

Maria P. Foschini · Alberto Righi · Maria C. Cucchi ·
Teresa Ragazzini · Stefano Merelli · Bruna Santeramo ·
Vincenzo Eusebi

The impact of large sections and 3D technique on the study of lobular in situ and invasive carcinoma of the breast

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Abstract The aim of the present study was to investigate the type of intraglandular spread of lobular neoplasia (LN) and its relationship with invasive lobular carcinoma (ILC) through three-dimensional (3D) stereomicroscopy and analyses of large histological sections (histological macrosections, HM). Fifteen cases showing multiple foci of in situ LN and/or ILC (1 pure LN, 12 LN+ILC, and 2 pure ILC) constituted the basis of the present study. Thirteen cases were treated with mastectomy (including the case of pure LN), and two cases were treated with quadrantectomy. In all cases, large parallel 5-mm-thick sections were em-

bedded in paraffin and stained with hematoxylin and eosin (H&E). Selected large paraffin blocks were investigated with stereomicroscopy. The H&E-stained HM were then compared with the corresponding tissues examined using stereomicroscopy. (1) LN was multicentric in nine cases. (2) The average maximum distance among LN foci was 37.9 mm, while the average maximum distance among ILC areas was 58.2 mm. (3) On 3D examination, LN-filled acini and ducts appeared dilated. When “Pagetoid spread” was present, the ducts were lined by a continuous layer of neoplastic epithelium. (4) No anastomoses between lobes were observed in the two cases where glandular trees were visualized. (5) In 12 cases, ILC areas enveloped ducts and acini affected by LN—an association that was more than coincidental. (6) Multicentric ILC areas not associated with LN indicated vascular spread. It is concluded that the information given in LN and ILC, obtained by analyses of large histological sections, is far superior than that obtained by analyses of conventional histological sections, which underestimate multiple distant small foci of invasion. 3D sections are useful in understanding the architecture of specific lesions.

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M. P. Foschini · A. Righi · T. Ragazzini · V. Eusebi
Section of Anatomic Pathology,
Department of Oncological Sciences,
University of Bologna, Bellaria Hospital,
Bologna, Italy

M. C. Cucchi
Section of Oncologic Surgery,
Department of Oncological Sciences,
University of Bologna, Bellaria Hospital,
Bologna, Italy

S. Merelli
Section of Plastic Surgery,
Department of Oncological Sciences,
University of Bologna, Bellaria Hospital,
Bologna, Italy

B. Santeramo
Department of Surgery,
Clinica Villa Laura,
Bologna, Italy

V. Eusebi (✉)
Anatomia Patologica,
Ospedale Bellaria,
Via Altura, 3,
40139 Bologna, Italy
e-mail: Vincenzo.Eusebi@ausl.bologna.it
Tel.: +39-51-6225523
Fax: +39-51-6225759

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Introduction

Large histological sections (histological macrosections, HM) and three-dimensional (3D) stereomicroscopy allow the visualization of the entire mammary glandular tree. 3D studies were originally applied to mouse breast tissues and gave relevant insights into the development of normal breasts [1, 9].

The site of origin of ductal carcinoma in situ (DCIS)/ductal intraepithelial neoplasia (DIN) is believed to be the terminal ductal lobular unit (TDLU) [21], and the type of intraglandular spread of DCIS/DIN in terms of unifocality or multifocality has been repeatedly shown [7, 11]. Accordingly, well-differentiated DCIS recognizes a multifocal

discontinuous type of growth, with gaps of noninvolved ductal walls located between two neoplastic foci. On the contrary, poorly differentiated DCIS spreads along the ducts in a continuous unifocal fashion without visible gaps [7, 11]. The correlation between mammography and pathology is also enhanced by HM and 3D studies [19]. 3D reconstruction of the breast glandular tree, recently aided by computers, has allowed a better understanding of the normal glandular structure of the breast, which is subdivided into lobes (anatomical structures that are independent of one another) [10, 13]. Lobes vary from 11 to 48 (median, 27) [10] and do not have apparent anastomoses among themselves.

To date, lobular carcinoma, either in situ (lobular neoplasia, LN) or invasive (invasive lobular carcinoma, ILC) [22], has never been studied at the 3D level and, to our surprise, there has only been one study that used HM on ILC, but it did not mention LN [17].

During routine practice, it is common to observe cases containing multiple foci of LN or ILC located far away from one another, but the multifocality (here defined according to Tot [17] as multiple foci in the same lobe) or multicentricity (here defined according to Tot [17] as multiple foci present in different lobes) of LN is a highly controversial issue in the literature. Foote and Stewart [8] stated that LN is always a disease of multiple foci. Subsequent reports have confirmed that multicentric LN is identified in 60–80% of mastectomy specimens [16]. These conflicting results probably are consequent to the fact that, in routine practice, biopsy provides only a limited tissue sample and, thus, the process cannot be visualized in its entire extension.

The purpose of the present study is to investigate the intraglandular spread, size, and multifocality and/or multicentricity of LN and ILC, using HM and 3D stereomicroscopy.

Materials and methods

Fourteen cases that have undergone preoperative diagnostic procedures [either fine needle aspiration cytology or core biopsy (CB)] for palpable nodules, which in turn have led to diagnostic categories of positive (13 cases) or suspicious for malignancy (1 case) [3], were randomly selected for this study from the files of the Section of Anatomic Pathology of the University of Bologna at Bellaria Hospital. Two cases were pure ILC and 12 cases showed LN and ILC simultaneously.

An additional case of pure LN diagnosed by histology of tissues excised for reduction mastoplasty was also included in the study.

On mammography, no calcifications were visible in all cases. The only criterion of selection was the processing of cases using the procedure for macrosections (see below).

The diagnostic criteria of LN (also called lobular in situ neoplasia, LIN) and ILC were in consonance with current histological criteria [22]. Accordingly, E-cadherin immunostaining (monoclonal, clone HECD-1, dilution 1:1,000; Zymed) yielded negative results in all cases. Twelve cases underwent mastectomy, the case of pure LN was treated with bilateral subcutaneous mastectomy, and two cases were treated with quadrantectomy. Axillary dissection was performed in 11 cases.

Specimens were received fresh. In all cases, large parallel 5-mm-thick slices (also called macrosections) were obtained. Care had been taken to slice each section, which included the nipple, perpendicularly to the skin during mastectomy. Slices were then formalin-fixed and paraffin-embedded. From each large block, hematoxylin and eosin (H&E)-stained HM were obtained [9] and used for routine histology.

For 3D stereomicroscopy study, large paraffin blocks were selected with the guidance of a related large H&E

Table 1 Clinical data

| Case | Age (years) | Surgical procedure | Laterality | Histological ILC subtype | Mets |
|------|-------------|--------------------|------------|--------------------------|--------------|
| 1 | 56 | Mastectomy | U R | Pleomorphic | Axly + |
| 2 | 44 | Mastectomy | U L | Pleomorphic | Axly + Men |
| 3 | 41 | SC mastectomy | B | | ND |
| 4 | 61 | Mastectomy | U L | Classic | Axly |
| 5 | 53 | Quadrantectomy | U L | Classic | No Axly mets |
| 6 | 63 | Mastectomy | U L | Pleomorphic | Axly + |
| 7 | 72 | Mastectomy | U L | Pleomorphic | ND |
| 8 | 69 | Mastectomy | U R | Pleomorphic | Axly + |
| 9 | 56 | Mastectomy | U L | Pleomorphic | Axly + |
| 10 | 74 | Mastectomy | U R | Classic | Axly + |
| 11 | 46 | Mastectomy | U R | Classic | Axly + |
| 12 | 85 | Quadrantectomy | U | Classic | ND |
| 13 | 77 | Mastectomy | U L | Pleomorphic | No Axly mets |
| 14 | 87 | Mastectomy | U L | Classic | ND |
| 15 | 50 | Mastectomy | U L | Pleomorphic | Axly + |

SC Subcutaneous, U unilateral, B bilateral, R right breast, L left breast, Mets metastases at presentation, Axly axillary lymph nodes, Men meningeal metastases, ND axillary dissection not performed

Table 2 Summary of pathological data

| Case | LN foci (<i>n</i>) | LN <i>D</i> (mm) | ILC foci (<i>n</i>) | ILC <i>D</i> (mm) | ILC (mm) ^a | LN in ILC |
|------|----------------------|------------------|-----------------------|-------------------|-----------------------|-----------|
| 1 | 49 | 60 | 4 | 15 | 47 | 39 |
| 2 | 9 | 32 | 19 | 67 | 20 | 4 |
| 3 R | 77 | 112 | – | – | | |
| 3 L | 1 | | – | – | | |
| 4 | 21 | 44 | 2 | 10 | 38 | 10 |
| 5 | 27 | 32 | 1 | 0 | 10 | 15 |
| 6 | 5 | 22 | >100 | 50 | | 5 |
| 7 | 3 | 24 | 1 | 0 | 30 | 3 |
| 8 | 2 | 5 | >100 | 130 | 70 | 2 |
| 9 | 3 | 12 | 9 | 85 | 68 | 3 |
| 10 | 49 | 44 | 2 | 13 | 40 | 49 |
| 11 | 2 | 17 | 21 | 55 | 30 | 2 |
| 12 | 50 | 36 | 4 | 70 | 40 | 38 |
| 13 | – | – | 19 | 64 | 16 | 0 |
| 14 | 14 | 15 | 1 | 0 | 45 | 14 |
| 15 | – | – | 8 | 82 | 21 | – |

R Right breast, *L* left breast, *n* number, *LN D* maximum distance between LN foci, *ILC D* maximum distance between ILC areas, *LN* lobular neoplasia, *ILC* invasive lobular carcinoma
^aThe size of the greatest area is reported

section. Selected blocks were then deparaffinized. Paraffin was melted in a hot bath at 60°C (3–4 h) and subsequently immersed in xylene (four times) for at least 24 h to remove residual paraffin [6]. Tissues were then rehydrated as follows: 50% absolute alcohol and 50% xylene (1 h), absolute alcohol (2 h), and 70% alcohol (2 h). Finally, blocks were washed overnight in distilled water, stained in Harris' hematoxylin for 4–5 min, rinsed in tap water for 10 min, and immersed in four baths of acid alcohol for 8 min each. Then tissues were dehydrated, through a graded series of alcohol to xylene, and finally immersed in methyl salicylate for one night.

3D examination was performed using a stereomicroscope (Nikon, Tokyo, Japan). The H&E-stained large histological slide was used to retrace the lesions to examine corresponding cleared tissues.

In all cases, the number of LN foci (focus is defined here as neoplastic cells located within, at least, a space of one another) and the number of ILC areas (area is defined here as a clump, sheet, or nest of ILC cells), independently of their size, were counted. The distance between foci and areas was assessed microscopically using calibrated lenses on large histological slides.

Results

Clinical data are summarized in Table 1. All patients were female, with age ranging from 41 to 87 years (mean, 62 years). Twelve cases showed LN and ILC at the same time, while one case (case 3) showed only bilateral LN. Pure ILC was present in two cases.

All LN foci showed features of LIN 2, as recently subtyped [22]. ILC was of the classic type in six cases and was of the pleomorphic type [5] in eight cases.

Number of LN foci With the exception of the two ILC cases that did not contain LN foci, all the other cases, as

seen in HM, ranged from a maximum of 77 LN foci randomly distributed throughout the whole section of the right breast in case 3 to a minimum of 2 foci in cases 8 and 11 (mean, 23.9 foci) (Table 2 and Fig. 1). Case 3, the only one that had undergone bilateral mastectomy, had only one focus of LN in the contralateral breast. In no fewer than seven cases was the number of LN foci higher than ten (53.8%).

Distance between LN foci as measured on HM The maximum space between the most distant LN foci ranged from 112 mm in the right breast of case 3 (Fig. 1) to 5 mm in the breast of case 8 (mean, 37.9 mm). The maximum space was greater than 20 mm in not less than nine cases (69%).

Number of ILC areas The number of ILC areas in the 14 cases ranged from 1 (in three cases, 21.4%) to more than 100 (in two cases, 20.7%). The areas were diffusely spread throughout the breast tissues of the various cases and, as general rule, there was a dominant larger-sized area in each case (see Table 2 and Fig. 2a). When multiple ILC areas were present in the same cases, diffuse vascular in-

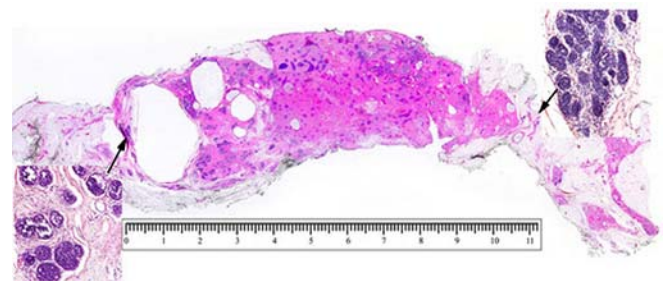


Fig. 1 Case 3: subcutaneous mastectomy. In this case, 77 foci of LN were observed. The maximum distance between LN foci was 112 mm. In both insets, LN involvement of acini is evident

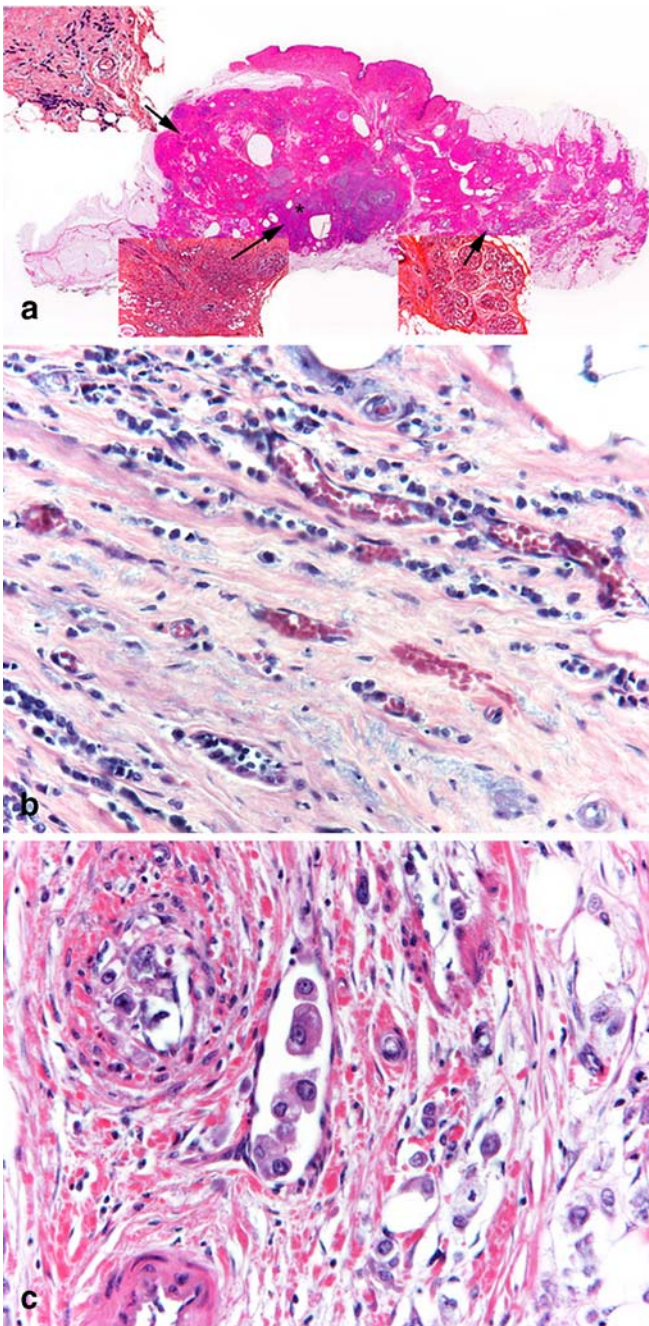


Fig. 2 Case 2. **a** In this case, LN foci (*lower right inset*), multiple ILC areas (*upper inset*), and ILC intermingled with LN (*lower left inset*) are present. (*) A dominant large area is evident. **b** Neoplastic invasive cells are located in perivascular spaces reminiscent of Hartveit's prelymphatic labyrinth. **c** In the center, neoplastic cells are clearly evident in a small vessel. *Right*: there are cells located in spaces suggestive of Hartveit's channels

vasion or invasion of Hartveit's pseudovascular channels [2] was observed (cases 2, 6, 8, 9, 11, 13, and 15) (Fig. 2b,c).

Distance between ILC areas as measured on HM The maximum distance of uninvolved tissue between the most distant ILC areas ranged from 10 to 130 mm (mean,

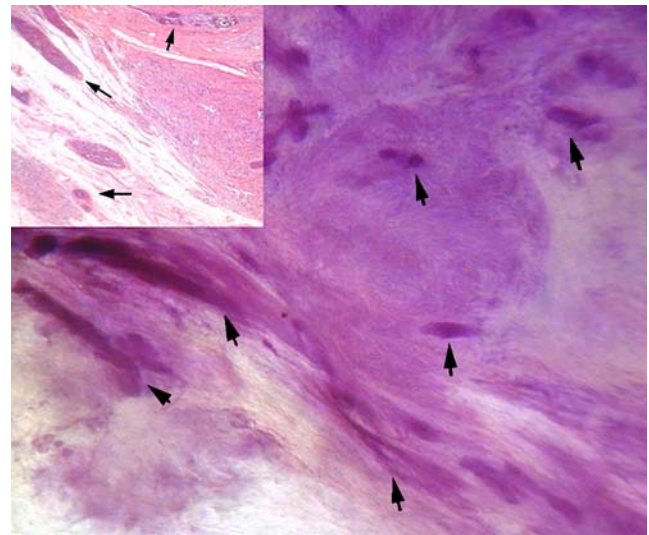


Fig. 3 Case 4: LN foci (*arrows*) immersed in ILC cells. On 3D stereomicroscopy, the invasive area shows a "cloudy" appearance with evanescent margins. *Inset*: the corresponding area at HM shows LN (*arrows*) intermingled with ILC

58.2 mm). The size of the largest ILC areas in each case ranged from 10 to 70 mm (mean, 36.7 mm).

Relationship between LN foci and ILC areas In no fewer than ten cases most of the LN foci were located within the largest sized area of ILC, enveloped by and immersed in invasive neoplastic cells (Fig. 3). Nevertheless, in five of such cases (cases 1, 2, 4, 5, and 12), some foci (5–12 foci) of LN were independent of ILC areas. On the contrary, in nine cases, several ILC areas located far from the largest area of ILC did not contain LN foci.

A total of 234 foci of LN were observed in the 12 cases where LN and ILC were present at the same time, and it appeared that 184 LN (78.6%) were located inside ILC areas.

Three-dimensional study On 3D examination, lobular acini of LN were enlarged and globoid, and the related TDLU

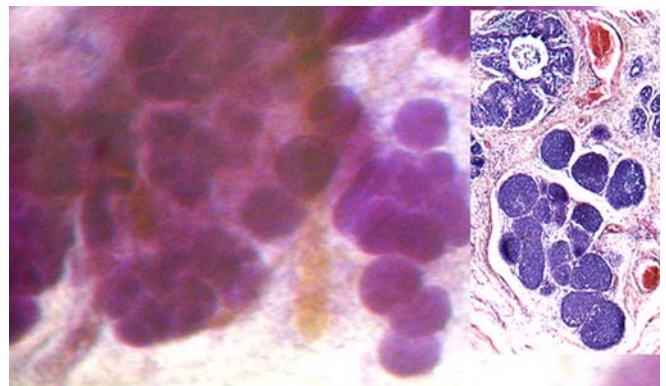


Fig. 4 Case 3: the 3D appearance of LN shows the uniform growth of neoplastic cells in acini (as also shown in the *inset* from the same area) as seen with HM

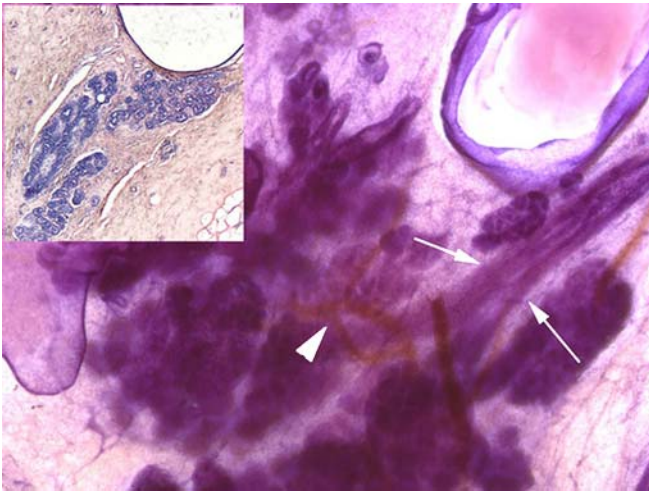


Fig. 5 Case 5: at 3D level, Pagetoid spread manifests as a continuous intramural growth along the duct (arrows). The arrowhead shows serpiginous vessels. Inset: the corresponding histological section as seen with HM

appeared completely filled by neoplastic cells (Fig. 4). No anastomoses between different subsegmental ducts were observed.

LN spread intramurally along larger ducts from acini, presenting the features of “Pagetoid spread” in two cases (cases 3 and 5) (Fig. 5). The spread showed a continuous pattern of growth, and no gaps of noninvolved glandular walls were visible. Duct walls appeared as thickened, bluish structures (Fig. 5). ILC appeared as finely granular, bluish areas that resembled clouds with fading margins. ILC “clouds” (Fig. 6) enveloped ducts and lobules affected by LN (Fig. 3) and also contained numerous vessels.

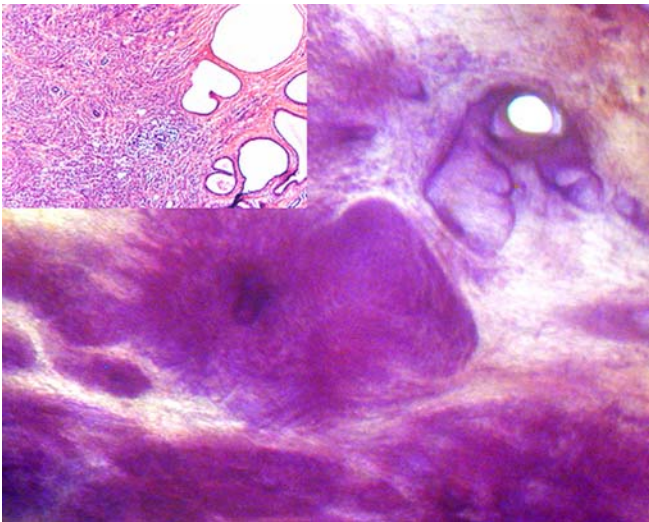


Fig. 6 Case 10: invasive carcinoma appears on 3D stereomicroscopy as bluish, irregular “clouds.” Inset: the same area as seen in “conventional” histology

Discussion

Since the seminal paper of Foote and Stewart [8] in which it was stated that all LN recognize multiple foci of growth, multicentricity in LN has been shown in as many as 85% of patients [15]. This is in keeping with the present data in which the 13 cases containing LN showed a wide range of multiple foci varying from 2 to 77 (mean, 23.9) in various cases, with no fewer than seven cases (53.8%) having ten or more foci.

Tot [18] has suggested that DCIS usually arises in one “sick” lobe only. This in view of the fact that the maximum distance between different foci of DCIS does not exceed 10 mm in most of the cases [7]—a measurement that probably corresponds to the width of most lobes, although it has been suggested that lobes are of different sizes and can extend from 2 to 23% of the breast volume [10].

The maximum distance between LN foci in the present cases varied from 5 to 112 mm (mean, 37 mm); in addition, in nine cases (69%), the maximum distance was no less than 20 mm. This probably indicates that, in most LN, more than one lobe is affected by the tumor (not to mention case 3 in which virtually all lobes from the same breast were involved).

No anastomoses between lobes were found in the 3D study of the cases in which entire lobes could be visualized—a finding also corroborated by previous works [10]. A continuous type of spread along the breast glandular tree, similar to that observed in poorly differentiated DCIS [7, 11], was seen in the two cases in which Pagetoid spread was found.

Invasive areas, with the exception of three cases that were unifocal (21.4%), were multiple in the same breast. There were as many as more than 100 areas in one case, and these averaged 20.7 in different patients. In cases with multiple involvement, the distance between the farthest-apart areas ranged from 10 to 130 mm. These data are in keeping with the only available study on macrosections of 130 cases of ILC, in which it was found that 39% of cases were unifocal, while the remaining cases were widespread lesions [17].

In the ILC areas of the present cases, the most frequent feature was that of LN immersed in and surrounded by ILC cells in 78.6% of the instances—an association so relevant that it cannot indicate a fortuitous phenomenon [14].

Furthermore, it has been shown in a literature review of 493 cases of pure LN diagnosed with CB that the presence of invasive breast cancer was underestimated in 18% of patients [4]. This further indicates, as shown in the present cases, that LN and LCI are widespread lesions that cannot all be represented in the limited materials obtained by CB. To adequately map a quadrant or a whole breast, numerous conventional histological blocks are required, making routine examination an expensive and time-consuming procedure [9]. Jackson et al. [12] compared two series: one studied with conventional histology and the other studied with HM. With the latter, the size of the invasive carcinoma could be determined for all cases. In contrast, size could be measured for only 63% of the 99 cases studied with

conventional small blocks. The size measured with HM was larger than that measured with conventional blocks; DCIS was also found more frequently, in association with invasive carcinomas, in specimens assessed by HM (80%) than in cases processed using small blocks (63%).

In nine cases in which ILC areas were devoid of LN, neoplastic cells were frequently seen in vessels or in spaces of Hartveit's prelymphatic labyrinth [2], suggesting that multicentric invasive carcinoma without LN is more probably an expression of intramammary metastases.

Admittedly, the number of cases studied here is small, and a selection bias of some sort is present, reflecting the high incidence of pleomorphic ILC observed in eight cases. Six cases were classic ILC, with an average of five ILC areas per case, while the eight pleomorphic cases showed, on average, 32 areas per case. Therefore, it appears that pleomorphic lobular carcinomas are neoplasms different from classic ILC, as shown by the fact they are aggressive tumors, as previously suggested [5, 20] and recently demonstrated by Tot [17].

Nevertheless, in spite of the limited number of cases, the data obtained in the present cases indicate the following:

1. LN equally involves ducts and acini, and Pagetoid spread appears as a thickening of the ductal walls in which the cells spread in a continuous fashion, as shown in 3D stereomicroscopy. No anastomoses between lobes were observed in the two cases in which the glandular trees were visualized.
2. LN observed in the present cases often appeared as a true multicentric lesion, affecting different breast lobes. This finding would probably indicate that there is a near-germline commitment of the breast epithelium to neoplastic transformation [18], although this view has to be further proven.
3. Conventional histological slides clearly would have underestimated ILC areas, as the mean distance between ILC areas was 5.8 cm.
4. ILC contained numerous LN foci (78.6% of LN), suggesting the existence of a direct relation between the two lesions in which LN might be the precursor of ILC.
5. In addition, the presence of multiple areas of pure ILC is probably the result of intramammary metastases in transit, as numerous cells are observed in vessels or Hartveit's spaces.

In conclusion, larger series of LN and ILC, which appear to be more and more necessary to better understand the extent of breast tumors, have to be studied using HM.

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