subtypes (luminal A and B, basal, Her2) differ not only by molecular markers but also by specific biology, which is supported

by clinical observations [27]. In particular, it has been shown that molecular subtype may serve as an independent prognostic criterion and a prediction factor of an effectiveness of the therapy [28].

In view of the foregoing, we attempted the prospective study of the relation between LF expression in tumors and clinical features of BC including molecular phenotype of the tumors. LF expression was about the same in the tumors of patients aged 42–50 and 61–89, i.e., at premenopausal and postmenopausal periods (Fig. 3). Nevertheless, the percentage of LF+ tumors decreased in the group of BC patients aged 51–60, i.e. at menopause. The stage I and II groups differed neither by H-score of LF positivity, nor by the percentage of LF+ tumors (Table 2). In both groups (stage I and II) the tumors with low LF expression were prevalent (65.8 and 58.7%, respectively) (see Table 2).

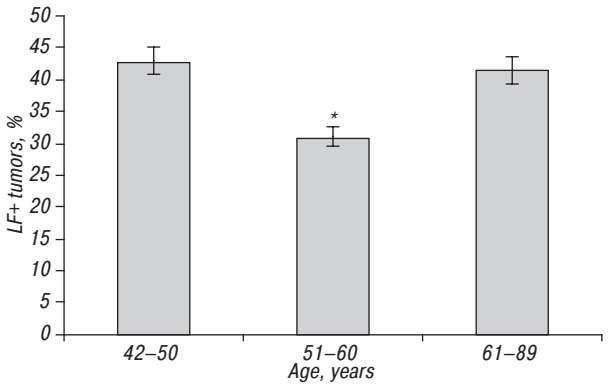

Fig. 3. Expression of LF depending on the age of BC patients and menstrual function. *The difference is significant compared to other groups ($p < 0.05$)

Table 2. Expression of LF in breast tumors of different histological type and at different stage of the disease

Clinical indexes	Number of patients		Expression patterns of LF in breast tumors		
	n	%	Total number of LF+ cells (by H-Score)	Number of tumors with low LF expression, %	Number of tumors with moderate and high LF expression, %
Stage of the disease					
I	35	31.8	172.3 ± 16.7	65.8	34.2
II	75	68.2	174.5 ± 14.4	58.7	41.3
Histological type of the tumor					
Invasive ductal carcinoma	75	70.9	187.2 ± 16.5*	55.12*	44.87*
Invasive lobular carcinoma	25	22.7	143.2 ± 18.3	76.92*	23.07

Note: *the difference is significant compared to invasive lobular carcinoma ($p < 0.05$)

Comparison of LF expression in the tumors of different histological type revealed significantly higher number of LF+ cells in invasive ductal carcinoma *vs* invasive lobular carcinoma (see Table 2). In invasive ductal carcinoma, the percent of the tumors with moderate and high LF expression was significantly higher than the percent of tumors with low LF expression (55.12 *vs* 44.87%). In invasive lobular carcinoma, the tumors with low LF expression were prevalent (76.92 *vs* 23.07%). Also, it was shown that in the tumors of low differentiation grade, LF expression was significantly higher than in the tumors of high or moderate differentiation grade (Table 3).

Table 3. Expression patterns of LF in breast tumors of different differentiation grade

Differentiation grade of the tumor	Number of patients		Expression patterns of LF in breast tumors		
	n	%	Total number of LF+ cells (by H-Score)	Number of tumors with low LF expression, %	Number of tumors with high LF expression, %
High	22	20.00	146 ± 21.5	63.4	36.6
Moderate	57	51.81	150 ± 16.2	62.0	38.0
Low	32	29.09	162 ± 15.1*,**	44.5	55.5

Note: *the difference is significant compared to the highly differentiated tumors ($p < 0.05$).

**The difference is significant compared to moderately differentiated tumors ($p < 0.05$)

An analysis of clinical data of BC patients in an aspect of molecular phenotype of the tumor has shown that median age of the patients with luminal A and B BC subtypes was somewhat higher than 55 years, while the patients with basal phenotype were significantly older (Table 4).

Table 4. Clinical characteristics of the BC patients with different molecular tumor subtypes

Clinical indexes	Molecular subtype of the tumor					
	Luminal A		Luminal B		Basal	
	n	%	n	%	n	%
Average age, years	59.57 ± 8.7		56.55 ± 5.4		67.16 ± 13.5	
Menstrual function:						
premenopause	21	26.25	6	33.33	3	8.3
menopause	23	28.75	5	27.75	3	8.3
postmenopause	36	45.00	7	40.92	29	83.3
Stage:						
I	27	33.75	3	16.65	15	42.85
II	53	66.25	15	83.25	20	57.41
Histological type:						
invasive lobular carcinoma	22	27.5	4	22.2	15	42.85
invasive ductal carcinoma	52	65	14	77.7	20	57.41
Differentiation grade						
high	17	21.25	3	16.65	13	37.14
moderate	43	53	14	77.7	10	28.57
low	20	25	1	5.55	12	34.28

The majority of the patients grouped by receptor status of the tumor were at postmenopausal period and with prevalently basal BC phenotype. A large majority of the patients with luminal or basal tumor subtype were at stage II, luminal B subtype being much more frequent. The percentage of luminal A and luminal B molecular subtypes in invasive ductal carcinoma was respectively 2.3 and 3.4 times higher than in invasive lobular carcinoma. Also, luminal A and luminal B subtypes occurred more frequently in moderately differentiated tumors compared to those with high or low differentiation grade, while the percentage of basal subtype did not differ significantly between the tumors of different differentiation grade (see Table 4).

An analysis of LF expression did not reveal significant differences in number of LF+ cells measured by H-Score between BC of different molecular phenotypes (Table 5).

We also analyzed expression of LF in BC with different molecular phenotype taking into acount the most important prognostic clinical indexes (menstrual function, disease stage, histological type and differentiation grade of a tumor). It has been shown that expression of LF in the luminal A tumors was equally frequent in the patients

at premenopausal and postmenopausal periods, and was at average higher than in the patients at menopause. Expression of LF in the luminal B tumors did not depend on menstrual function and was significantly higher than in the luminal A tumors. In the tumors with basal phenotype, no expression of LF was registered in the patients at premenopausal period while in the patients at menopause or postmenopausal period high expression levels of LF were observed which were somewhat lower than that in luminal B tumors (Table 6).

Table 5. Expression of LF in breast tumors of different molecular subtypes

Molecular subtype of the tumor	Number of tumors		Indexes of LF expression in the tumors	
	n	%	Number of LF+ cells (by H-Score)	Number of tumors with moderate and high LF expression, %
Luminal A	81	53.0	174.5 ± 18.2	35.7
Luminal B	35	23.5	168.0 ± 13.2	46.4
Basal	35	23.5	175.2 ± 16.7	41.6

Comparison of LF expression in the tumors of different molecular phenotype between the groups with different disease stage did not reveal the differences between the stages I and II, but it has been noted that at both stages expression of LF in tumors with luminal B and basal subtypes was higher compared to subtype A tumors.

In cases of invasive ductal BC, expression of LF was significantly higher in luminal B tumors than luminal A, and this index was equal in basal and luminal A subtypes. In the case of invasive lobular carcinoma, expression of LF was significantly lower in basal subtype compared to luminal B subtype, but was significantly higher than in luminal A subtype (see Table 6).

An analysis of LF expression in tumors of different differentiation grade has shown that in the groups with moderate and low differentiation grades the highest expression of LF was present in luminal B subtype compared to subtype A. In luminal subtype A tumors of high differentiation grade the indexes of LF expression were slightly lower than these in tumors of low and moderated differentiation grades and equal to these in basal tumors (see Table 6).

The correlation analysis did not demonstrate correlation of LF expression with the stage of the disease (I or II) in all molecular subtypes. In invasive ductal carcinoma LF expression correlated with occurrence of luminal A subtype while in invasive lobular carcinoma expression of LF showed a positive correlation with occurrence of luminal B subtype. In the tumors of both histological types with basal molecular phenotype no correlation was found (Table 7). LF expression was in a strong positive correlation with occurrence of low and moderate differentiation grade of luminal B or basal tumors.

Therefore, the present research has shown that expression of LF in BC correlated with some important physiologic and clinical indexes of the disease. The patterns of correlation strongly depended on molecular phenotype of this pathology, i.e. luminal A, luminal B or basal.

Table 7. Correlation between expression of LF in breast tumors of different molecular subtype and clinical and morphological characteristics of BC patients

Molecular subtype of the tumor	Stage of the disease		Histological type of carcinoma		Differentiation grade		
	I	II	Invasive ductal	Invasive lobular	High	Moderate	Low
Luminal A	0.11	0.2	0.43	0.22	0.27	0.18	0.16
Luminal B	0.17	0.23	0.14	0.71	–0.57	0.68	0.23
Basal	0.026	0.14	0.24	0.21	–0.63	0.73	0.06

According to the data of epidemiologic studies, the development of each particular molecular BC phenotype could be affected by many factors including physiologic, constitutive and genetic ones [29]. For example, high waist-to-hip ratio is a risk factor for postmenopausal luminal and basal carcinoma. It is known that basal BC subtype develops more frequently in young Afro-American women compared to white women. There are some studies reporting that basal carcinoma development could be prevented by weight control and prolonged breast feeding [30]. By the data of genetic studies, the majority of *BRCA1*-associated tumors are of basal subtype, while in the cases with mutated *BRCA2* the tumors are mostly of luminal A and B subtypes [31]. In general, the data of literature evidence that molecular pathogenesis of BC strongly depends on physiologic and genetic factors [24], and molecular BC subtypes represent different forms of the disease with different etiology and pathogenesis.

Along with this, some recent publications demonstrate that biologic heterogeneity of BC could as well be affected by the heterogeneity of expression of molecular markers that determine BC phenotype, and important signal pathways involved in the development and progression of the tumors [32, 33]. In particular, by the data of cluster microanalysis, it has been considered reasonable to classify Her2-positive subtype into three separate subtypes because one of them is characterized by more unfavorable prognosis than two others [34]. Also, one should take into account the existence of two forms of ER — ERα and ERβ, as far as the latter is differently expressed in molecular subtypes of BC [35]. Hyperexpression of ERβ is the highest in basal BC subtype and serves as a negative prognostic factor; by multifactor analysis, it is considered to be an independent risk factor of BC. It is supposed that screening of BC patients by expression of ERβ and ERα may help to assess proliferative activity of the tumors and to make prognostic indexes more accurate.

Table 6. Expression of LF in breast tumors of different molecular subtypes and clinical and morphological characteristics of BC patients

Molecular subtype of the tumor	Menstrual function			Stage of the disease		Tumor morphology				
						Histological type of carcinoma		Differntiation grade		
	Premenopause	Menopause	Postmenopause	I	II	Invasive ductal	Invasive lobular	High	Moderate	Low
Luminal A	181.3 ± 15.2	160.1 ± 8.0	184.2 ± 17.2	186.3 ± 14.8	180.3 ± 14.4	172.3 ± 21.8	152.4 ± 10.8	183.2 ± 15.3	176.2 ± 17.6	172.4 ± 16.1
Luminal B	235.4 ± 10.2	241.3 ± 16.5	221.5 ± 14.2	216.4 ± 14.0	221.3 ± 12.1	206.1 ± 17.4*	231.2 ± 13.3*	198.2 ± 18.2	223.4 ± 14.2*	229.1 ± 11.7*
Basal	–	215.2 ± 18.3	210.7 ± 15.7	198.5 ± 13.7	203.9 ± 18.1	175.1 ± 12.1	182.1 ± 19.6	180.0 ± 15.6	210.0 ± 12.6*	215.6 ± 14.2*

Note: *the difference is significant compared to other molecular subtypes ($p < 0.05$).

Taking into account biological properties of LF and wide range of its functional activities, we propose to consider LF as an integral biological index reflecting different changes associated with tumor cell heterogeneity in a neoplasm, and with individual response of an organism on particular tumor phenotype. Such idea is supported by the recent data on capability of DNA, different nucleotides and oligosaccharides to affect the formation of LF oligomers that differ by antioxidant and antitumor activities and by their interaction with the components of immune system [36].

The results of the present study on the correlation between the expression of LF in breast tumors with life expectancy of BC patients are of special importance. It seems reasonable to find the ways of LF correction in BC patients taking into account the individual patterns of clinical course of the disease and tumor heterogeneity.

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