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Review

Invasive lobular carcinoma of the breast: we diagnose it, but do we know what it is?

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Summary
Invasive lobular carcinoma of the breast is the most common special type breast cancer. It has been defined using morphological features, has a characteristic immunophenotype associated with the loss of E-cadherin mediated intercellular adhesion, and the background of this immunohistochemistry and morphology is generally a biallelic genetic alteration of the *CDH-1* gene coding E-cadherin. However, the morphology may often deviate from the classical, and immunohistochemistry may also deviate from the typical, and then the diagnosis of invasive lobular carcinoma becomes less straight forward. Eventually, the definitions of this histological type, although similar, are not identical and this may also give ground to occasional different interpretations. This review summarizes different approaches to invasive lobular carcinomas and the deviations from what is considered normal.

Key words: breast, *CDH-1* gene, E-cadherin, immunohistochemistry, invasive lobular carcinoma

Introduction

In medias res (starting in the middle, with the relevant, without much introduction): lobular carcinoma is not properly defined, and therefore its diagnosis does not stand on firm ground. Before you immediately refute this statement, please, read the arguments behind it.

In a recent review article, Kuba and Brogi concluded that there is „variability in the histopathologic criteria used for diagnosis of ILC, and particularly tumors with mixed morphology" [1]. They also stated that a clinical behavior-oriented classification was needed, as well as consensus guidelines for making the diagnosis [1]. I can only agree with this.

If you are asked, whether you, as pathologist, or just a resident in pathology, know what lobular carcinoma is, the answer is obviously yes. And what you would say would probably include that this is the most common special type of breast carcinomas, a tumor characterized by non-cohesive tumor cells arranged in single files or dispersed as single cells in the stroma; often this is associated with lobular neoplasia (LN), and the characteristic morphology is explained by a loss of E-cadherin function which is responsible for the dyscohesive morphology. And of course, you would mention that this is recognized as the classical form, but there are also other patterns recognized by the World Health Organization (WHO) classification [2], and these include the solid, the alveolar, the tubulolobular and the pleomorphic ones, which can be admixed with the classic pattern giving ground to mixed forms. Perhaps, you would also mention aberrant

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E-cadherin staining as a possibility, for cases that show some E-cadherin staining either at the wrong place (i.e. cytoplasm) or with low intensity and focal nature (i.e. not strong circumferential), as staining patterns deviating from the simplistic "black and white" (negative: lobular; positive: non-lobular) textbook style examples. Let's go through this knowledge step by step.

In a past issue of the journal, I wrote about the classification of breast cancers according to five editions of the WHO blue book [3]. It was obvious that histological appearances formed the basis of the classification, but considering the expanding knowledge from molecular and genetic backgrounds, the last three editions of the WHO classification included many additional data concerning the molecular basis or genomic background of various types of tumors or tumor-like lesions [2,4,5].

ILC by HE morphology

Invasive lobular carcinoma (ILC) of the breast is a well-recognized histological type of breast cancer. It first entered the classifications of breast tumors in 1950 in the fascicle of the Armed Forces Institute of Pathology (AFIP) written by Fred Stewart [6], as accompanying lobular carcinoma in situ (LCIS) and showing transition from this latter. It was also depicted a few years earlier as associated with LCIS in a paper introducing this name for the precancerous lesion [7]. Earlier depictions without this specific name date back to 1850, when Longman, London published *The diseases of the breast and their treatment* written by John Birkett [8].

ILC was recognized and precisely described as a tumor consisting of rather uniform cells infiltrating in cords and loosely dispersed in the connective tissue matrix, not forming nests or sheets [7]. In earlier texts, this classical morphology and invasive pattern was referred to as Indian filing to reflect the smart movements of North American indigenous tribe warriors stepping one after the other into the same footprints to hide the exact number of people in motion to tracereaders; this is at least the analogy one can have from teenage readings. Another picturesque analogy is to refer to this pattern as geese line, a term used in middle European countries where geese are still encountered in rural conditions, and often march one after the other. Linguistic and cultural development has led to call this pattern "single filing" (Fig. 1A).

As nicely reviewed by Christgen et al. [9], this first recognized and classical pattern was later supplement-

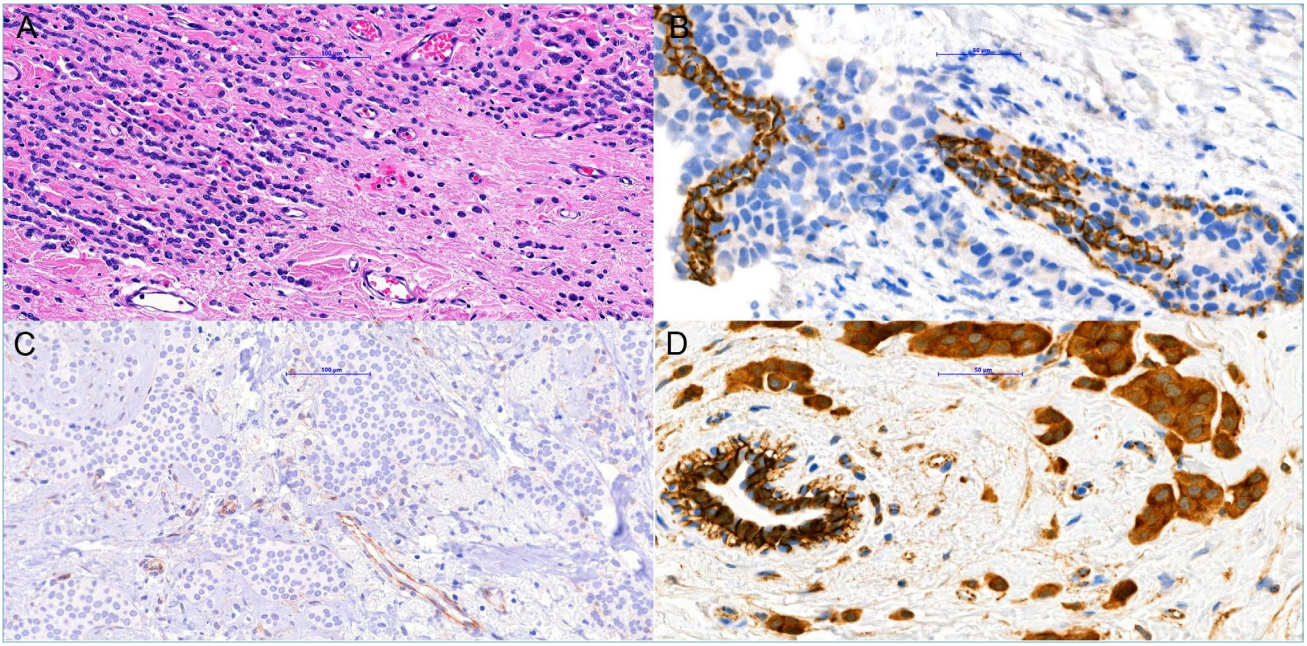

Figure 1. Typical features of ILC. A: Dyscohesive cells with single filing on the left, and as dispersed isolated cells on the right (HE x20); B: The tumor cells lack E-cadherin staining; note the pagetoid spread of E-cadherin-negative lobular neoplasia on the right (E-cadherin x40); C: In this ILC with alveolar pattern, the tumor cells lack β-catenin staining; note positivity in vessels that can also serve as control when normal breast epithelium is missing (β-catenin x20); D: p120 catenin shows cytoplasmic staining in ILC cells in contrast to the membranous staining of the normal duct and myoepithelium (p120 x40).

ed by numerous other morphological variants which have been described one after the other. Some of these subtypes reflected a different infiltrative pattern (solid, alveolar, trabecular, plexiform, solid papillary), whereas others referred to cellular features (histiocytoid, signet-ring cell, pleomorphic) or various differentiation aspects (tubulolobular, with tubular elements, with extracellular mucin production, with neuroendocrine or apocrine differentiation). As with many other classifications, when non-exclusive criteria are used, there is a possibility for falling into multiple categories, e.g. an ILC can be predominantly solid according to its infiltration pattern, pleomorphic according to nuclear size [10], and additionally forming extracellular mucin [11]. At present, it is unclear whether such combinations of morphology along different features should be or are adequately entered into the mixed form of ILC [2].

The current WHO classification [2] recognizes some of these morphologies as patterns of ILC: namely the solid, the alveolar, the pleomorphic and the mixed (generally consisting of the classic with any of the other recognized patterns); it also lists the tubulolobular variant, which will be discussed later. Finally, it also mentions extracellular mucin production by ILC under mucinous carcinomas, stating that "it is unknown whether these tumors represent a subtype of ILC or MC" (mucinous carcinoma). In fact, these tumors fulfil the definitions of both types, and accordingly can be classified as either. The present author favors their classification as ILC, if one histological type needs to be selected.

In a recent analysis of nearly 150 ILCs, it was recognised that at least minor areas of both classical and non-classical ILC are present in most tumors, making the mixture of patterns the most common manifestation of ILCs [12]; this feature is rarely stressed in definitions and descriptions of ILC, and quantitative requirements for identifying variant morphologies of the disease are seldom given. The fourth series of the AFIP fascicles mentioned the common admixture of ILC patterns and suggested at least 80% dominant pattern for a given classification [13]. As a consequence of variable composition, the search for a classical pattern area in variant ILCs may help the diagnosis [12,14].

Cellular features also help to define ILCs, notably the non-cohesive nature of the cells (with intercellular gaps), their relative uniformity, nuclear shapes consistent with cellular compression (concavity and/or angulation), the common presence of intracytoplasmic vacuoles sometimes giving rise to signet ring cells. Often, there is accompanying lobular neoplasia. All of these are mentioned with different stresses in descriptions of ILC (Tab. I) [2,4-6,13,15-20].

On the other hand, it is well acknowledged that invasive breast cancers of no special type (NST) may also infiltrate in cords, some of which may be a single cell thick, and a few single cells may also be seen. This feature makes it difficult at low power to distinguish NST carcinoma with some lobular infiltrative pattern from predominantly non-classical ILCs with minor classical pattern or without it. Features more perceptible at high power include the cellular morphology and lack of cohesion. NST carcinomas may have cellular dyscohesion due to suboptimal fixation in larger tumors or mechanical disruption in core biopsy samples taken with a biopsy gun. Signet ring cells and intracytoplasmic vacuoles are not specific to ILC, and may also be seen in other types of breast cancer. Therefore, no single features enable the morphological diagnosis of ILC, but their combination is quite distinctive and allows for a proper diagnosis most of the time. In a series of 524 breast cancers, the HE distinction of ILC from other histological types was made with 14% (73 cases) uncertainty, reflecting any doubt in these cases, and in practice, a request for E-cadherin immunohistochemistry (IHC). The unanimous addition of E-cadherin IHC resulted in 17 histological type changes, including mainly cases where uncertainty on type was present, and the favored diagnosis was not correct, but also 3 cases confidently typed on HE [14].

As a conclusion, using only HE morphology may lead to the mistyping of some cases; especially those with non-classical pattern [21].

ILC with the help of immunohistochemistry

Since the discovery of E-cadherin playing a role in the morphology of ILCs [22-24], the diagnosis of ILC has become easier. E-cadherin is a transmembrane calcium-dependent cell adhesion molecule found in most epithelial cells being a part of their adherens junctions. While its extracellular domain links cells to cells, its intracellular domain fixes the molecule to the cytoskeletal actin via the E-cadherin-catenin complex, including β-catenin and α-catenin; E-cadherin-bound p120 catenin being also part of the complex [25]. Under normal conditions, all of these proteins are arranged along the cell membranes. Whenever E-cadherin or part of it is lost, the loss of the anchoring element leads to the catenins also lacking from the cell membranes [26]. Diagnostic immunohistochemistry includes loss of E-cadherin (Fig. 1B), β-catenin (Fig. 1C), α-catenin and p120-catenin (Fig. 1D) from the cell membranes in ILC cells, although α-catenin is rarely used and reported in this respect. Typically, the small size p120-catenin is displaced to the cytoplasm, and gives not only the lack of a membranous staining, but also

Table I. Key elements of different definitions of invasive lobular carcinomas (ILCs)

Reference	Definition	Cellular shape	Mitotic rate	Dyscohesion	Classical morphology (single files, single cells)	Variant morphology	LN	IC lumina	E-cadherin	CDH1 mutation
15	No	No	No	No	No	No	"Mainly intralobular epithelial overgrowth"	No	No (NA)	No (NA)
16	Yes	**Yes** (uniform)	**Yes** (Low)	No	(Yes)	(Yes) tubulo-lobular, solid	(LCIS)	(Yes)	No (NA)	No (NA)
4	Yes	(Small, round or notched ovoid nuclei, thin rim of cytoplasm)	(Low in classic)	**Yes**	**Yes**	(Yes) solid, alveolar, pleomorphic, tubulo-lobular	**Yes** (usually)	(Yes)	(Yes)	(Mentioned)
5	Yes	(Small, round or notched ovoid nuclei, thin rim of cytoplasm)	(Low in classic)	**Yes**	**Yes**	(Yes) solid, alveolar, pleomorphic, tubulo-lobular, mixed	**Yes** (usually)	(Yes)	(Yes; 15% positive)	(Described)
2	Yes	(Small, round or notched ovoid nuclei, thin rim of cytoplasm)	(Low in classic)	**Yes**	**Yes**	(Yes) solid, alveolar, pleomorphic, tubulo-lobular, mixed	(Yes)	(Yes)	(Yes; 15% positive)	(Mentioned, as background information)
6, 17	No	(Small or medium size, uniform)	No	(Yes: "loosely dispersed)	(Yes)	No	(Yes)	No	No (NA)	No (NA)
18	No	**Yes** (uniform)	**Yes** (Low)	**Yes**	(Yes)	(Yes) tubulo-lobular, solid, alveolar, pleomororphic, histiocytoid variants; trabecular pattern	(Yes)	(Yes)	No (NA)	No (NA)
13	Yes	**Yes** (similar to LIN)	(Low in classic)	**Yes**	(Yes)	(Yes) alveolar, tubulolobular, mixed, pleomororphic, apocrinem histiocytoid, signet ring cell, myoepithelial cell variants; solid	(Yes)	(Yes)	Yes	No
19	(Yes)	**Yes** (regular small)	**Low**	**Yes**	**Yes**	(Yes) solid, alveolar, pleomorphic, tubulo-lobular, mixed	No	**Yes**	(Yes; aberrant expression)	No
20	No	(Yes - similar to LIN)	No	(Yes)	(Yes)	(Yes) solid, alveolar, pleomorphic, tubulo-lobular, mixed	No	No	No	No

Features in bold represent worded elements of the definitions with optional explanations in parentheses, whereas features in parenthesis without bold characters reflect parts of the more detailed descriptions. IC: intracytoplasmatic, LIN: lobular intraepithelial neoplasia, LN: lobular neoplasia, NA: not applicable.

a positive stain in the cytoplasm (Fig. 1D). This IHC panel has largely helped to establish the diagnosis of ILC in doubtful cases.

Unfortunately, loss of E-cadherin is not only seen in ILCs and lobular neoplasia, but is an event that has been associated with increased cell motility, invasion and epithelial-mesenchymal transition [22,25]. In breast pathology, it is widely accepted that lack of E-cadherin immunostaining alone is not sufficient to make the diagnosis of ILC, since non-lobular carcinomas may also occasionally demonstrate this feature [2]. Conversely, ILCs may show E-cadherin staining in about 15% of cases and this has been stressed in the WHO classifications, too, and therefore, E-cadherin positivity should not deviate one from the diagnosis of ILC, if the morphology is typical [2,5].

This E-cadherin positivity seen in ILC has been referred to as aberrant, since it should not be present, but it is there. Aberrant positivity can relate to the presence of the staining at the wrong place, e.g. the cytoplasm, or being weaker and segmental, fragmented on the membrane. Unfortunately, some ILCs aberrantly display a clear-cut positive staining. The addition of other elements of the E-cadherin-catenin complex to the IHC panel may help in clarifying the E-cadherin positive ILCs and distinguish them from NST invasive carcinomas. The catenins often show a typical ILC supportive staining pattern (loss of membranous β-catenin with either no staining or cytoplasmic staining, and displacement of p120 from the membrane to the cytoplasm) [27] (Fig. 1), but there are also cases where the staining becomes more equivocal: weaker, fragmented, but membranous β-catenin staining and membranous plus cytoplasmic p120 staining (Fig. 2), which may still be supportive of the diagnosis of ILC.

ILC and molecular analysis

The lack of E-cadherin function on cell membranes can theoretically derive from several genomic events. One of these is biallelic alteration, mutation of the *CDH1* gene, which can result in no protein in the cell membrane or a protein without adhesive function; such missense mutations have been recognized to be the sources of erroneous classifications as NST carcinomas [21].

Although promoter hypermethylation has been described as a potential way of silencing the *CDH1* gene, a recent review of previous reports points to methodological issues (the use of non-quantitative methylation specific PCR, contamination by cells harbouring methylated *CDH1*) behind the results of earlier studies, and suggest that aberrant DNA methylation is not a mechanism for depriving the cells from E-cadherin [28].

On this basis, *CDH1* mutational analysis has been suggested as an adjunct to make the diagnosis of ILC, and has helped in clarifying some cases, where the morphology and IHC were not convincing enough and/or showed overlapping features [14], [Matthias Christgen oral communication at the 34th European Congress of Pathology, Excerpts from Breast Pathology Symposium, Reproducibility in the diagnosis of invasive lobular carcinoma (ILC)]. *CDH1* mutational analysis is a method not as vastly available as IHC, and therefore, it is not mentioned as a diagnostic criterion of ILC in current recommendations, although it was mentioned earlier as an ancillary test helping the identification of ILCs along with E-cadherin IHC [4]. In a larger series of ILCs, 25/364 cases (7%) were found to lack *CDH1* biallelic alterations, but alterations in other adhesion molecules were detected instead; these involved the *CTNND1* (p120) or *AXIN2 genes,* of which the latter is also reported to play a role in cellular adhesion along its role in the Wnt-signaling [29]. Rarely (with a figure of 14/1101 quoted from The Cancer Genome Atlas) *CTNNA1* somatic mutations or biallelic deletions and consequent loss of the coded α-catenin protein may also be encountered [30], and this may also underlie the ILC morphology. For the completeness of the analysis, *CTNNA1* mutational analysis may also complement the diagnostic work-up of uncertain ILCs. In contrast, it seems that *CTNNB1* (the gene coding β-catenin) mutations are not underlying loss or reduction of β-catenin expression in ILCs or its nuclear translocation and the activation of the Wnt pathway in mainly triple negative NST carcinomas [31].

Very rarely (7/5842, 0.1%), biallelic *CDH1* genetic alterations may be encountered in tumors without lobular features, i.e. in non-ILCs [32].

ILC and tubules, the catenin switch and inconsistencies in the nomenclature

Pathologists noted the presence of tubules in ILC a long time ago. Martinez and Azzopardi mentioned this in their paper assessing the patterns of ILC in 1979 [33]. This is also mentioned in other papers, but as this is in great contradiction with the classical pattern of ILC where the cells are dyscohesive and arranged isolated or in single files, this manifestation has caused trouble ever since. Several scenarios may explain tubules in ILC; these are 1) mixed ductal (NST) and lobular carcinomas, 2) tubulolobular carcinomas and 3) ILC with

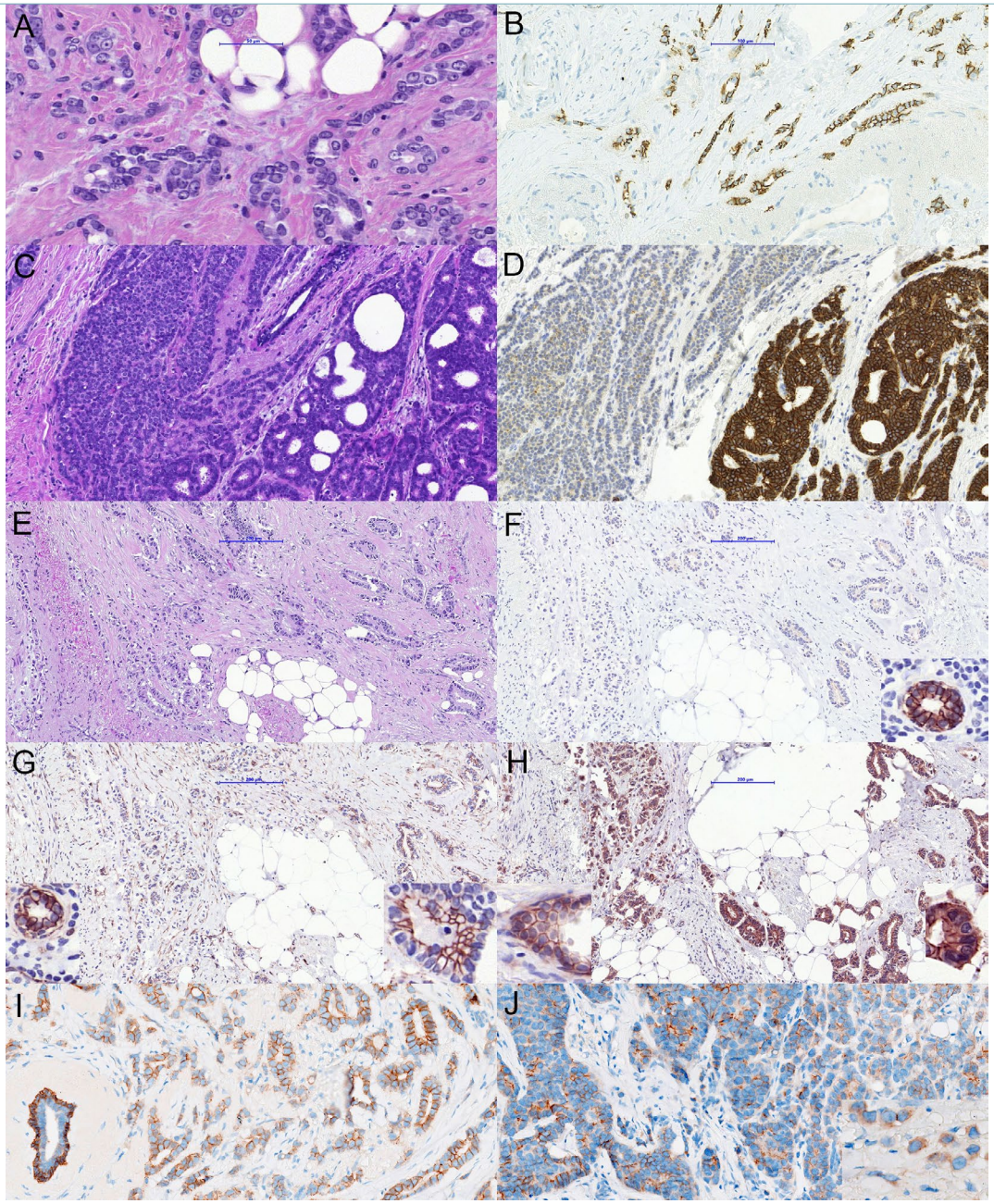

Figure 2. Tubules and invasive lobular carcinoma (ILC). A, B: tubulolobular carcinoma with (generally small sized) tubules and cords reminiscent of lobular carcinoma, all being positive for E-cadherin; this is best regarded as a "ductal" (NST) carcinoma with lobular infiltration pattern / features (A: HE, x40; B: E-cadherin, x20); C-D: Mixed lobular (left) and NST (right) carcinoma with distinct morphological and immunohistochemical dichotomy: dyscohesive E-cadherin-negative cells in ILC, and cohesive, E-cadherin-positive cell in the NST part make up this collision tumor (C: HE, x20; D: E-cadherin, x20); E-J: ILC with tubular elements: easily discernible glandular structures (tubules) are seen along classical or trabecular patterns of invasion of classical ILC (E: HE, x10), both being negative for E-cadherin - the insert in F shows a normal ductal structure being positive (F: E-cadherin, x10, insert: x40); G: The classical ILC area is b-catenin-negative, whereas the normal ducts are positive (left inset) and the tubular element display partial membranous staining (right inset) (b-catenin, x10; insets: x40); H: there is cytoplasmic p120 staining, which is complemented with membranous labelling in the more cohesive areas, including the tubules (right inset) – this is in contrast with the typical staining of normal ducts being only membranous (left inset) (p120 catenin, x10; insets: x40). I: There is also strong membranous labelling of the tubules with P-cadherin, with the myoepithelial cells of a normal duct serving as control, whereas the areas with no tubules but closely arranged, more cohesive cells (J) also show focal, partial membranous labelling; the inset shows non-cohesive ILC cell with no membranous P-cadherin but some cytoplasmic background staining (P-cadherin, I and J: x20, insets x40 – staining, digital slides courtesy of Drs Matthias Christgen and Leonie Kandt).

tubular elements, as nicely reviewed by Christgen et al [9] (Fig. 2).

Tubulolobular (in some texts transcribed as tubulo-lobular) carcinomas (TLCs) have been introduced into the terminology of breast cancer types by Fisher et al in 1977 [34]. These tumors are characterized by the mixture of ILC-like single cell cords, trabeculae and small neoplastic glandular structures, tubules. Importantly, a small series of patients with this type of tumor (24 of 1665 breast cancers; 1.4%) had an intermediate prognosis when compared to tubular carcinomas (with better prognosis) and ILCs (with worse prognosis). Although Fisher et al. stated that the classification as tubular or lobular was a philosophical issue, based on whether one puts more stress on the "structural configuration" (i.e. tubules) or the "growth pattern" (single files, targetoid arrangement), they decided to identify TLCs as a subset of ILCs, and despite contradictory published evidence from a few studies, these tumors are still classified as a subset of ILCs [2,4,5,19], probably because of tradition and/or unclarified terminology and definitions. The last edition of the AFIP fascicle on breast tumors discusses TLCs under separate cover [13]. Indeed, since the discovery of the diagnostic role of E-cadherin and the associated catenins in the differential diagnosis of ILCs, a few studies have examined TLCs, and found that the majority displayed a strong membranous staining with E-cadherin [35], and additionally α-catenin, β-catenin [36,37] plus p120 catenin [38], and concluded that TLC had an overlapping [35] or "ductal" phenotype [36], or that it was a ductal cancer growing in a lobular pattern [36]. This is further reinforced by 3D reconstruction of TLCs, demonstrating the connection between the cords and the tubules making it a network like spatial structure [38]. Therefore, TLC is best regarded as an NST carcinoma with lobular-like growth [9], or synonymously as NST with lobular features or NST with lobular infiltration pattern (Fig. 2AB). However, it is not sure that everyone understands the term of TLC the same way; the tubulolobular pattern of ILC may well refer to the third category discussed under this heading, i.e. ILC with tubular elements.

Mixed carcinomas demonstrating a lobular phenotype with E-cadherin-negative dyscohesive cells and an admixture of E-cadherin-positive tubules, solid nests and/or cords in various proportions are classified as mixed (NST and lobular) carcinomas (Fig. 2CD). The rules for classifying carcinomas as mixed have changed over time. Probably, the era before quantitative rules have come into play allowed any proportion of mixed morphologies to be labelled as mixed carcinoma, but common sense might have made minor components to be ignored, or just mentioned in lengthy phenotypical descriptions of the histological pattern. With quantitative rules for classification, a pure special type carcinoma is generally required to have 90% or more of that special type histology, whereas mixed carcinomas had to have between 50 and 90% special type morphology [5,19]. Currently, a mixed carcinoma must have at least 10% of the special type histology to qualify as such [2]. It goes with this, that carcinomas with < 10% tubule formation are ignored from classification rules, and might be / are probably unanimously reported as pure ILCs. According to the interpretation of this mixture of morphologies as a collision tumor, the dual components show diverse E-cadherin staining, being negative in the lobular and positive in the non-lobular area (Fig. 2CD). Although the collision interpretation is pragmatic, the collision of histological types would suggest two parallelly developing tumors merging with each other. Evidence from molecular studies would rather suggest these components to derive from the same neoplastic clone, and therefore divergent subclonal evolution explains the dual histological type [39]. It is likely that many of the other 2 entities discussed under this heading are also categorized as mixed ILC and NST if E-cadherin IHC is not used as an adjunct in their proper classification. Finally, ILC with tubular elements is a cancer with cells and the infiltration pattern of ILC, with the presence of tubules. Before the widespread use of E-cadherin IHC, these might have been recognized as mixed lobular and ductal/NST carcinomas, but E-cadherin is generally negative in the tubules. The *CDH1* gene is mutated, E-cadherin is missing as a protein, but functionally, it is replaced by P-cadherin and this allows cellular cohesion to return, a phenomenon that defeats the essential diagnostic criteria of ILC [2], as "dispersed or linear dyscohesive cells" are missing from the area involved. This phenomenon of switching to another adhesion molecule, notably P-cadherin in order to replace the missing E-cadherin was described in 2020 by Christgen et al, and explains the molecular mechanism of tubule formation in ILC [40]. However, some P-cadherin staining and cohesion may appear in ILCs without tubules but with other variant patterns (Matthias Christgen personal communication, Fig. 2 IJ). This phenomenon may even occur at metastatic sites, like the colon mucosa, where the microenvironment may play a role in its development [41]. Therefore, P-cadherin IHC may have a role in making the diagnosis of ILC with no E-cadherin staining but some cohesion, depending on how the definitions evolve. Little is known about the behavior of ILCs with tubular structures (or other cohesive areas without tubules). A recent study found that tubule formation was a relatively rare pattern in ILC, and most of the time it involved < 10% of the

tumor, which means that it would be ignored by most pathologists for typing purposes. Certainly, without E-cadherin staining, some ILC with tubular elements would be diagnosed as mixed carcinomas or TLCs. A basis for this latter classification may be the less than clear separation of ILC with tubule formation and non-ILC (NSTs) with lobular infiltration pattern. The current WHO classification defines the tubulolobular pattern of ILC as being "composed of the admixture of a tubular growth pattern and small uniform cells arranged in a linear pattern" [2], and this definition can fit both the Fisherian TLCs which – as stated above – are best regarded as NST carcinomas with lobular infiltration pattern – and ILC with tubular elements. Without further clarifications, there is clearly a possibility of using a single term for two different diseases.

In summary, from a pragmatic point, E-cadherin IHC may be of help to separate the 3 entities with tubules and an ILC like infiltration motif as discussed above. Collision tumors with separate ILC and NST components show a dual E-cadherin IHC phenotype (Fig. 2CD). ILCs with tubular elements are E-cadherin negative (and P-cadherin positive) (Fig. 2EFIJ), whereas NST cancers with lobular growth pattern are E-cadherin positive (Fig. 2AB). There may still be a chance for missense *CDH1* mutations and E-cadherin positivity in this context too, but this will remain a potential pitfall as far as molecular analysis is not part of the work-up and classification. I believe that it would be best to abandon the term tubulolobular, as it may cover both E-cadherin positive tumors (as exemplified by some publications) [12,34-37] and E-cadherin negative ILCs with tubular elements [9,40], or alternately, to categorically define it for current use. Unfortunately, it is not known, how many of the 24 cases identified by Fisher et al. in their series [34] belonged to the first or the second category. The last edition of the WHO classification suggests that E-cadherin loss is a desirable diagnostic criterion for ILCs, including all patterns, i.e. the tubulolobular pattern, too. Therefore, it may suggest that the tubulolobular pattern is what others mean by ILC with tubular elements, but not what others mean by TLC being a cancer with membranous staining for E-cadherin and related catenins. In the third edition of the blue book [4], a proposal was made to use E-cadherin and determine its categorization as tubular (NST) or lobular on this basis, and whenever this is not available, to classify the cases as ILC. This is the approach that can still be suggested today, although it has received less attention in later editions [2,5].

ILC and its definitions

Whenever a single feature is not sufficient to define an entity, and as discussed above, this is the case with ILC (e.g. single filing alone, the presence of associated LN alone… etc are not sufficient on their own to diagnose ILC), a combination of features can / should be used to define it. The present and past "official" classification and other examples of classifications use different stresses on diagnostic components, but most criteria and essential requisites are based on HE morphology (Tab. I) [2,4-6,13,15-20]. The combination of diagnostic criteria is a nice thing, and it is fine when all features are present; this makes the diagnosis of ILC obvious. However, one is left in doubt when only some of the requisites are present and the rest is not, despite the fact that many of us tend to pragmatically ignore minor deviations from the typical. This comes when the morphology leaves the classical pattern, and e.g. trabecular and tubule forming components are part of the tumor. These have been documented to predispose to diagnose ILC as non-ILC [21]. In fact, the HE diagnostic criteria result in moderate inter-observer agreement for making the diagnosis of ILC with a median pairwise kappa of 0.58 [21]. The addition of E-cadherin improves reproducibility, and resulted in a median pairwise kappa of 0.75, reflecting substantial agreement [21]. A histological typing based on HE sections resulted in 14% uncertainty concerning lobular vs non-lobular typing; this was reduced to 5% with the routine use of E-cadherin IHC [14]. This latter approach, i.e. routine E-cadherin staining for diagnosing ILC of breast carcinomas has been reported to be part of histological typing in about half of the 153 pathologists responding to an international survey, but variations in antibodies and protocols may have a great influence on staining and interpreting the results [42]. It has been quoted several times that on the basis of the data from 2 large clinical trials, the MINDACT [43] and the West German Study Group Plan B [44], ILC can be overdiagnosed, since only about 60% of the locally diagnosed cases were accepted as ILC on central review [9,42]. This might probably be due to the lack of E-cadherin IHC, although little is known on the frequency of using this IHC in the routine diagnostic work at the participating local pathology laboratories. The figures point to the fact that the diagnostic criteria laid down are not sufficient to reliably diagnose ILC, and wider use of IHC may help. With the use of IHC, aberrant E-cadherin staining, the possibility of NST carcinomas to lack strong circumferential E-cadherin staining, and technical issues relating to antibody types and staining protocols, novel diagnostic dilemmas may arise. This problem can be illustrated by the figure of 5% uncertainty in type following E-cadherin IHC and a 2% change in histological type (lobular vs non-lobular) or lack of consensus even after expert

review of cases with uncertainty or difficulty in classification [14]. In such cases, further IHC with P-cadherin or catenin members of the E-cadherin-catenin complex [27,40], or CDH1 mutational analysis [14,40,45], may help in sorting out whether the carcinoma studied can be an ILC or not, although this latter approach was mentioned only in the 3rd edition of the WHO blue book ("A combination of mutation analysis and E-cadherin protein expression may offer a method for identification of lobular carcinoma.") [4], and was discontinued in the later editions; therefore, this is not included in the current diagnostic criteria [2,5] (Tab. I).

A practical approach

This is a summary of personal practice considering the doubts described in previous sections of this manuscript.

When the infiltration pattern, cellular composition, associated lobular neoplasia and clinical presentation are typical, i.e. classical pattern ILC is encountered, make the diagnosis of ILC (classical pattern). Remember, this is often associated with at least minor areas of cells not arranged in single files or isolated in the stroma [12,13]. E-cadherin IHC is advised against by several authors [1,27], but if ever done, consider its results only if there is no staining or the staining is clearly aberrant, and tend to ignore positivity (also a rarer form of aberrant staining in this context). In the latter case, weigh your opinion in the context of the whole picture (e.g. presence of LCIS, additional IHC, mutational analyses if available), but give HE morphology high priority. To illustrate this, our series of 1001 breast cancers included a single case where all six pathology opinions were for the case being an ILC, despite a completely "ductal" type E-cadherin and β-catenin stain and lack of *CDH1* alterations [14].

In variant morphologies, when a tumor is likely to represent ILC, E-cadherin IHC may help confirming the diagnosis, and I prefer to use it on a regular basis. If cellular dyscohesion is present, the morphology is lobular (angulated, compressed nuclei, vacuoles), the E-cadherin stain is missing from the cell membranes, ILC with recognized non-classical patterns can be diagnosed. Although the WHO blue book [2] recognizes only some of the patterns that have ever been described as variations on the theme of ILC (See e.g. Christgen et al for review [9], other morphological variants may be mentioned in the report, or until further clarification of the definitions, these can be lumped under the classical form without mentioning them specifically. One pattern requires special attention, and this is when neoplastic glandular structures, tubules are also present, and there are areas with cellular cohesion. From a pragmatic point, 3 scenarios should be considered: E-cadherin (and β-catenin, p120 catenin) positive and negative areas ("collision" tumor consisting of lobular and non-lobular, generally NST components); E-cadherin positive tumors representing NST carcinomas with lobular infiltrative pattern, inclusive of the rare TLC described by Fisher; and finally E-cadherin negative tumors, which are a subset of ILC but form tubules ("with tubular elements") and have also been referred to as tubulolobular pattern in the WHO blue books. This latter pattern typically shows an E- to P-cadherin swich, therefore P-cadherin positivity may be a supportive additional IHC, as well as the catenins, where typical staining (β-catenin negativity and cytoplasmic p120 staining) may occur, but may also appear as hybrid staining (aberrant β-catenin, and combined cytoplasmatic and membranous p120 labelling).

Additional IHC (P-cadherin, catenins) may also be useful when in doubt on the basis of HE morphology (e.g. not a clearcut lobular cellular shape, but a lobular infiltration pattern at least partially, or dyscohesion being partial) or/and non-obvious E-cadherin IHC (Fig. 3). In such doubtful cases, it is pragmatic to rely on IHC and classify the tumor as ILC or non-ILC on the basis of the IHC staining pattern. If doubt remains or in any conditions where contradiction is experienced between morphology and IHC staining patterns, *CDH1* mutational analysis by next generation sequencing may shift the diagnosis from one entity to the other (ILC in cases of mutations with protein changes or lack of protein) [14] [Matthias Christgen oral communication at the 34th European Congress of Pathology, Excerpts from Breast Pathology Symposium, Reproducibility in the diagnosis of invasive lobular carcinoma (ILC)], but this approach is not widely available. Consultation with peers might be an additional step, but two people can be simultaneously wrong (or right or discrepant) and the majority may not always give the ground truth (e.g. Figs. 2I-J are from case 146 of a previous study [14], with 3 opinions for NST, 2 for ILC with tubular elements and 1 for uncertainty on type).

In a few cases, one might be left with uncertainty in type, and in such cases, it might be wise to follow the protocols for ILC (e.g. MRI for extent and contralateral disease, considering it less suitable for neoadjuvant chemotherapy unless pleomorphic and high grade).

With all this, a diagnosis regarding lobular histological type (i.e. ILC) may be established in the great majority of the cases, but whether this diagnosis has the same impact on prognosis and treatment decision remains to be shown. *CDH1* mutations maybe associated with targetable pathways resulting in synthetic lethality, and this may be a novel treatment modality which is tested

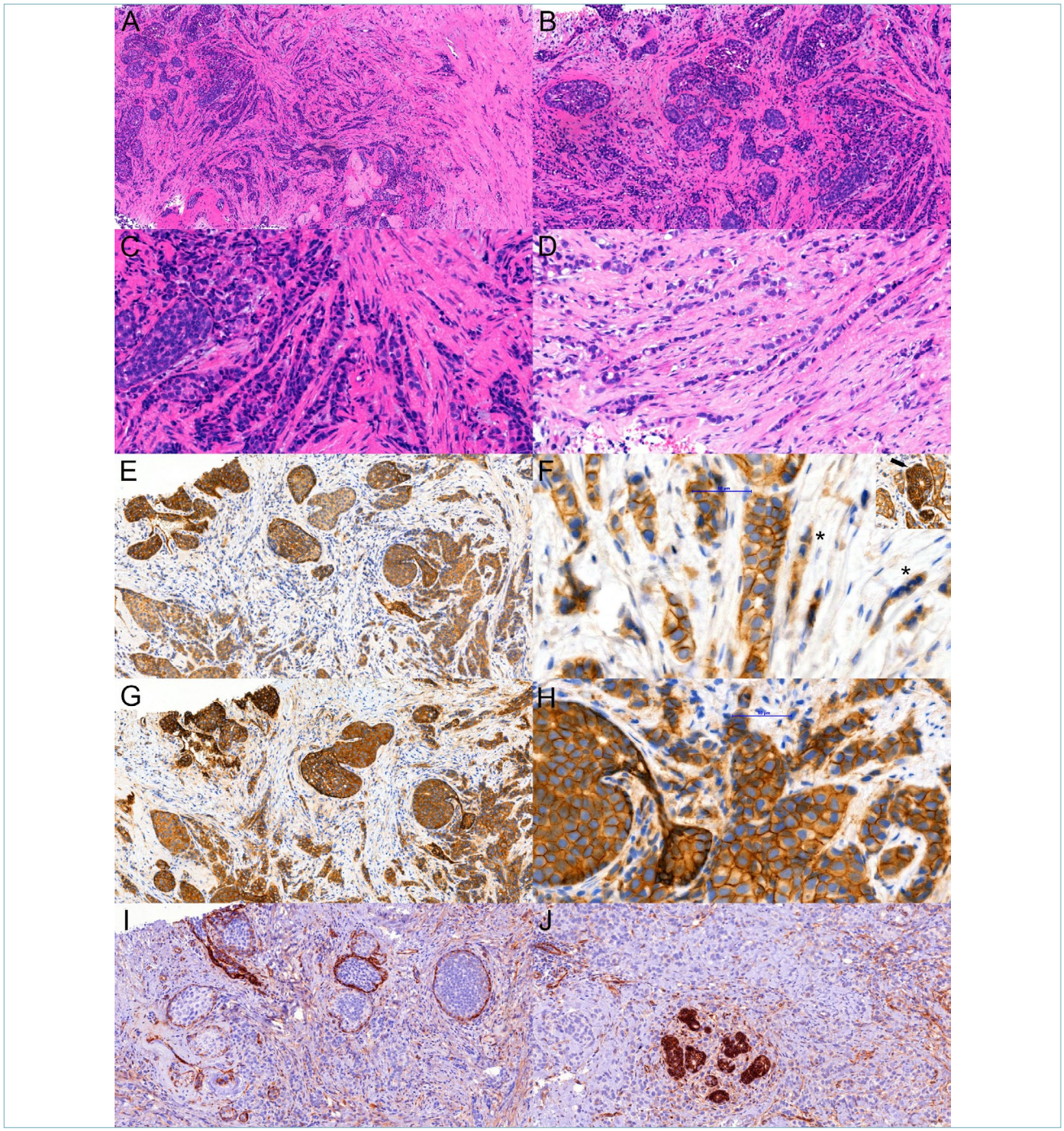

Figure 3. A controversial case analysed step-by-step. Step 1: HE morphology (A-D). This is a core needle biopsy from a palpable tumor manifesting as a 6-cm-large architectural distortion, visible on ultrasound as a 3.3 cm hypoechoic mass with shadow, classified as malignant on the basis of imaging (R5, U5). A, B and C show the same area with increasing magnifications, and represent some trabecular infiltration pattern giving about 80-90% of the specimen, some cellular dyscohesion is perceptible at high power (C); D shows and area with a more classical pattern, although not specific, some intracellular vacuolisation also suggests the possibility of a variant ILC (HE, A: x10, B: x20, C & D: x40). In situ component (left of A and B) is also compatible with classical LCIS. Step 2: E-cadherin IHC (E-F). According to personal experience such cases are better reinforced by E-cadherin IHC. However, the cytoplasmic and membranous positivity of the case may question the true histological type. Some single files (*) on part F do not show membranous staining (E-cadherin, E: x20, F: x40). Step 3. Further IHC (G-J). A p120 stain shows both cytoplasmic and membranous labelling (p120, G: x20, H: x40). Such staining is compatible with aberrant E-cadherin staining and a lobular histological type, but can also be seen in non-lobular cancers with not perfectly titrated monoclonal antibody and cytoplasmic background staining. The problem of overlapping features and not straight forward IHCs is finally solved by the β-catenin IHC, which shows the absence of this protein from the membranes (β-catenin, I: x20, J: x25); note positivity of myoepithelial cells around LCIS (I), vessels (I, J) and normal acini in a lobule (J). On this basis, this is an ILC.

in clinical trials (ROLo, ROSALINE) [46,47]. But what are the consequences of a cadherin-switch returning some cells to the cohesive state remains to be clarified.

Finally, a note needs to be inserted. The definitions currently available do not give a 100% key to the identification of ILCs, and the features that should be given more or less weigh in making this diagnosis. It is even not evident if E-cadherin immunophenotype should be included in the definition or not, and when it should be part of the diagnostic work-up. Until the definitions are improved, one should navigate carefully between the interpretational possibilities, and this review aimed to help this navigation. This is a personal approach at present time in a field that is dynamically changing. There are several groups aiming at better characterising ILC (European Lobular Beast Cancer Consortium, Lobular Breast Cancer Alliance), and therefore novel results may alter the definitions of ILC, its diagnostic requirements, and therefore the approach may change over time; we are also awaiting the publication of the 6th edition of the WHO blue book.

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