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Histology and Immunophenotype of Invasive Lobular Breast Cancer. Daily practice and pitfalls

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Abstract. Invasive lobular carcinomas (ILC) represent the most common subtype of invasive breast cancer and account for about 5–15% of all breast cancer cases. Invasive lobular carcinoma is often accompanied by in situ lesions, by lobular neoplasia (LN). Invasive lobular carcinomas display diverse histologic patterns varying from classical through solid to pleomorphic subtypes. When analyzing histological subtypes, the classical variant is reported to have a more favorable outcome. The majority of invasive lobular carcinomas are hormone receptor positive, overexpression and/or amplification of the Her2 gene is lower than in carcinomas of invasive ductal type. Loss of heterozygosity of the 16q chromosomal regions and the consequent lack of E-Cadherin expression are common findings in invasive lobular carcinomas. Intra-operative evaluation of resection margins in ILC is often unsatisfactory due to the diffuse nature of the tumor. Size estimation of the invasive component poses a similar challenge in daily practice.

GROSS PATHOLOGY

Invasive lobular carcinomas present mostly as an irregular, infiltrating, poorly delineated mass rather than a sharply demarcated lesion. Invasive lobular carcinomas often display multifocality in the ipsilateral breast and have a higher tendency to develop bilaterality than other tumor types. The size of the invasive component varies considerably (from millimeters to several centimeters). The edges of the tumor mass are often difficult to evaluate and may be best appreciated by palpation. The cut surface often appears as an inconspicuous, grey or white area. In other cases there is no grossly visible mass and the tumor can only be appreciated by palpation or not at all [1–4].

GENERAL HISTOLOGICAL ASPECTS

The original definition of invasive lobular carcinoma comes from Stewart and Foote, who described the classical infiltrative subtype [1–4]. Invasive lobular carcinomas display characteristic cytologic features and a distinct infiltration pattern of the stroma. Classical invasive lobular carcinomas are composed of single cells, infiltrating in strands. In some cases focal tubule formation is seen. The classic pattern of invasive lobular carcinomas are characterized by a small uniform cell population, lacking cohesion and invading the stroma as individual tumor cells, resulting in a 'single-file' or 'Indian-file' pattern of growth. Sometimes tumor cells are located around benign ducts in a circular fashion resulting in a targetoid appearance. Tumor cells usually have a round nucleus, thin cytoplasm with occasional cytoplasmic vacuoles. The nuclei are usually small, eccentric and show little variation in size, nucleoli are absent or inconspicuous. Mitotic figures are rare. The classical form of invasive lobular carcinoma is often accompanied by in situ lesions of lobular neoplasia occurring in 40–60% of invasive carcinomas. Invasive lobular carcinoma is sometimes accompanied by a prominent lymphocytic or granulomatous reaction. The histological diagnosis of classical lobular

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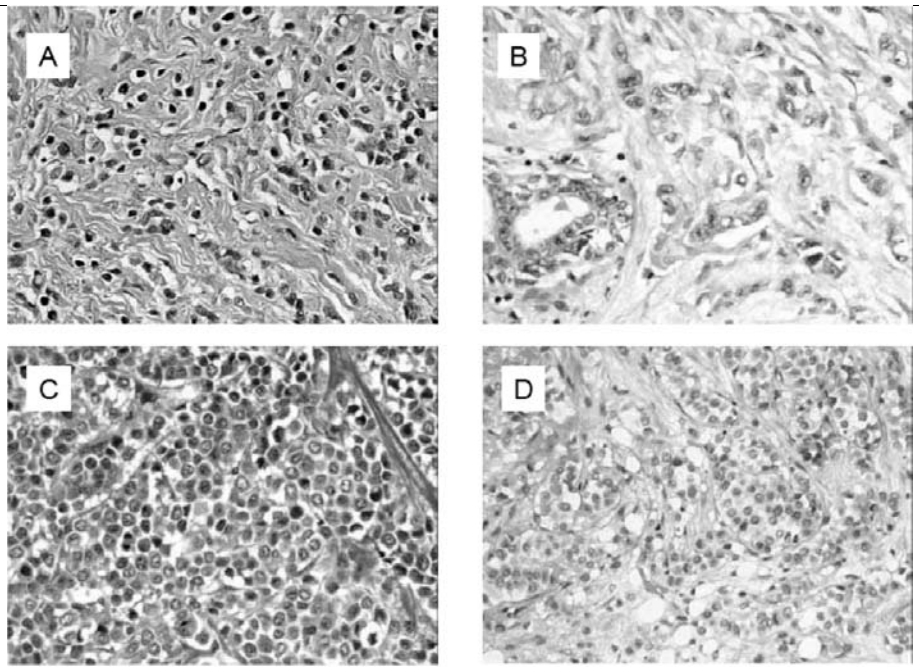

Fig. 1. Special types of invasive lobular breast cancer. A) classical type (HE). B) histiocytoid variant (HE). C) pleomorphic variant (HE). D) alveolar variant (HE). Magnification: 300X.

carcinomas requires at least 70% of single-file growth pattern [1–4].

SPECIAL SUBTYPES OF INVASIVE LOBULAR CARCINOMA (see Figs 1 and 2)

Several histological variants of invasive lobular carcinoma have been described.

Solid pattern: this pattern is characterized by sheets of uniform cells, lacking cohesion, resulting in a growth pattern in large confluent areas with little or absent stroma in between.

Alveolar pattern: in this variation tumor cells are arranged in small clusters or nests of approximately 20 cells, separated by delicate fibrovascular septa.

Trabecular pattern: this variant represents a histological overlap between classical and so called trabecular filing pattern. This morphological coincidence is due to the morphological similarity between 'single file' seen in the classical variant and 'one-cell-thick-trabecules' observed in the trabecular variant.

Tubulolobular pattern: this lesion is composed of small tubular structures as well of single cell files seen in classical lobular carcinomas. This category represents a morphological overlap between invasive tubular and invasive classical lobular carcinoma, traditionally categorized as a variant of invasive lobular carcinoma.

Pleomorphic variant: this variant represents a distinct subtype of invasive lobular carcinoma with similar growth pattern and stroma infiltration as seen in other subtypes. However, the tumor cells are larger and exhibit more cellular atypia and pleomorphism. The cytoplasm is more abundant and it often shows some eosinophilia. Recurrence free survival is reportedly poorer in pleomoprhic lobular carcinomas than in classical lobular carcinomas. Pleomorphic lobular carcinomas can display apocrine, signet ring cell and histicytoid differentiation. Apocrine differentiation in invasive lobular carcinomas has been found to have an especially aggressive clinical course.

Histiocytoid variant: in this variant tumor cells have a pale appearance with foamy cytoplasm and mild nuclear variation.

Signet cell variant: this subtype occurs in invasive breast carcinomas with a growth pattern of a lobular carcinoma, consisting of a prominent component of signet ring cells.

Mixed lobular carcinomas: this group shows an admixture of classical lobular carcinoma accompanied by at least one more additional pattern, which could be either a special type of invasive lobular carcinoma or of invasive ductal carcinoma [1–4].

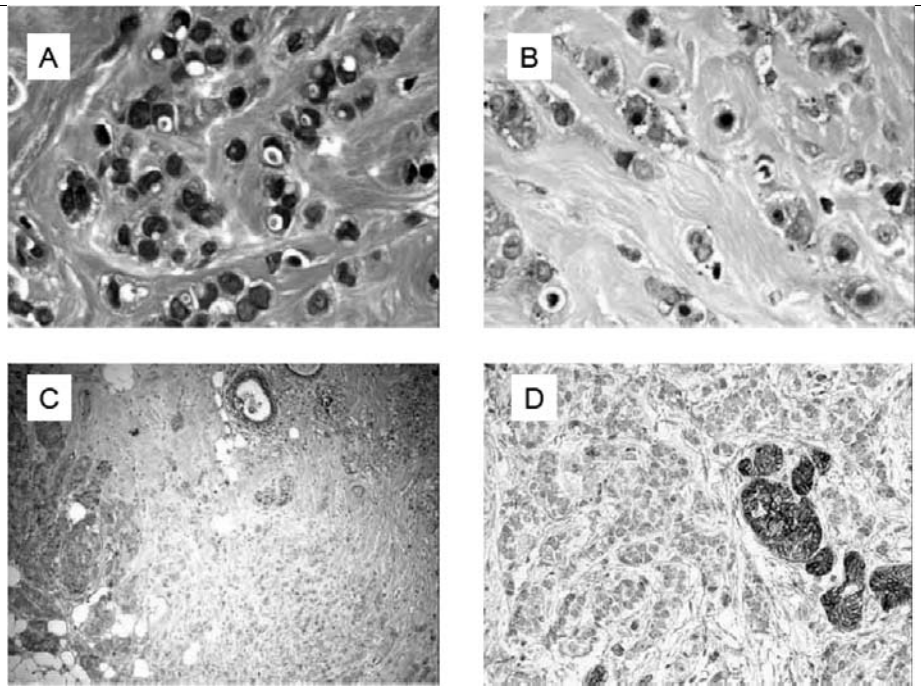

Fig. 2. Special types of invasive lobular breast cancer. A) intracellular mucin production (HE). B) intracellular mucin production (Alcian blue PAS). C) Mixed carcinoma, ductal (left) and lobular (right) (HE). D) Negative immunoreactivity for E-Cadherin (E-Cadherin). Magnification: 300X (A,B,D) 50X (C).

GRADING OF INVASIVE LOBULAR CARCINOMA

Grading of invasive lobular carcinomas is widely accepted in routine surgical pathology despite the fact, that the application of BRE Score is somewhat special in this tumor type. There is no tubular formation in invasive lobular carcinomas, therefore, a score of 3 has to be attributed for tubule formation. Mitotic activity is usually low (score 1 or 2), nuclear pleomorphism can vary from monotonous to largely pleomorphic nuclei (score 1 to 3). Therefore, the majority of invasive lobular carcinomas are classified as grade 2, due to moderate nuclear pleomorphism and low mitotic rate. Classical lobular carcinomas can be graded as grade 1, if tumor cells exhibit round small monotonous nuclei. Pleomorphic lobular carcinomas can be also graded as grade 3 if sufficient mitotic figures are present [3,5–7].

DIFFERENTIAL DIAGNOSIS

Lymphatic proliferation

Invasive lobular carcinomas can closely resemble an atypical lymphatic proliferation. This diagnostic challenge preferentially occurs with suboptimal tissue fixation. The use of immunohistochemistry (cytokeratin in the neoplastic cells) or the detection of cytoplasmic vacuoles containing mucin, should easily identify the lesion as invasive lobular carcinoma. Rarely, inflammatory cells can mimic invasive lobular cancer cells, which can be challenging particularly on frozen sections. The evidence of in situ components, lobular neoplasia, may be helpful in such settings [1,2,4,5].

Invasive ductal carcinoma

The distinction between lobular and ductal carcinomas can pose a diagnostic difficulty based on histology alone. Invasive ductal carcinomas can display a growth pattern similar to invasive lobular carcinomas since tumor cells spreading in single files can occur in invasive ductal carcinomas. The evidence of peri- or intratumoral lobular neoplasia (LN) or ductal carcinoma in situ (DCIS) may be of some help, however this is of limited value as in situ components can be present independently of the histology of the invasive component. Intracytoplasmic vacuoles and areas of single cell infiltration favor the diagnosis of invasive lobular carcinoma. Immunostains for E-Cadherin, hormone

receptors, gross-cystic disease fluid protein, p120 can support the correct diagnosis. Generally, the absence of E-Cadherin expression on the cell membrane and in most cases strong hormone receptor expression favor the diagnosis of invasive lobular carcinoma (see also paragraph below) [2,8–13].

Fat necrosis

The histiocytoid variant of invasive lobular carcinoma may be particularly difficult to differentiate from fat necrosis in some instances, as tumor cells can mimic reactive histiocytic infiltration and vica versa. Immunohistochemistry for macrophage markers (e.g. CD68) and cytokeratins should lead to the correct diagnosis [4, 5].

IMMUNOHISTOCHEMISTRY

Hormone receptors

The large majority of invasive lobular carcinomas show strong expression of estrogen and progesterone receptors, a higher rate than in invasive ductal carcinomas [2–4].

Her2 gene and protein

Her2 protein expression and/or gene amplification are rare events in invasive lobular carcinomas. The pleomorphic variant of invasive lobular carcinomas represents an exception to this rule, as Her2 gene amplification and protein expression, particularly in grade 3 cases have been described in the literature [2,14].

Proliferation index (*MIB-1*)

Proliferation index is generally low in invasive lobular carcinomas. In some studies distinct differences in proliferation activity were detected in classical versus pleomorphic subtypes varying from 2.9% (classical type) to 8–11% (pleomorphic variants). The proliferation fraction of invasive ductal carcinomas is usually higher, in grade 3 cases the MIB-1 labeling index often reaches 25–30% or even higher [15].

E-Cadherin

The transmembrane protein E-Cadherin which mediates cell-cell adhesion and acts as an invasion suppressor factor, had been reported to be a reliable marker in the differentiation of ductal and lobular breast carcinomas. Ductal carcinomas usually express membranous E-Cadherin reactivity, while invasive lobular carcinomas are mostly negative. Therefore, the absence of E-Cadherin positivity is a significant finding in the diagnosis of invasive lobular carcinomas. Together with conventional histology, a negative E-Cadherin reaction is the most helpful immunhistochemical marker. Conversely, in a subset of poorly differentiated invasive ductal carcinomas (less than 15%), E-Cadherin may be absent. In these cases other morphological features such as tubule formation, hormone receptor status and additional immunohistochemistry are needed to confirm the diagnosis of an E-Cadherin negative invasive ductal carcinoma [8,12,13,16,17].

P120

The interaction of E-Cadherin with catenins such as p120 has been documented in previous studies. Cytoplasmic positivity for p120 generally favors the diagnosis of lobular carcinoma, as membranous reaction has mostly been reported in invasive ductal carcinoma [10, 17].

GCDPF-15 (*gross cystic disease fluid protein*)

Approximately one third of the invasive lobular carcinomas are positive for GCDFP-15, a marker for apocrine differentiation. Invasive lobular carcinomas with histiocytoid, pleomorphic or signet ring cell components tend to express GCDFP-15. Positivity for this marker however does not necessarily confirms the diagnosis of invasive lobular carcinoma, as GCDFP-15 can be seen in other breast cancer subtypes such as apocrine, endocrine, oncocytic and acinic cell carcinomas and some positivity has been reported in invasive ductal carcinomas as well [2–4].

Cytokeratin 5/6

Invasive lobular carcinomas are reportedly negative for cytokeratin 5/6. In recent studies a subset (15%) of invasive lobular carcinomas tested positive for cytokeratin 5/6, which also were preferentially hormone receptor negative, leading to postulating a distinct basal like ILC subtype. However, no definite morphological differences could be found upon cytokeratin 5/6 positivity among invasive lobular carcinomas [11,16,17].

Cyclin D

Cyclin D1 can evoke hormone receptor activation through binding to estrogen receptors. Overexpression of Cyclin D1 has been reported in over 80% of invasive lobular carcinomas. However, this positivity is not specific for a definitive lobular differentiation, as it can occur in other subtypes as well [3,4].

PROGNOSIS

In general, prognosis of invasive lobular carcinomas does not consistently differ from invasive ductal carcinomas. However when histological subtypes of ILC are analyzed one by one, a more favorable outcome has been reported for the classical type of invasive lobular carcinoma. In some studies, the pleomorphic and the signet ring cell variants have been associated with poorer outcome. Also, the metastatic pattern of invasive lobular carcinomas differs from invasive ductal breast carcinomas. Higher rates of bone, gastro-intestinal tract, meningeal and/or ovarian metastases have been documented in invasive lobular carcinomas [3,18,19].

PITFALLS IN ROUTINE PRAXIS

Frozen sections

Differentiation of invasive lobular carcinoma cells from inflammatory cells, fibroblasts or cells from hematological malignancies can be very difficult on frozen sections. Searching for other features such as lobular neoplasia, characteristic growth pattern, stromal infiltration or overtly malignant epithelial cells may be helpful. If available, cytokeratin immunohistocemistry adapted for frozen sections, can be applied as well.

The intra-operative assessment of involvement of surgical margins by invasive lobular carcinoma is often unsatisfactory as no palpable tumor mass is present at gross examination for the correct selection of suspicious edges.

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