

HISTOLOGIC SUBTYPES OF INVASIVE LOBULAR CARCINOMA IN CORRELATION WITH TUMOR STATUS AND HORMONE RECEPTORS*

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SUMMARY – Invasive lobular carcinoma is a distinct biological entity with several histologic variants the prognostic value of which is not fully elucidated. The aim of this study was to determine the immunohistochemical expression of estrogen receptors (ER) and progesterone receptors (PR) in subtypes of lobular breast carcinoma (classic and variant types) and to correlate them with clinical and tumor parameters. Fifty lobular carcinomas, i.e. 33 classic and 17 variant, were analyzed. The classic type was more common (66%) and found in older women (61 years of age), smaller in size (1.5 cm), with N0 status and modestly positive ER and PR. The variant types were more frequent in younger women (55 years of age), larger in size (2.5 cm), with N1 status and maximal positive ER and PR. ER was expressed in 82% and PR in 78% of all tumors, mostly with maximal expression. Only one tumor of the classic type was HER/2neu 3+, ER and PR-. ER expression was significantly associated with a decreased incidence of other proliferative lesions ($P=0.043$). The total sample and the classic type were characterized by a significant positive correlation between ER and PR ($P=0.004$) and between tumor size and N status ($P=0.005$), and by negative correlation between HER/2neu and N status ($P=0.042$). In variant types, larger tumors were related to higher N ($P=0.021$), while higher ER expression was associated with smaller tumors ($P=0.044$). The study indicated distinct biological features of lobular carcinoma subtypes and the need for a more selective therapeutic approach.

Key words: *Breast neoplasms – diagnosis; Breast neoplasms – pathology; Carcinoma, lobular – diagnosis; Carcinoma, lobular – pathology; Neoplasm staging; Aged; Female*

Introduction

Invasive lobular carcinoma (ILC), as reported by different authors, accounts for 0.6%–20% of all invasive breast carcinomas¹⁻³. In the last 20 years, the incidence of ILC has increased in women over 50 years of age, which may be related to their postmenopausal

status^{4,5}. Because of the lack of desmoplastic reaction of the adjacent stroma, lobular tumors are often clinically nonpalpable³; it is one of the reasons for the high number of false-negative mammographic and diagnostic cytology reports, so it is important to find and determine new, more reproducible clinical and morphological parameters in the current diagnostic approach. It seems that ILC has a distinct biological behavior compared with invasive ductal carcinoma (IDC) and other special types of breast carcinoma. Some authors suggest that ILC is more dependent on hormones than IDC^{6,7}. Despite its less aggressive biological phenotype, the average survival and recurrence rates are similar between women with ILC and those

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with IDC⁷. ILC shows both diversity in grade and morphology, with a number of distinct variants. The prognostic utility of grading ILC, however, has not yet been fully elucidated.

The main purpose of our study was to determine the expression of estrogen and progesterone hormone receptors (ER and PR) in different histologic subtypes of lobular carcinoma and to correlate ER and PR status with epidemiological, clinical and morphological features of ILC.

Patients and Methods

The study was performed by examining 50 cases of ILC diagnosed from the surgically removed specimens at Ljudevit Jurak University Department of Pathology in the period from January 1, 2003 to December 31, 2008. Tumor tissue was fixed in 10% formalin, embedded in paraffin, cut in 5- μ m sections, deparaffinized and stained with hematoxylin and eosin. ILC was subclassified as classic type and variant types (including solid, pleomorphic, alveolar and mixed types). The classic pattern of ILC was characterized by proliferation of small round or oval cells without cohesion between them, dispersed individually through the stroma, arranged in single file linear cords known as 'Indian's file' or concentric 'targetoid' pattern around normal ducts³. The solid subtype was characterized by sheets of cells with lobular morphology but they were often more pleomorphic and had a higher frequency of mitoses than the classic type. Alveolar pattern was made of similar cells, which were arranged in globular aggregates of at least 20 cells. In pleomorphic variant, tumor cells showed a typical growth pattern of lobular carcinoma but with a greater degree of cellular atypia and pleomorphism than the classic or other types of ILC and often included signet ring cells or pleomorphic cells³. Mixed group was made of an admixture of the classic type with one or more of other patterns.

Immunohistochemistry for ER, PR and the oncoprotein HER2/neu was performed with standardized kits (DAKO, Glostrup, Denmark). Tissue sections were subjected to antigen retrieval in a microwave 2x5 min in citrate buffer solution pH 6.0, Chem Mate Buffer for Antigen Retrieval diluted 1:10. Upon termination of the antigen retrieval step, the slides were left in the buffer for at least 20 minutes at room tem-

perature. The slides were stained immunohistochemically by labeled streptavidin biotin method (LSAB) as visualization system on a Dako Tech Mate automatic immunostainer using the microwave streptavidin immunoperoxidase (MSIP) protocol. Scoring for estrogen and progesterone receptors was done by counting positive tumor cells in 'hot spot' and grading as negative (<5% positive tumor cells), 1+ (5%-10% positive tumor cells), 2+ (10%-50% positive tumor cells) and 3+ (>50% positive tumor cells). Scoring for HER2/neu was done using the guidelines for scoring HercepTest, Dako.

Data were analyzed by descriptive statistics and checked for normal distribution by the Kolmogorov-Smirnov test. Differences in qualitative data were analyzed by χ^2 -test, whereas quantitative differences were determined by Mann-Whitney U test. Furthermore, Spearman's correlation coefficient was calculated on comparing ordinal variables, whereas the contingency coefficient phi was used on assessing correlations between nominal variables. A value of $P < 0.05$ was considered significant. Statistical analyses were performed by using the SPSS v 17.01 statistical package (Chicago, IL).

Results

During the 6-year study period, 50 lobular breast carcinomas were diagnosed, accounting for 8.08% of all breast carcinomas. All patients were women aged 33-83, ten (20%) of them premenopausal and 40 (80%) postmenopausal. There was no difference in the prevalence of ILC between the left and the right breast. Histologic features of classic type ILC were recorded in 66% and one of the variant types of ILC (5 pleomorphic, 2 solid, 2 alveolar and 8 mixed types) in 34% of cases. The size of invasive tumor foci ranged from 0.2 to 7 cm and the number of tumor foci varied from one to six. In most cases, i.e. in 74% of ILC analyzed, there was one tumor focus, showing mostly classic histologic type. Also, the variant types of ILC most commonly presented as one tumor focus. More than three tumor foci were found in 10% of ILC, with a similar ratio in the classic and variant types.

Concomitant lobular intraepithelial neoplasia (LIN) was found in 35 (70%) ILC, with the majority

Table 1. Distribution of quantitative variables in classic and variant histologic types of invasive lobular breast carcinoma: Mann-Whitney U test

Variable	Histologic type								P
	Classic				Variant				
	N	Percentile			n	Percentile			
	25	50 (Median)	75		25	50 (Median)	75		
Age	33	55.5	61.0	69.5	17	48.5	55.0	63.0	0.069
Tumor size (cm)	33	0.9	1.5	2.7	17	1.8	2.5	4.0	0.001
Focus (N)	33	1.0	1.0	2.0	17	1.0	1.0	1.5	0.821
Status T	33	1.0	1.0	2.0	17	1.0	2.0	2.0	0.012
Status N	33	0.0	0.0	1.0	17	0.0	1.0	2.0	0.028
Positive lymph node (N)	33	0.0	0.0	4.0	17	0.0	2.0	7.0	0.044
ER	33	1.0	2.0	3.0	17	2.5	3.0	3.0	0.047
PR	33	0.5	2.0	3.0	17	1.0	3.0	3.0	0.473
HER2/neu	33	0.0	0.0	1.0	17	0.0	0.0	0.0	0.031

of them being defined as a classic type of ILC. Ductal component including *in situ* and invasive forms was present in 7 (14%) ILC, mostly of classic type. Other proliferative and preneoplastic breast lesions, such as different degrees of ductal hyperplasia with and without atypia, intraductal papilloma and papillomatosis were found in 10 (20%) cases. There was a significant negative correlation between histologic subtypes and presence of other proliferative lesions in the classic

type in comparison with variant types of ILC (27.3% vs. 5.9%; $P=0.046$). In 18% of patients, a multicentric, bilateral or mixed type of breast cancer (lobular and ductal simultaneously) was diagnosed.

ILC, unclassified in subtypes, was found in women aged 58 as one tumor focus, without axillary metastases, with maximum (3+) of both ER and PR expression and negative HER2/neu. However, Mann-Whitney U test yielded differences in some quantitative variables between the classic and variant subtypes of ILC. The median tumor size, T and N status, number of positive lymph nodes and estrogen receptors were significantly higher in variant types of ILC (Table 1).

ER was positive in 41 (82%) cases of ILC (Fig. 1). In 54% of carcinomas, both histologic groups showed maximal expression (3+), (Table 2). PR was also positive in the majority of tumors ($n=39$, 78%) (Fig. 2), with maximum level of expression (3+) in 44% of carcinomas of both histologic groups (Table 3). In most cases (68%), ER and PR showed simultaneous expression.

Only one tumor was HER2/neu 3+; it was ILC of the classic type, ER and PR negative.

In the total sample, not divided by different histologic subtypes, we found a positive correlation between ER and PR ($P=0.004$) as well as between tumor size and N status ($P=0.005$). There was a negative correla-

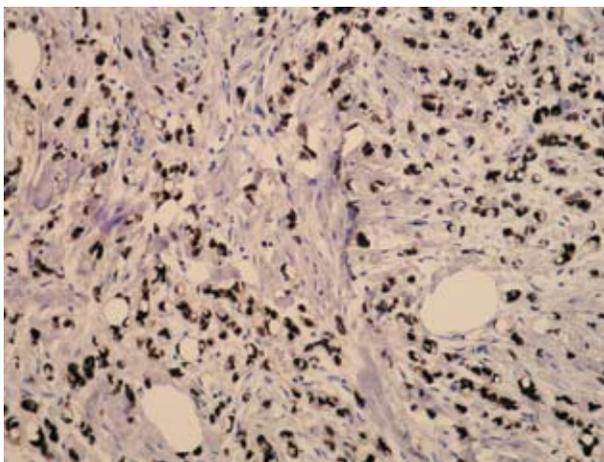


Fig. 1. Positive estrogen receptors (+++) in invasive lobular carcinoma determined by immunohistochemistry (MSIP, X200).

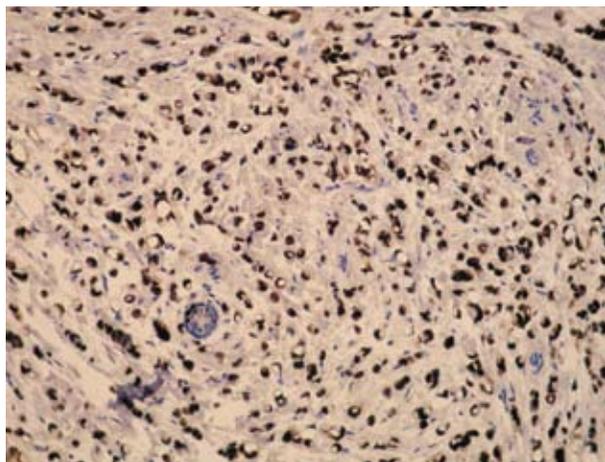


Fig. 2. Positive progesterone receptors (+++) in invasive lobular carcinoma determined by immunohistochemistry (MSIP, X200).

tion between HER2/neu and N status ($P=0.042$) in the total sample. The aforementioned correlations also applied to the classic histologic type. In variant types, we found a significant positive correlation between T status and N status ($P=0.021$) and negative correlation between ER expression and T status ($P=0.044$). A significant positive correlation ($P=0.003$) was observed between the patient menopausal status and ER expression. Another interesting result emerged from this study, namely, that in premenopausal women younger than 50, the ratio between ER positive and

ER negative ILC was the same (with five patients in each group). LIN had a higher prevalence in ER positive ILC (in 27 of 41 ER positive tumors); concomitant ductal component was also more frequently found in ER positive tumors and in only one ER negative tumor. Our results showed a significant negative correlation between ER expression and presence of other proliferative and precancerous breast lesions ($P=0.043$); ER expression in ILC correlated significantly with a lower prevalence of other proliferative breast lesions.

PR was also more frequently expressed in postmenopausal patients (in 31 of 40 patients in postmenopause), however, without any statistical significance, which, in contrast, was found in ER expression. It is interesting that PR was more often expressed in premenopausal women (in 8 of 10 patients), while ER was positive and negative in the same ratio in this group of patients. LIN also had a higher prevalence in PR positive ILC (in 29 of 39 PR positive ILC) as well as concomitant ductal component, which was found in only one PR negative ILC. Other proliferative breast lesions also were more frequently detected in PR positive (9 of 10 ILC with the presence of other proliferative lesions) than in PR negative ILC, whereas its presence in ER positive tumors showed a statistically significant negative correlation.

Table 2. Intensity of estrogen receptor (ER) expression in histologic subtypes of invasive lobular breast carcinoma

		Histologic subtype		Total	
		Classic	Variant		
ER	0	n	7	2	9
		%	21.2	11.8	18.0
	1	n	4	1	5
		%	12.1	5.9	10.0
	2	n	8	1	9
		%	24.2	5.9	18.0
	3	n	14	13	27
		%	42.4	76.5	54.0
	Total	N	33	17	50
		%	100.0	100.0	100.0

χ^2 -test, $P=0.138$

Table 3. Intensity of progesterone receptor (PR) expression in histologic subtypes of invasive lobular breast carcinoma

		Histologic subtype		Total	
		Classic	Variant		
PR	0	n	8	3	11
		%	24.2	17.6	22.0
	1	n	2	2	4
		%	6.1	11.8	8.0
	2	n	10	3	13
		%	30.3	17.6	26.0
	3	n	13	9	22
		%	39.4	52.9	44.0
	Total	N	33	17	50
		%	100.0	100.0	100.0

χ^2 -test, $P=0.607$

Discussion

A review of recent literature revealed just few studies that focused on different features of histologic subtypes of lobular carcinoma through possible epidemiological and clinical differences among them, as in the expression of hormone receptors and HER2/neu oncoprotein.

The prevalence of ILC recorded in our study for the 2003-2008 period (8.08%) is in line with comparable data already reported in the literature, e.g., 5%-15% according to WHO³. Eighty percent of women with ILC were postmenopausal, which is in agreement with data from the literature^{3,7,8}. In the last twenty years, an increasing trend in the prevalence of ILC in women over 50 years of age has been noticed, which can partly be explained by a growing number of women receiving hormonal therapy. Thus, the theory of a greater hormone dependence of lobular in comparison with ductal carcinoma, as advocated by some authors, can be corroborated^{3,6,7}.

DiCostanzo *et al.* revealed an 8%-19% incidence of contralateral ILC, which is higher than the incidence of contralateral IDC⁹. Arpino *et al.* emphasize that the incidence of bilateral ILC is twice as high as the incidence of bilateral IDC⁶. Thus, they point to the importance of taking tamoxifen in women with ILC to prevent contralateral carcinoma. In the present study, 18% of our patients developed bilateral or mixed carcinoma during the study period. Dixon *et al.* found the classic type of ILC to include LCIS in more than 90% of cases¹⁰. However, our study provided evidence for not only of LCIS, but also for LIN.

ER positive tumors are more likely to occur in older women, they are often of a lower histologic grade, usually diploid, with lower mitotic activity, and they rarely show expression of p53 and HER2/neu¹¹. Our results showed that ER was more frequently expressed in ILC, i.e. in 41 (82%) carcinomas. Sastre-Garau *et al.* found that 70%-95% of ILC cases were ER positive, which is higher than 70%-80% ER positive IDC cases¹². In the study by Bane *et al.*, based on 50 ILCs, all ILCs observed were ER positive irrespective of the patient age or size and grade of tumors, as usually found in the literature⁷. Previous data suggest the role of ER as a nondiscriminative prognostic factor in the treatment of ILC. Our study showed that in the classic and variant types of ILC, maximal or 3+ level of

ER was most frequently observed. Nesland *et al.* also confirmed the existence of ER in the classic and variant types of ILC, with almost 100% of ER positivity in the alveolar type, whereas the ratio of ER positivity in the pleomorphic variant was lower than in the classic type¹³.

PR expression also has a prognostic as well as predictive value in the treatment of breast carcinomas. PR expression seems to be the reflection of functional ER in tumors, and in this way PR predicts patient response to adjuvant endocrine therapy^{11,14}. However, its role as an independent prognostic marker does not seem to be so important. According to our results, PR was expressed in 39 (78%) cases of ILC. Sastre-Garau *et al.* found PR expression in 60%-70% of ILC cases, which is slightly lower in comparison with our results¹². Bane *et al.* demonstrated a high incidence of PR positive ILCs (85%-90%), without any correlation between PR status and tumor size⁷. Our study indicated that the classic and variant types of ILC most often showed maximal or 3+ PR expression. Classic ILC, which correlates with grade I ILC, is known to have a high incidence (>90%) of PR expression^{7,15}.

The higher expression of HER2/neu is associated with higher grade and bigger tumors, with negative ER and PR, and with positive axillary lymph nodes; therefore, it correlates with poor disease prognosis^{16,17}. It is significantly more often expressed in IDC than ILC, where it is most often negative³. In 50 ILC cases presented in our study, only one was HER2/neu positive (3+); it was an ILC of the classic type, with simultaneous ER and PR negative expression. Porter *et al.* report that HER2/neu is significantly more often expressed in pleomorphic types in comparison with other ILC types¹⁸. Hoff *et al.* found HER2/neu amplification in 0% of special types of breast carcinomas and in less than 1% of ILCs, almost always in those of pleomorphic type with high nuclear grade¹⁹.

Conclusion

Our results revealed statistically significant differences in tumor status between histologic subtypes of ILC, whereas ER and PR expression was equally distributed among the classic and variant types. This study as well as relevant data from recent literature proves that ILC is a distinct biological and clinical entity that needs to be further investigated, so that

thorough understanding of its biology might enable selective approach in choosing the most appropriate surgical and oncologic treatment. The results presented in this paper will surely contribute to the modest insights into the biological and morphological organization of lobular breast carcinoma and its morphological subtypes available so far. They will aim at possible differential therapeutic directions in women with this type of breast cancer.

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Sažetak

HISTOLOŠKI PODTIPOVI INVAZIVNOG LOBULARNOG KARCINOMA U KORELACIJI
S TUMORSKIM STATUSOM I HORMONSKIM RECEPTORIMA*I. Miše, M. Vučić, I. Maričević, M. Šokčević i S. Čurić-Jurić*

Invazivni lobularni karcinom je zaseban biološki i klinički entitet s nekoliko različitih histoloških podtipova prognostička vrijednost kojih je još uvijek nedovoljno jasna. Cilj rada bio je odrediti imunohistokemijsku izražajnost ER i PR u podtipovima lobularnog karcinoma dojke (klasični i varijantni) i korelirati ih s kliničkim i tumorskim parametrima. Analizirano je 50 invazivnih lobularnih karcinoma, 33 klasičnog i 17 varijantnog tipa. Klasični tip bio je češći (66%), prosječno u nešto starijih žena (61 god.), manje veličine (1,5 cm), statusa N0 i s umjereno pozitivnim ER i PR. Varijantni tipovi bili su češći kod nešto mlađih žena (55 god.), prosječno veći (2,5 cm), statusa N1 i s maksimalno pozitivnim ER i PR. ER je bio izražen u 82%, PR u 78% svih tumora, oba receptora najčešće s maksimalnom ekspresijom. Svega jedan tumor bio je HER2/neu 3+, istodobno ER i PR-, po tipu klasičan. Izražajnost ER bila je značajno povezana s manjom učestalošću drugih proliferirajućih lezija ($P=0,043$). Za cijeli uzorak i klasični tip bila je značajna pozitivna korelacija ER i PR ($P=0,004$), također između veličine tumora i statusa N ($P=0,005$) te negativna korelacija HER/2neu i statusa N ($P=0,042$). U varijantnim tipovima veći tumori značili su veći N ($P=0,021$), a veća izražajnost ER bila je povezana s manjim tumorima ($P=0,044$). Rad je pokazao jasna biološka obilježja podtipova lobularnog karcinoma i potrebu selektivnijeg terapijskog pristupa.

Ključne riječi: Novotvorine dojke – dijagnostika; Novotvorine dojke – patologija; Karcinom, lobularni – dijagnostika; Karcinom, lobularni – patologija; Određivanje stadija novotvorine; Starenje; Žene

