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Lobular neoplasia and invasive lobular breast cancer: Inter-observer agreement for histological grading and subclassification

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ABSTRACT

Lobular neoplasia (LN), invasive lobular breast cancer (ILBC) and related pleomorphic variants represent a distinct group of neoplastic mammary gland lesions. This study assessed the inter-observer agreement of histological grading in a series of ILBC and LN.

54 cases (36x ILBC, 18x LN) were evaluated by 17 observers. 3978 classification calls on various histological features, including nuclear grade, proliferative activity (Ki67 immunohistochemistry, categorical scoring), histological grade and pleomorphism were obtained. Pairwise Cohen's kappa values were calculated and compared between various features and different observer subsets with variable histomorphological experience.

In ILBC, pairwise inter-observer agreement for histological grade ranged from poor to almost perfect concordance and was higher in advanced and experienced histopathologists compared with beginners ($P < 0.001$). Agreement for proliferation (Ki67) ranged from slight to almost perfect concordance and was also higher in advanced and experienced histopathologists ($P < 0.001$). Considering different features, agreement for proliferation (Ki67) was superior to agreement for histological grade and nuclear grade, even among advanced and experienced histopathologists ($P < 0.001$). In LN, agreement for B-classification ranged from poor to almost perfect concordance and was higher in advanced and experienced histopathologists ($P < 0.001$). Considering different features, agreement for proliferation (Ki67 in LN) was superior to subclassification agreement based on conventional features, such as acinar distention and nuclear grade ($P < 0.001$).

In summary, pairwise inter-observer concordance of histological grading of ILBC and LN is dependent on histomorphological experience. Assessment of proliferation by Ki67 immunohistochemistry is associated with favorable inter-observer agreement and can improve histological grading of ILBC as well as LN.

1. Introduction

Lobular neoplasia (LN), invasive lobular breast cancer (ILBC) and related pleomorphic variants represent a distinct group of neoplastic mammary gland lesions. These lesions arise from mammary epithelial cells, which have lost the cell adhesion molecule E-cadherin by somatic mutation or epigenetic inactivation [1,2]. Loss of Ecadherin renders tumor cells resistant against anoikis, a form of anchorage-dependent programmed cell death, and abrogates cell adhesion [3]. ILBC and LN can show a wide spectrum of different histomorphological presentations [4]. Nuclear atypia, proliferative activity, growth pattern and tumor cell burden can differ significantly in different patients and in separate lesions within the same mammary gland [4,5].

As any other type of invasive breast cancer, ILBC is subjected to

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standardized histological grading using the modified Scarff-Bloom-Richardson (mSBR) score [6–8]. Histological grade is a proven prognostic factor in ILBC [9,10]. However, assessment of the mSBR score, based on the degree of tubule formation, nuclear atypia and proliferation, can be difficult in ILBC. The scoring point value for histoarchitecture is invariable (3 points), because ILBCs essentially lack tubule formation [11,12]. Assessment of nuclear atypia, however, is complicated by nuclear compression artifacts, which are inherent to this tumor entity and may reflect altered cytoskeleton stability and constitutive actin/myosin filament contraction [4]. Assessment of proliferation by mitotic counts or immunohistochemistry for Ki67 may be complicated by comparatively low cellularity [13]. Two previous studies have shown, that pairwise inter-observer agreement for histological grade is significantly lower in ILBC compared with breast cancer of no special type [14,15]. In extremely poorly differentiated ILBCs, histology can resemble undifferentiated pleomorphic sarcomas. These cases are traditionally termed pleomorphic ILBC [16]. Pleomorphic ILBCs may display altered oncogene activation pattern and higher oncotype DX recurrence scores [10,17–20]. However, no attempts for a definition or morphological standardization of pleomorphic ILBC have been published yet. Prognostic and therapeutic implications remain controversial, probably because diagnostic criteria for pleomorphic ILBC are not well standardized [21].

LN can show a particularly wide spectrum of histomorphological presentations, ranging from diminutive lesion with hardly any nuclear atypia to carcinoma in situ with high grade nuclear atypia. For LN, several histological classification schemes are used in clinical diagnostics. Their prognostic and biological relevance is controversially discussed [22–24]. The World Health Organization (WHO) currently classifies LN as atypical lobular hyperplasia (ALH) or lobular carcinoma in situ (LCIS), depending on the degree of acinar filling and distention [11]. This classification, termed WHO scheme herein, was first proposed by Haagensen and colleagues [25]. A modified classification scheme was proposed by Bratthauer and Tavassoli [26]. According to the Bratthauer scheme, lobular intraepithelial neoplasia (LIN) is divided into grades 1-3 (LIN 1-3) and florid LIN, depending on the degree of lobular distention and other features [26,27]. The Bratthauer scheme was adopted by many histopathologists, but it is currently not endorsed by the WHO [11]. A third classification has evolved in the context of mammography screening [28]. According to the B(biopsy)-classification, which stratifies cases for further diagnostics or surgical intervention, LN or LIN can be classified as a B3-type lesion (facultative cancer precursor with uncertain malignant potential) or a B5a-type lesion (obligate cancer precursor equivalent with in situ malignancy) [29]. LN with pronounced nuclear atypia is well documented [16]. Depending on the preferred classification, these cases are termed pleomorphic LCIS (pLCIS) or pleomorphic LIN (pLIN) and are included within B5a-type lesions [30,31]. The B-classification has clinical relevance. B3-type LN or LIN can be managed by clinical observation without surgery, under certain circumstances [30]. B5a-type LN or LIN warrants surgical resection [30]. A fourth approach to the classification of LN has been suggested by Andrade and colleagues. Microarray gene expression profiling revealed two distinct clusters of LN cases, which were not related to morphological LN sub-entities, but differed in the expression of proliferation-associated genes, most notably Ki67 [32]. Accordingly, Andrade et al. discussed that immunohistochemistry for Ki67 may have the potential to differentiate between proliferating versus growth-suppressed LN [32].

The present work aimed to assess the pairwise inter-observer agreement for histological grading and subclassification of ILBC and LN. Particular attention was dedicated to pleomorphic ILBC, B5a-type LN and the impact of histomorphological experience on grading agreement.

Table 1
Tissue specimen.

	number	percent
LN and ILBC, all cases	54	100
tissue format		
core needle biopsy	26	48
resection	28	52
histology		
LN, pure	15	28
LN in complex sclerosing lesion	3	5
ILBC with LN	7	13
ILBC without LN	29	54
E-cadherin status		
negative	54	100
positive	0	0

2. Materials and methods

2.1. Tissue specimens

Tissue specimens included n = 54 cases (Table 1). All specimens were selected from consecutive cases encountered in routine clinical diagnostics. Poorly differentiated ILBCs with features attributable to pleomorphic ILBC were preferentially selected. In the original diagnostic reports, 15/36 (42%) ILBCs were classified as G3-differentiated ILBC and the median Ki67 index was 20% (range 1% to 90%) (Supplemental Data Table 1). Standard formalin-fixed paraffin-embedded tissue blocks were retrieved from the archive of the Institute of Pathology of the Hannover Medical School (MHH, Hannover, Germany). All specimens were made anonymous for subsequent scientific purposes. All cases were subjected to immunohistochemistry for E-Cadherin (ECH-6 antibody, Zytomed Systems, Berlin, Germany) and Ki67 (rabbit 30-9 antibody, Ventana, Tuscon, U.S.A.) [5]. Complete loss of E-cadherin expression was confirmed in all cases.

2.2. Histological evaluation

For histological evaluation, haematoxylin-eosin (HE)-stained and Ki67-stained sections were provided together. All observers received the same set of slides. Observers could opt for virtual microscopy or glass slides. HE-stained and Ki67-stained sections were scanned using a dotSlide scanner microscope (Olympus Soft Imaging Solutions GmbH, Münster, Germany) and are available for virtual microscopy (http://patserv01.mh-hannover.de/). All observers were provided with a study handbook, which included detailed evaluation instructions, all classification criteria in tabular form as published in the 2012 edition of the WHO classification of tumors of the breast and relevant literature references [11,16,25–28,32,33].

Cases of ILBC were evaluated for nuclear grade (ordinal, 1, 2 or 3), proliferation (ordinal, 1, 2, or 3, corresponding to Ki67 indices of 0–10%, 11–25%, and > 25% for low, intermediate and high proliferation), histological grade using a mSBR score (ordinal, 1, 2, or 3), composed of architecture, nuclear grade and Ki67-based proliferative activity instead of mitotic counts, and the histological variant (ordinal, non-pleomorphic versus pleomorphic) [33]. Ki67 assessment technique (eyeballing versus counting) was not regulated. Of note, no attempts for a definition or morphological standardization of pleomorphic ILBC have been published yet. Therefore, for the diagnosis of pleomorphic ILBC, all observers were instructed to adhere to the phenotypic features or criteria described by Eusebi and colleagues [16].

Cases of LN were evaluated for nuclear grade (ordinal, 1, 2, of 3), proliferation (ordinal, 1, 2, or 3, corresponding to Ki67 indices of 0–10%, 11–20%, and > 20%) and were subclassified according to the current WHO scheme (ordinal, 1, 2 or 3, corresponding to ALH, LCIS or pleomorphic LCIS), the Bratthauer scheme (ordinal, 1, 2 or 3,

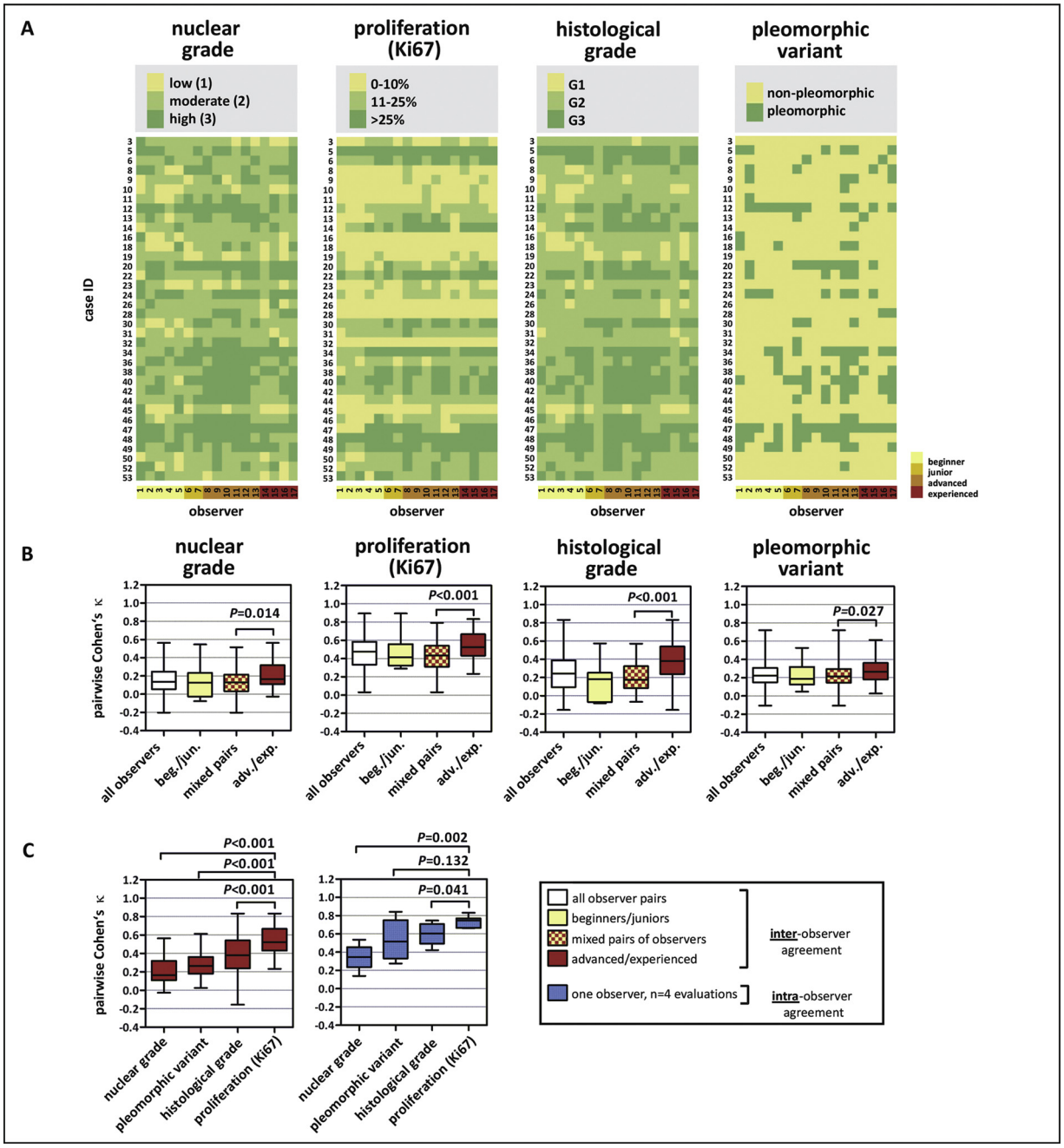

Fig. 1. Histological evaluation of ILBC by different observers. **(A)** Two-dimensional presentation of n = 2448 classification calls. Each row represents an ILBC specimen and each column represents an individual observer. Study IDs and observer IDs are indicated at the y- and x-axis, respectively. Classification calls are coded by color, as indicated in the legend. **(B)** Pairwise Cohen's kappa values for assessment of nuclear grade, proliferation (Ki67), histological grade and the pleomorphic ILBC variant. Whiskers indicate the minimal and maximal kappa value. Boxes indicate the 25- and 75-percentiles. Horizontal lines in the box centers indicate the median. Data obtained in different observer subsets (all observers, beginners/juniors, mixed pairs, advanced/experienced histopathologists) are coded by color, as indicated in the legend. **(C)** Pairwise Cohen's kappa values reflecting inter-observer agreement in advanced/experienced histopathologists (left) and intra-observer agreement (right).

corresponding to LIN1/LIN2, LIN3, and pleomorphic LIN/florid LIN/LIN3 with necrosis), and the B-classification (nominal, B3, B5a, or other B-category) [11,26,28]. B-classification was applied to core needle biopsy specimens and resection specimens alike.

2.3. Observer characteristics

Between 2016 and 2018, histological evaluation was completed by n = 17 observers, who were blinded to classification results of other participants. Observers were from different institutes and had different levels of histomorphological experience. Recruitment of heterogeneous observer groups with different levels of experience has previously been described for the assessment of pairwise inter-observer agreement for scoring chronic viral hepatitis [34]. This approach was adopted here [34]. Observers included n = 4 experienced histopathologists (diagnostic experience at the time of the evaluation ≥15 years, from three different institutes), n = 6 advanced histopathologists (diagnostic experience of 6 to 14 years, from five different institutes), n = 2 junior histopathologists (diagnostic experience ≤5 years, from two different institutes), and n = 5 beginners in histopathology (four medical

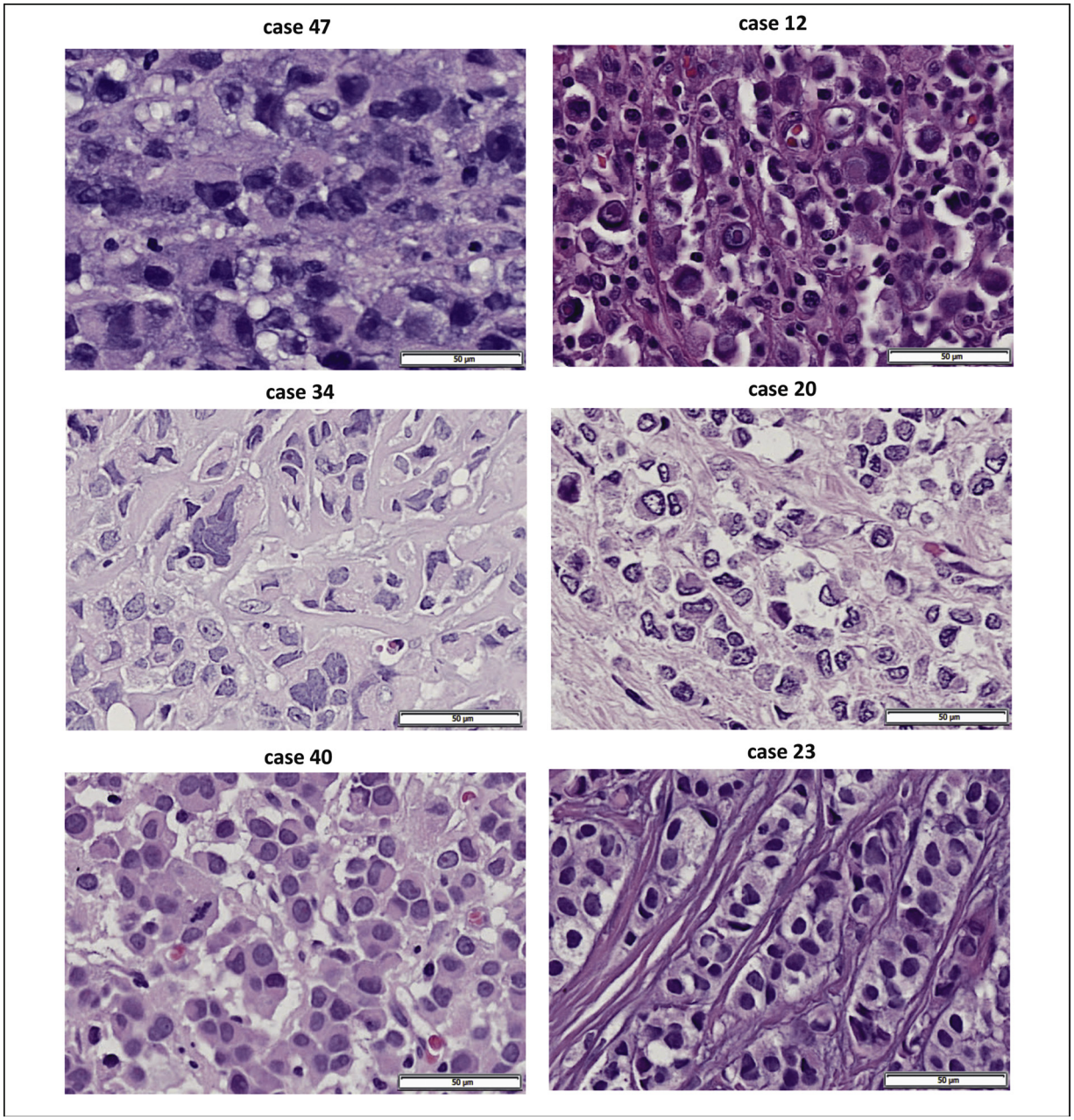

Fig. 2. Histology of cases classified as pleomorphic ILBC by the majority of observers. For details see text.

students after a 30-h course in histopathology, and one histology technician educated in cytopathology). Experienced and advanced histopathologists had different sub-specializations. 6/10 of the experienced and advanced histopathologist were specialized on breast pathology. Before participating in the study, all beginners attended a 1-h crash course and photomicrograph presentation on ILBC and LN.

2.4. Data collection and statistics

Classification data were collected using study handbooks with checkmark matrices for each of the n = 54 cases, which was completed by all participants. A total of n = 3978 classification calls were obtained (36 × 17 × 4 = 2448 for ILBC, and 18 × 17 × 5 = 1530 for LN) and were subsequently transcribed in tables using Microsoft Excel software (Excel 2016, Microsoft Corporation, Redmond, Washington, U.S.A.) (Fig. 1A and 3 A). For statistical analysis of inter-observer agreement, pairwise Cohen's kappa values (ranging from -1 to 1) were calculated for each pair of observers (n = 136 observer pairs in total) using JMP software (JMP 11, SAS Institute Corporation, Cary, North Carolina, U.S.A.). As the group of observers was heterogeneous in terms of histological experience and to enable comparison with similar studies, conservative pairwise Cohen's kappa statistics were favored over other statistical methods, such as Fleiss' kappa statistics or weighted kappa statistics [14,15,34,35]. GraphPad Prism software (GraphPad Prism 5, GraphPad Software, Inc., San Diego, California, U.S.A.) was used to calculate the median of pairwise Cohen's kappa values and to determine statistical significance using the Mann-Whitney test [34]. Pairwise Cohen's kappa values were also evaluated separately for observer pairs composed of only experienced/advanced histopathologists (n = 45 pairs), as well as only beginners/junior histopathologists (n = 21 pairs), and for mixed pairs composed of a beginner/junior and an experienced/advanced histopathologist (n = 70 pairs). Intra-observer agreement was assessed based on data of one advanced observer trained in breast pathology, who completed the series four times. Kappa

Table 2
Features of cases classified as pleomorphic ILBC.

observers classifying as pILBC	case 47 15/17 (88%)	case 12 11/17 (64%)	case 34 10/17 (58%)	case 20 9/17(52%)	case 40 9/17(52%)	case 23 0/17 (0%)
features attributable to pILBC						
overall impression: sarcomatoid	1					
growth pattern: vague trabecular	1			1	1	
growth pattern: loose solid or solid		1	1	1	1	
nuclear size: strongly enlarged	1	1	1			
nuclear size: variable	1	1	1	1		
nuclear shape: squeezed to triangles			1	1		
chromatin: deep-dark hyperchromatic	1	1				
chromatin: vesicular, clumped				1		
chromatin: variable chromatin			1	1		
multiple apoptotic figure per high power field	1		1		1	
multiple mitotic figure per high power field	1				1	
proliferation: Ki67 index > 25%	1		1		1	
cytoplasm: wide eosinophilic			1		1	

1: feature present; pILBC: pleomorphic ILBC.

values for agreement were interpreted as follows: < 0.0 (poor), 0.0–0.20 (slight), 0.21–0.40 (fair), 0.41–0.60 (moderate), 0.61–0.80 (substantial), and 0.81–1.00 (almost perfect) [36].

3. Results

3.1. Inter-observer agreement for ILBC

In ILBC, pairwise inter-observer agreement for nuclear grade ranged from poor to moderate concordance (pairwise Cohen's kappa −0.20 to 0.56, median 0.14) (Fig. 1B). Agreement for proliferation (Ki67) ranged from slight to almost perfect concordance (Cohen's kappa 0.03 to 0.89, median 0.48). Agreement for histological grade varied between poor and almost perfect concordance (Cohen's kappa–0.16 to 0.83, median 0.24). Agreement for pleomorphic ILBC did not exceed substantial concordance (Cohen's kappa −0.10 to 0.72, median 0.22). Pairwise Cohen's kappa values were higher in observer pairs composed of advanced/experienced histopathologists compared with beginners/juniors or mixed pairs (Fig. 1B). For instance, the median Cohen's kappa value for histological grade among advanced/experienced histopathologists was twice as high as among beginners/juniors or mixed pairs of observers (median Cohen's kappa 0.38 versus 0.18, $P < 0.001$) (Fig. 1B). Among advanced/experienced histopathologists, the median Cohen's kappa value for proliferation (Ki67) was higher than the median Cohen's kappa value for histological grade, pleomorphic variant and nuclear grade (median Cohen's kappa 0.52 versus 0.38 versus 0.27 versus 0.16, $P < 0.001$) (Fig. 1C, left panel). Intra-observer agreement was generally higher than inter-observer agreement (Fig. 1C and Supplemental Data Fig. 1, Supplemental Data Table 2 and 3).

3.2. Histology of cases classified as pleomorphic ILBC

The ILBCs included n = 5 cases (IDs 47, 12, 34, 20, 40) which were classified as pleomorphic by the majority of observers. These cases were re-reviewed retrospectively (Fig. 2). Case 47 was classified as pleomorphic by 15/17 (88%) observers, including all advanced and experienced histopathologists. Case 47 featured a vaguely trabecular growth pattern and a sarcomatoid appearance with numerous apoptotic bodies. Nuclei were strongly enlarged and hyperchromatic. Nucleoli were highly variable in size. However, case 47 lacked the wide, granular eosinophilic cytoplasm originally described for pleomorphic ILBC [16]. Case 34 was classified as pleomorphic by 10/17 (59%) observers, including 4/6 of the advanced histopathologists and 3/4 of the experienced histopathologists. Case 34 featured a loose solid growth pattern. Nuclei were massively enlarged and individual nuclear figures were wrinkled or squeezed into rectangular or triangular shape. Nuclei displayed different chromatin qualities. Contrary to case 47, case 34 featured wide slightly eosinophilic cytoplasm. The Ki67 proliferation index of cases 47, 34 and 40 was classified as high (> 25%) by the majority of observers. The Ki67 proliferation index of cases 12 and 20 was classified as intermediate (11%–25%) by the majority of observers. Features attributable to pleomorphic ILBCs were present in these cases in different mosaic patterns (Table 2).

3.3. Inter-observer agreement for LN

Pairwise inter-observer agreement for subclassification of LN according to the WHO scheme, including pleomorphic LCIS, ranged from poor to almost perfect concordance (pairwise Cohen's kappa −0.21 to 1.00, median 0.06) (Fig. 3B). Classification according to the Bratthauer scheme, including pleomorphic and florid LIN, ranged from poor to substantial concordance (Cohen's kappa–0.07 to 0.63, median 0.19). Agreement for nuclear grade ranged from poor to moderate concordance (Cohen's kappa −0.34 to 0.50, median 0.08). Agreement for B-classification ranged from poor to almost perfect concordance (Cohen's kappa −0.35 to 1.00, median 0.21). Agreement for proliferation (Ki67) ranged from poor to almost perfect concordance and achieved the highest median Cohen's kappa value in LN (Cohen's kappa −0.09 to 1.00, median 0.42) (Fig. 3B)). Pairwise Cohen's kappa values were higher in observer pairs composed of advanced/experienced histopathologists compared with mixed pairs of observers, but only regarding the Bratthauer scheme and the B-classification (Fig. 3B). Among advanced/experienced histopathologists, the median Cohen's kappa value for proliferation (Ki67) was higher than the median Cohen's kappa value for the Bratthauer and WHO classification schemes and nuclear grade (median Cohen's kappa 0.42 versus 0.24 versus 0.00 versus 0.08, $P < 0.001$) (Fig. 3C, left panel). Agreement for proliferation (Ki67) and B-classification were not different (median Cohen's kappa 0.42 versus 0.29, $P = 0.107$) (Fig. 3C, left panel). Intra-observer agreement was higher than inter-observer agreement, but only for the WHO classification scheme and B-classification (Fig. 3C, right panel and Supplemental Data Fig. 1).

3.4. Histology of LN cases classified as B5a lesions

The LN specimens included n = 4 cases (IDs 4, 7, 33 and 1) which were informative on histological features influencing B-classification calls.

Case 4 was classified as a B5a-type lesion (in situ malignancy) by 15/17 (88%) observers, including 5/6 advanced and 4/4 experienced histopathologists. This case featured pronounced acinar and ductal distention, multiple foci with comedo-type necrosis (> 200 µm

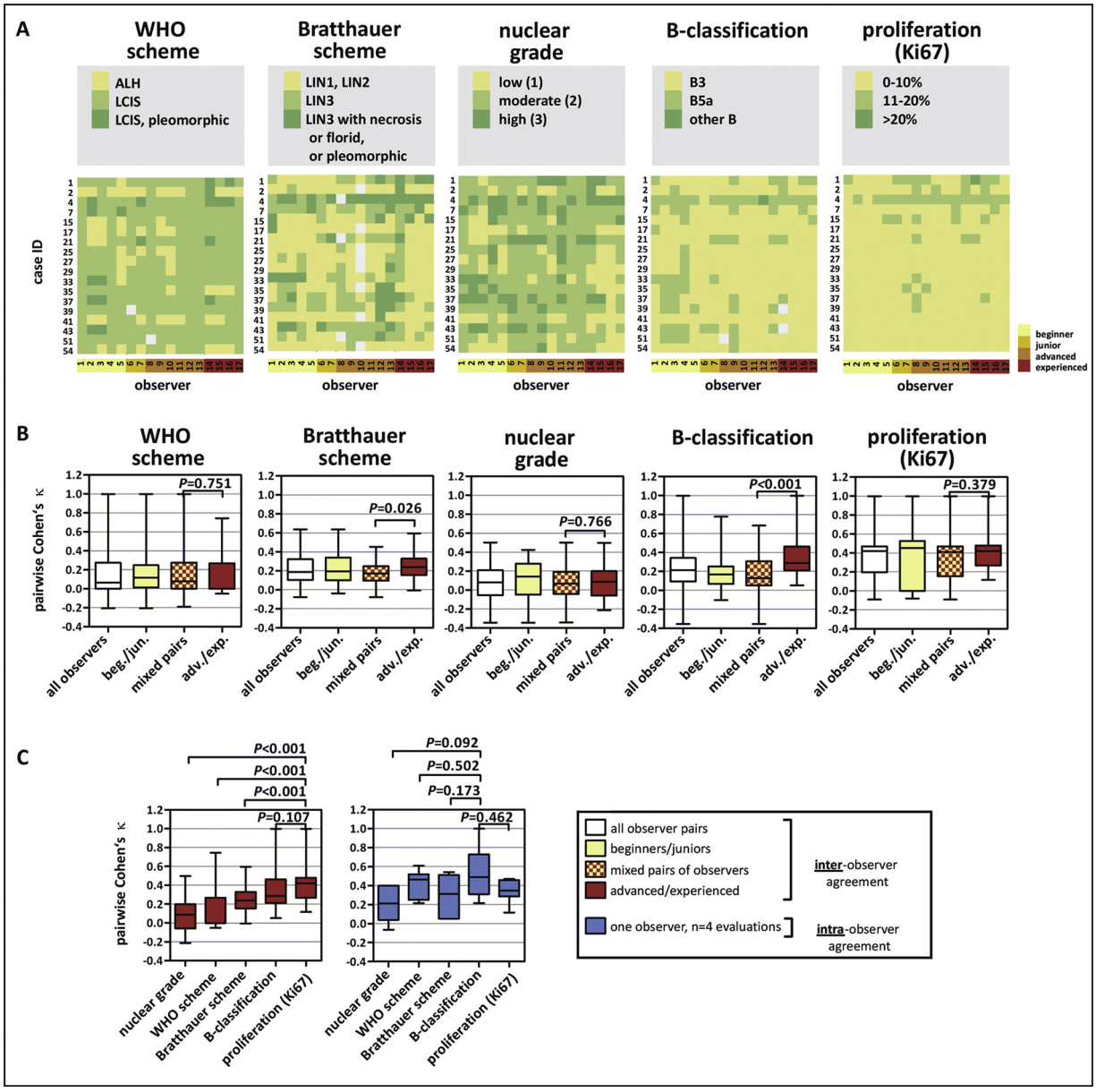

Fig. 3. Histological evaluation of LN or LIN by different observers. **(A)** Two-dimensional presentation of n = 1530 classification calls. Each row represents a case of LN and each column represents an individual observer. Study IDs and observer IDs are indicated at the y- and x-axis, respectively. Classification calls are coded by color, as indicated in the legend. **(B)** Pairwise Cohen's kappa values for classification according the WHO scheme, the Bratthauer scheme, nuclear grade, B-classification and proliferation (Ki67). Whiskers indicate the minimal and maximal kappa value. Boxes indicate the 25- and 75-percentiles. Horizontal lines in the box centers indicate the median. Data obtained in different observer subsets (all observers, beginners/juniors, mixed pairs, advanced/experienced histopathologists) are coded by color, as indicated in the legend. **(C)** Pairwise Cohen's kappa values reflecting inter-observer agreement in advanced/experienced histopathologists (left) and intra-observer agreement (right).

diameter and with prominent calcifications), and moderate to high nuclear grade. The Ki67 proliferation index was classified as 11–20% by 15/17 (88%) observers. Ki67-positive nuclei were randomly distributed over cross-sections of distended ducts and acini, a pattern not seen in other cases of LN in this study (Fig. 4). In other words, the inner and the outer half of distended ducts and acini showed similar percentages of Ki67-stained nuclei (no central growth suppression) (Fig. 4).

Case 7 was classified as a B5a-type lesion by 6/17 (35%) observers, including 2/6 advanced and 1/4 experienced histopathologists. It lacked comedo-type necrosis and featured pronounced acinar distension. The Ki67 index was concordantly classified as 0–10% by 15/17 (88%) observers. Case 7 showed a peculiar Ki67 staining pattern with Ki67-positive nuclei along the basement membrane and only few Ki67-positive nuclei in the center of distended acini (Fig. 5). In other words, the inner and the outer half of distended ducts and acini showed markedly different percentages of Ki67-stained nuclei (pronounced central growth suppression) (Fig. 5). A similar Ki67 staining pattern was present in other cases of pure LN (e.g. cases 17, 21 and others), and was also seen in cases of LN associated with ILBC (e.g. case 45).

Case 33 was classified as a B5a-type lesion by 5/17 (29%) observers, all of whom were beginners or junior histopathologists. In case 33, LN involved an area of sclerosing adenosis within a complex sclerosing lesion (CSL). Experienced histopathologists interpreted this case as a LIN1/2 with nuclear grade 1, corresponding to a B3-type lesion. Surprisingly, most beginners classified this lesion as a LIN3 with nuclear grade 3. This suggests that the complex architectural pattern of this composite lesion had interfered with the perception of the nuclear grade in beginners. 16/17 (94%) observers classified proliferation of

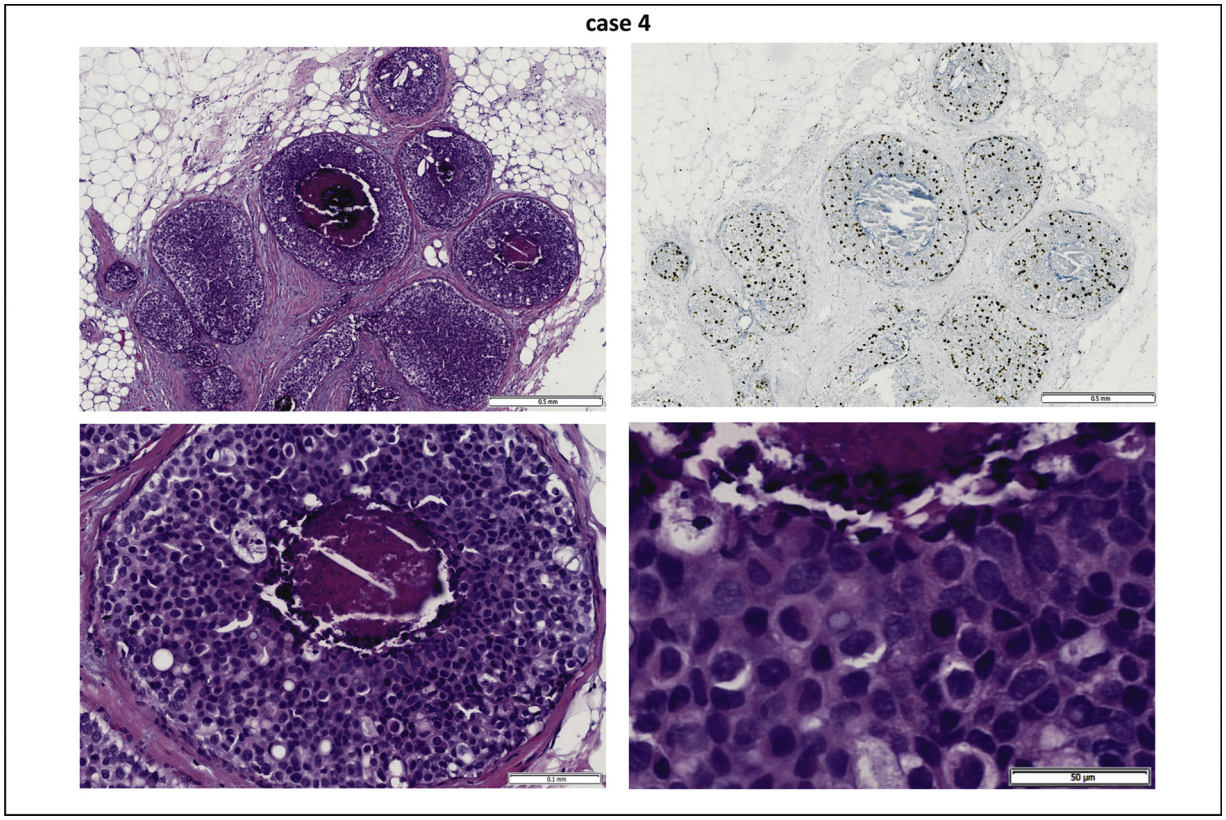

Fig. 4. Histology of LN case 4, classified as a B5a lesion by the majority of observers. Note that Ki67-positive nuclei are randomly distributed over cross-sections of distended ducts and acini.

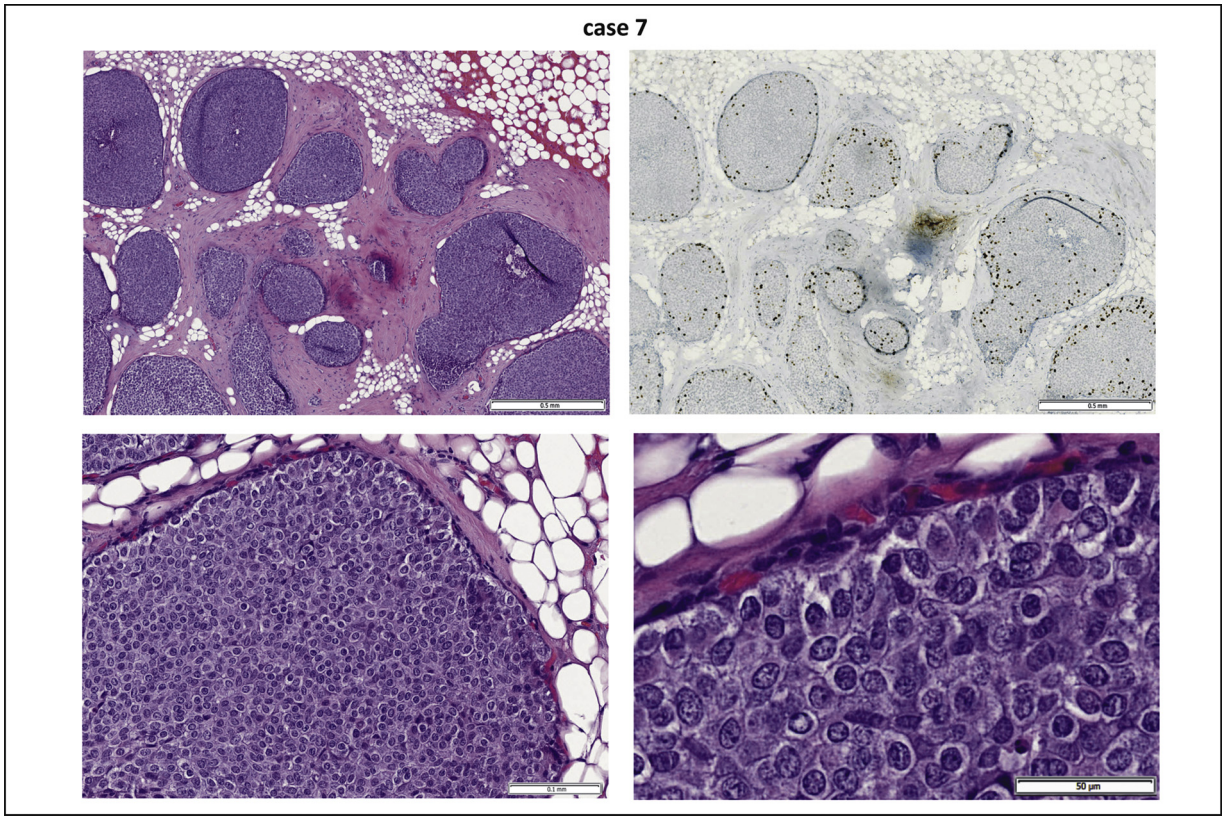

Fig. 5. Histology of LN case 7. Note the central growth suppression in distended ducts and acini. Ki67-positive nuclei are located in the periphery of distended ducts and acini.

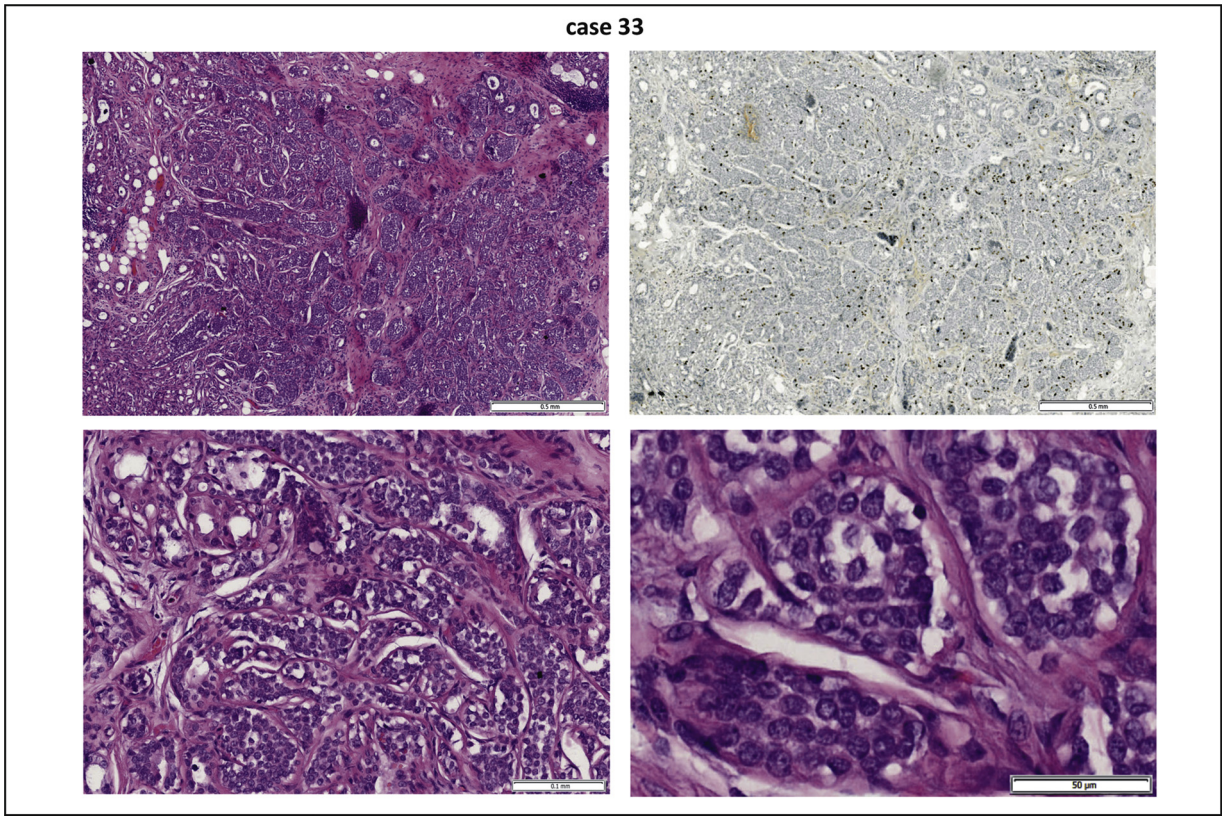

Fig. 6. Histology of LN case 33. Composite lesion of LN within a sclerosing adenosis.

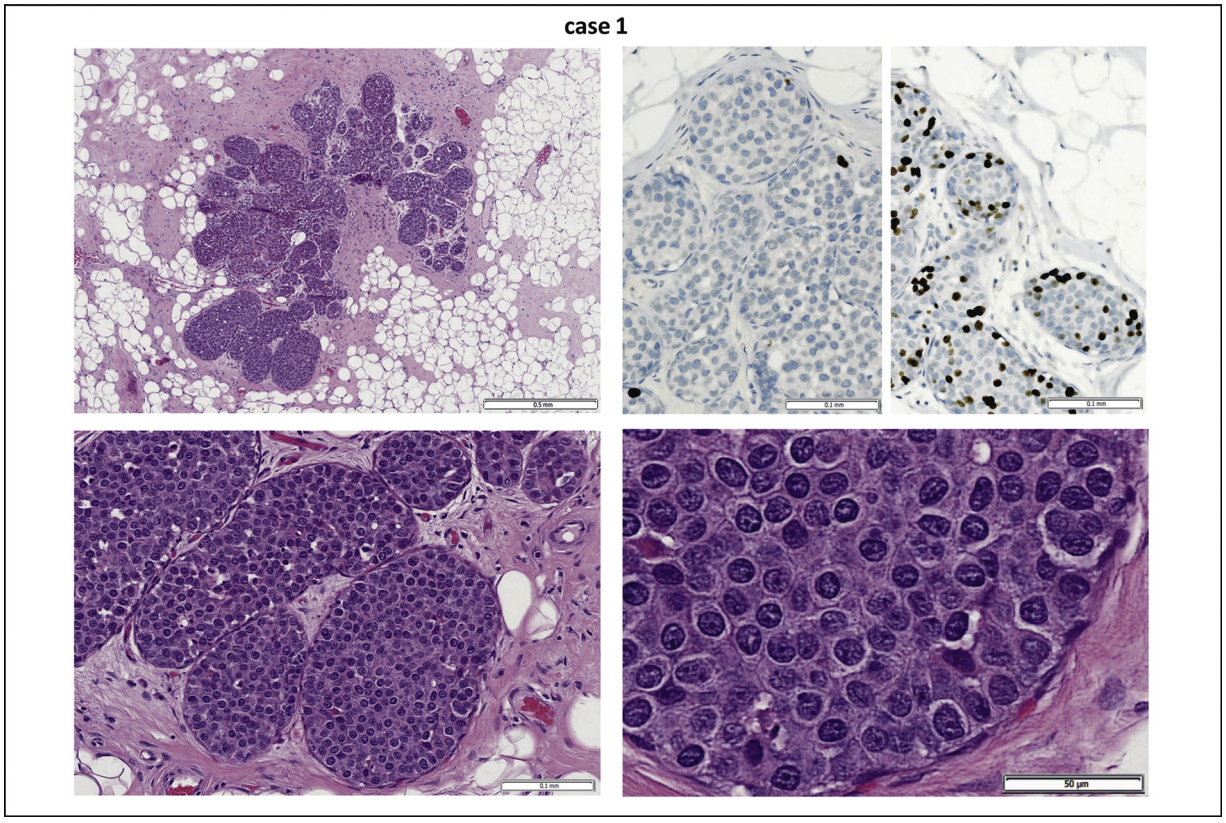

Fig. 7. Histology of LN case 1. Proliferative activity varied between different affected lobules.

this case of LN as low (Ki67 < 10%) (Fig. 6).

Case 1 was classified as a B5a-type lesion by 6/17 (35%) observers, including 2/6 advanced and 3/4 experienced histopathologists. In this specimen, LN affected several ductal-lobular units with either moderate or pronounced acinar distention and pagetoid extension into mammary ducts. Comedo-type necrosis or calcifications were not present. Nuclear atypia varied from lobule to lobule. 2/4 experienced histopathologists decided for nuclear grade 2, and another 2/4 experienced histopathologists decided for nuclear grade 3. Proliferation (Ki67) also varied from lobule to lobule. Experienced histopathologists classified the Ki67 index as 0–10% (1x), 11–20% (2x) or > 20% (1x). Multifocality and morphological heterogeneity may therefore have influenced the classification agreement in this case. Of note, some foci of this LN showed a beginning loss of the above-described central growth suppression (Fig. 7).

4. Discussion

Inter-observer agreement for histological grading and subclassification of invasive lobular breast cancer and lobular neoplasia is rarely investigated apart from ductal breast cancer of no special type. This study assessed the pairwise inter-observer agreement for histological grading in a series of n = 54 ILBC and LN specimens. Particular attention was dedicated to pleomorphic ILBC, B5a-type LN or LIN and the relevance of histomorphological experience for classification agreement.

In ILBC, pairwise inter-observer agreement for any histological feature (nuclear grade, Ki67, histological grade, pleomorphic variant) was higher in advanced/experienced histopathologists compared with beginners/junior histopathologists. This exemplifies that histological grading requires year-long training, which is a truism for histopathologists. However, even among advanced/experienced histopathologists, overall agreement for histological grade was only fair (median Cohen's kappa 0.38). This is in line with a previous study by Adams and colleagues. In their study, n = 5 experienced histopathologists had graded a series of n = 38 ILBCs and had achieved a median pairwise Cohen's kappa value of 0.37 [14]. Concerning pleomorphic ILBC, inter-observer agreement was also only fair (median Cohen's kappa 0.27). Immunohistochemistry for Ki67 was used to assess proliferative activity in ILBC. The technique of Ki67 evaluation (eyeballing versus counting) was not regulated, but the Ki67 index was scored in a three-tiered fashion (0–10%, 11–25%, and > 25%). Strikingly, Ki67 achieved the highest median Cohen's kappa values, independent of histomorphological experience (median Cohen's kappa values of 0.52 for advanced/experienced histopathologists and 0.48 for all observers). This indicates that assessment of tumor cell proliferation by Ki67 immunohistochemistry is comparatively robust and has favorable agreement characteristics in ILBC.

Concerning LN or LIN, subclassification agreement was slightly higher in advanced/experienced histopathologists, but only for the Bratthauer classification scheme and the Bclassification. Regarding the B-classification, overall inter-observer agreement for B5a-type LN showed only fair concordance (median Cohen's kappa 0.29 for advanced/experienced histopathologists). Of note, this is still better than the result in the only comparable study, which has recently been reported by Singh and colleagues. In their study, n = 6 breast histopathologists from two institutions had evaluated n = 25 LN specimens for pleomorphic (B5a)-type features and had achieved a median pairwise Cohen's kappa value of 0.16 [37]. Therefore, in clinical diagnostics, debatable cases of LN should be submitted for second opinion consultation without hesitation.

In the present study, immunohistochemistry for Ki67 was employed to assess proliferation of LN or LIN, as has recently been suggested by Andrade et al. based on microarray gene expression data [32]. Assessment of Ki67 achieved the highest inter-observer agreement in LN (median Cohen's kappa 0.42) and was superior to agreement based on conventional features, such as acinar distention or nuclear grade. Cases of LN with moderate to pronounced acinar distention mostly showed central growth suppression in Ki67-stained section. B5a-type LN showed a loss of this central growth suppression (id est equal percentages of Ki67-stained nuclei in the inner and outer half of distended ducts and acini) (Fig. 4). Currently, there is no data available regarding the frequency of ILBC adjacent to LN with or without this central growth suppression. Nonetheless, loss of central growth suppression, as determined by Ki67 immunohistochemistry, may be considered an additional criterion for classifying LN cases as B5a-type lesions.

As every study of this kind, this work has to be interpreted with caution. First of all, not all observers were histopathologists with experience in clinical diagnostics and the cutoffs for categorization of the experience level based on years of clinical practice were arbitrary. Inexperienced observers were included to enable a comparison against untrained individuals. Second, the overall distribution and frequency of certain histological features within the case collection was not exactly the same as seen in clinical routine. In fact, ILBCs were enriched in G3-differentiated cases and LN cases included complex composite lesions. Also, controversial cases were not discussed among participating observers. Despite these limitations, the present study may allow for some conclusions regarding the inter-observer agreement of histological grading and the robustness of grading-relevant histological features in ILBC and LN.

In summary, concordance of histological grading of ILBC and LN is fair and depends on histomorphological experience. Assessment of proliferation by Ki67 immunohistochemistry is associated with favorable inter-observer agreement and can improve histological grading of ILBC as well as LN.

Statement of author contributions

Nora Schaumann, Matthias Christgen and Hans Kreipe designed the study. All authors contributed to data collection and analysis. Nora Schaumann and Matthias Christgen wrote the manuscript. All authors approved the final version of the manuscript.

Declaration of Competing Interest

All authors declared no conflict of interest. This manuscript comprises only original, unpublished work and is not under consideration of publication anywhere else.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.152611.

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