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DIFFERENT BIOLOGIC BEHAVIORS AND RISK PROFILES AMONG B3 PROLIFERATIVE BREAST LESIONS

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Introduction

Breast cancer (BC) is the most common neoplasia diagnosed among women all over the world, with a 1 in 8 lifetime risk in our country. Screening programs and the most recent technological innovations have led to an improvement in the rates of early diagnosis, which is usually made with targeted image-guided core needle biopsy (CNB).

Although most CNB samples can be readily categorized as normal, benign or malignant, it must be recognized that a small proportion (probably less than 10%) of samples cannot (1). For all screen-detected and symptomatic lesions (microcalcification, architectural deformities and mass lesions) there are five reporting categories similar to those used in fine needle aspiration cytology, but these are not equivalent, potentially identifying morphological criteria of uncertain malignant potential (B3). The "B3 category" comprehends a groups of lesions for which findings to date do not clearly show whether surgical excision is necessary to properly rule out cancer diagnosis; consequently undersampling can result in missed cancers.

They include a range of epithelial proliferative lesions extending from atypical ductal hyperplasia (ADH), lobular neoplasia (LN) and flat epithelial atypia (FEA) but also papillary lesions (PL), radial scars (RS) or potential phyllodes tumors (PT). This view is further supported by the observation that FEA, ADH/low grade ductal carcinoma in situ (DCIS), LN and invasive low grade breast cancer (BC) have similar molecular genetic and immunophenotype characteristics which are distinct from those seen in high grade breast cancers, as well as their significant coexistence with invasive tubular and lobular carcinoma (ILC) (2). In addition, studies of loss of heterozygosity (LOH) in low-grade DCIS and ADH have shown similar genetic lesions that is interpreted as confirmatory evidence that these are

clonal processes and both therefore fulfill the basic concept of neoplasia (3). However the importance of this potentially useful classification system, lies in the lack of clarity regarding optimal management with ongoing fluctuations in trends of treatment or perceived associations with malignancy. For instance, earlier reports suggested a strong association of RS with carcinoma, but more recent studies concentrating on those diagnosed preoperatively by CNB describe low associated malignancy rates of 0% to 8% (4,5). Against this, increasing numbers of studies are recommending routine excision of LN by CNB since is found to be associated with high rates of malignancy at excision (range, 6%–53%), in addition to being often multicentric and carrying a risk of both ductal and lobular invasive carcinoma in the contralateral as well as ipsilateral breast (6,7,8).

As in all evaluations of the breast specimens obtained, radiologic and histopathologic correlation is essential (9). Cellular monotony, lack of myoepithelial cells, and cytological atypia in CNB specimens are accurate features helpful in differentiating papillary lesions, but also the imaging characteristics can aid in predicting malignancy, more likely associated with well circumscribed and lobulated irregular mass and associated microcalcifications (10). Furthermore, accurate identification of phyllodes neoplasms without surgical intervention is difficult, reducing the ability to manage "benign" lumps non-operatively and impacting on the open benign biopsy rate, particularly in case of dense and hypercellular stromal lesions from which tissue capture may be difficult (11). However, while there are rare reports of carcinoma arising within fibroadenomas and phyllodes neoplasms, there is no convincing evidence that these lesions are precursors to breast carcinoma (12, 13). An ideally future classification systems for epithelial proliferative diseases of the breast will be reproducible between centers, and take account of clinical, morphological, phenotypic and genetic evidence. The level of chromosomal alterations and genomic loss observed by molecular detection correlates with the degree of proliferation, complex

architectural patterns and cytologic atypia in the more advanced lesions (14). In this context previously published data demonstrate that these categories, according to comparative genomic hybridization and immunohistochemistry data show distinct risk profiles with divergent molecular pathways of development (15).

It is important to emphasize that these recommendations cannot exclude a false-negative (FN) diagnosis in every individual patient and each case should be discussed on an individual basis with a multidisciplinary team taking into account the imaging features, lesion size, practicality and technical feasibility of minimally invasive management, patient demographics, and patient preference. When calcification is the main radiological abnormality, the lesion diagnosed on CNB could be frequently epithelial atypia or lobular neoplasia, each with variable rates of upgrade or long-term increased risk of breast cancer (16). Furthermore higher malignant rates are associated with cases that demonstrated mass lesions and calcification imaging or with radiopathological discordance.

On the other hand, for many B3 lesions, instead of surgical excision, VAB may be sufficient for therapeutic excision which would benefit the patient and save on healthcare costs by obviating the need for surgery (17). Positive predictive value (PPV) for malignancy has been falling (from 29 to 10 %) in the last few years showing a gradual increase in the number of CNBs with an improvement of their performance on screen detected breast lesions (18). Therefore it is becoming clear that these different borderline categories should be subjected to further investigation for updating quality assurance targets and moving forward to a more conservative approach in selected cases, as a part of the screening assessment. According to recent findings, such a procedure should be specifically discussed when the lesion was not completely removed by morphological imaging means (for example in the case of residual calcification), and the finding was marked using a clip (19).

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Atypical Ductal Hyperplasia (ADH)

ADH represents a neoplastic intraductal proliferation, characterized by monotonous cells forming cribiform-like and micropapillary formations, growing in bridges, arcades or full-thickness bars. In this pathologic finding ducts are partially filled with abnormal cell proliferations evenly spaced. The histological features to define an ADH can be divided in quantitative and qualitative criteria

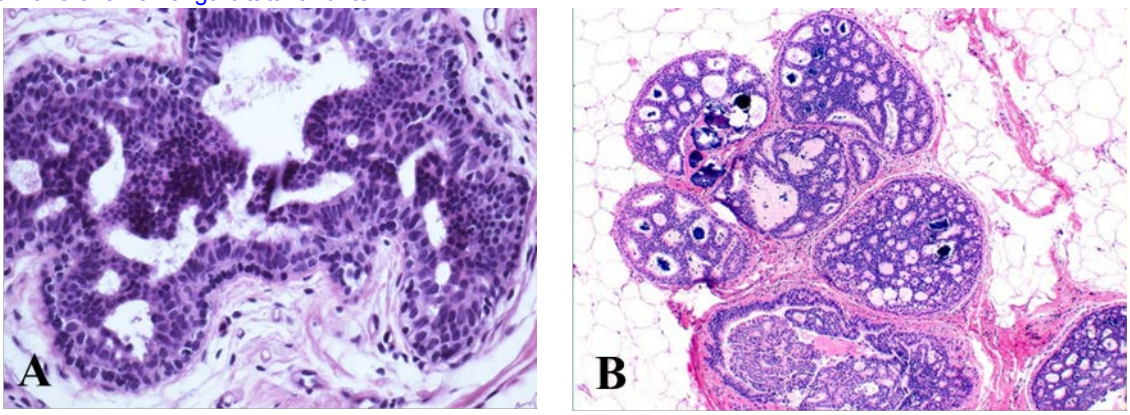
Quantitative criteria:

- Low grade atypia
- Involvement of one terminal ductal-lobular unit (TDLU) $\leq$ 2 mm
- Monomorphic nuclei with clear membranous borders

Qualitative criteria:

- Secondary intraluminal adenoid architecture
- Absence of expression of high molecular weight cytokeratins
- High expression of estrogen receptors
- No expression of HER2

ADH and low grade ductal carcinoma in situ (LG DCIS) share similar morphological features and they are often difficult to be distinguished; therefore the differential criteria has been defined arbitrarily on the basis of extension only: ADH has a size of $\leq$ 2 mm and it doesn't show the central necrosis, typical from LG DCIS.



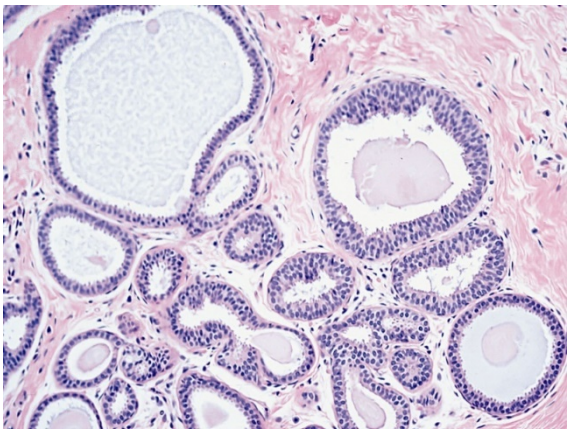
Atypical Ductal Hyperplasia (A) and Ductal Carcinoma In Situ (B

The dilemma in proper management of ADH derives from the lack of reliable clinical information that would help in predicting whether it is a part of larger LG DCIS or just an atypical cells proliferation. There are some features that can be considered suggestive, but not diagnostic, for an underestimated malignancy, such as multifocality (more than 2 foci on CNB findings), central necrosis, conflicting pathological-radiological findings (i.e. lack of calcifications in specimens on CNB performed for microcalcifications in mammograms)

ADH is considered a direct but non-obligate precursor to carcinoma, carrying a four-fivefold increased risk of developing cancer. The occurrence of ADH in the population ranges from 3% to 23%, depending on the series. This great variability can be explained by several factors: the increased number of CNB in the screening era, the increased use of hormonal replace therapy, the different definitions deriving from variation in sliding samples of ADH among different diagnostic centers. Some studies show the conflicting definition of ADH and intraobserver variability, demonstrating the lack of universal consensus, especially in differentiating ADH from LG DCIS.

Flat Epithelial Atyphia (FEA)

This category was defined for the first time in 2003 by the World Health Organization (WHO) as a presumably neoplastic intraductal proliferation characterized by the replacement of native epithelial cells with up to five layers of mildly atypical columnar cells without any secondary achitectural atypia, for this reason it's called flat. This pathological finding is fairly uncommon (1-2% of benign breast biopsy) and it's often associated with other high-risk lesions (e.g. atypical ductal hyperplasia, atypical lobular hyperplasia, lobular carcinoma in situ) and sometimes with carcinoma. A recent review and meta-analysis by Anatoliy et al. from Mayo Clinic of Rochester, has investigated the upgrade to cancer or to ADH rate in 32 studies (for a total of 1966 showing FEA). The authors found that the upgrade rate for cancer and for ADH was of 11% and 17,9% respectively, and it was lower (7,5% and 18,6%) if the analysis was restricted to higher quality studies, highlighting some variation across studies. As 20% of patients with FEA found at CNB will upgrade to cancer or to ADH, this potential change in the patient management has led the authors to recommend surgical excision for this diagnostic category. The current National Comprehensive Cancer Center guidelines suggest that FEA is a lesion that may not require surgical excision, although the details identifying patients suitable for observation are unspecified.



Flat Epithelial Atyphia

LOBULAR NEOPLASIA (LN)

The term Lobular Neoplasia (LN) refers to morphologic spectrum of lesions including Atypical Lobular Hyperplasia (ALH) and Classic Lobular Carcinoma In Situ (LCIS), both showing a population of non-cohesive cells. They often coexist.

Classic Lobular Carcinoma in Situ (LCIS)

This lesions was defined for the first time in 1941 by Foote and Stewart who described a rare form of cancer originating in lobules and terminal ducts, consisting of neoplastic cells that fill and expand most of the acini. It represents a risk factor and a non-obligate morphologic precursor of invasive breast carcinoma, characterized by some features:

- It's an incidental finding at CNB: 0,5%-1,5% of benign breast biopsy and 1,8%-2.5% of all breast biopsies. The increased use of mammographic screening has led to an increased rate of LCIS; it
- It's a multicentric disease in 60-80% of patients and bilateral in 20-60%
- It's clinically and mammographically occult, sometimes associated with amorphous or granular mammographic calcifications;

LCIS is characterized by a proliferation of non-polarized neoplastic cells with a round or oval shaped, inconspicuous cytoplasm and not clear borders, that can fill more than 505 of acini, commonly associated with a pagetoid extension into the terminal ducts.

The relative risk of breast cancer in patients with LCIS is 9-10 times higher than in the general population. In a recent analysis of 1060 patients with LCIS with a median follow up of 81 months, 14% of patients developed cancer (63% bilateral, 25% contralateral, 12% bilateral) and the annual incidence of breast carcinoma was 2%.

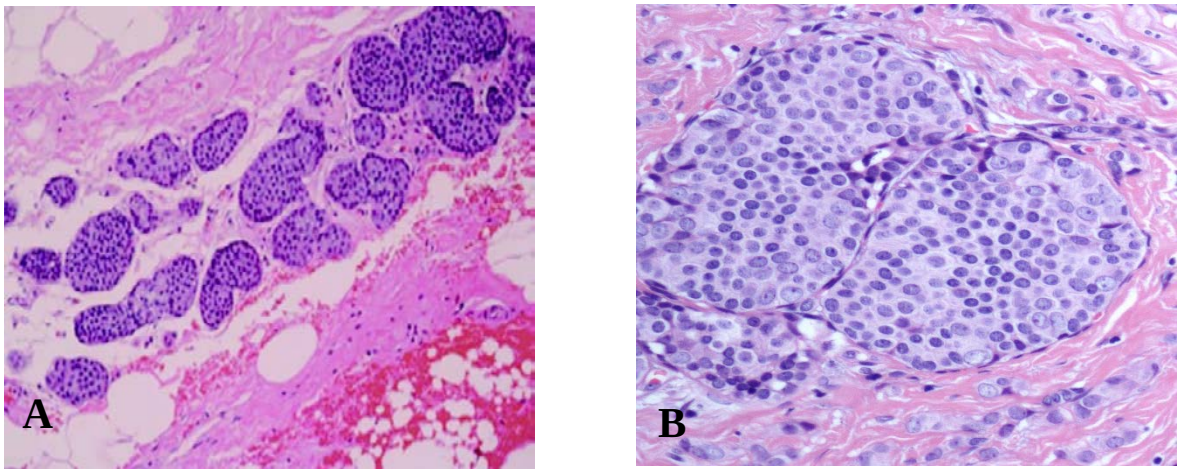
In the past years mastectomy was the treatment option for women receiving diagnosis of LCIS. Nowadays there is a general consensus that LCIS represents a benign disease and observation alone is the preferred option. The National Comprehensive Cancer Network guidelines recommend clinical examination every 6-12 months and annual mammograms. In the eight edition of the TNM tagingbi the American Joint Committee on Cancer LCIS is no longer staged as in situ tumor category.

Atypical Lobular Hyperplasia (ALH)

The cells composing ALH are morphologically identical to those of classic LCIS, but they interest less than 50% of the acini of the TDLUs and none of the acini is considerably expanded.

The relative risk of invasive breast carcinoma after diagnosis of ALH is 3-5 times higher than in the general population, roughly one-half that LCIS. Most invasive carcinoma that developed in patients with ALH have low nuclear grade and excellent survival outcome.

The management of patients with classic LCIS or ALH at CNB doesn't require routine excision, when there is concordance between pathological and radiological findings. A recent prospective multi-institutional trial with a central pathology review showed an upgrade to cancer rate of 1%.



Atypical Lobular Hyperplasia (A) and Classic Lobular Carcinoma in Situ (B)

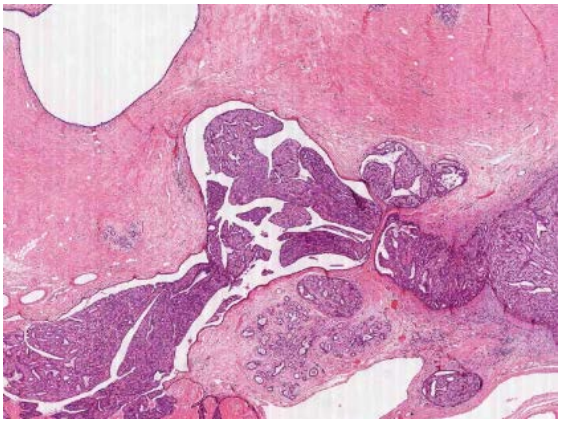
Papillary Lesions (PL)

Papillary Lesions of the breast are a heterogeneous groups of alteration characterized by the presence of epithelial proliferation supported by fibrovascular cores, which grow within the ducto-tubular units of the breast showing a cystic structure but solid forms. They can be classified in several subtypes depending on:

- Localization: they can arise from large ducts (a solitary mass or nipple discharge) or small ducts (often multiple, presenting as calcification oran incidental finding)
- Nature of proliferation: hyperplastic, neoplastic, or both
- Presence of myoepithelial cells useful in differential diagnosis between malignant and benign
- Morphology, including margins with or without peripheral fibrous capsule, focal stromal invasion, ragged edge, jigsaw pattern.

Most papillary lesions are benign, the so called intraductal papilloma or solitary/central papilloma. The diagnostic work-up of papillary lesions comprehends a spectrum of disease with typical benign intraductal papilloma at

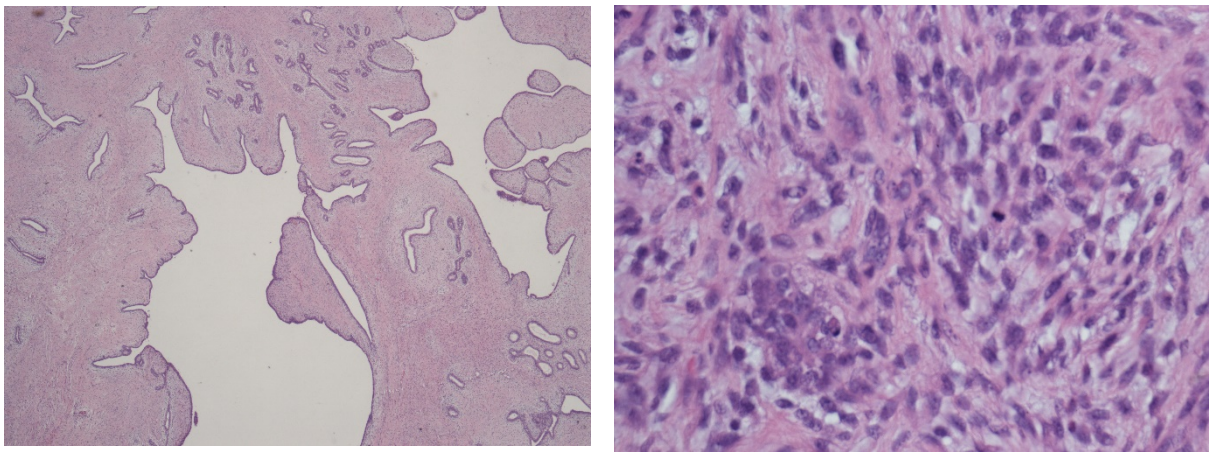
one end and invasive papillary carcinoma at the other end. The lack of reliable and reproducible criteria to differentiate certain papillary lesions can make diagnosis challenging.



Central papilloma

Phyllodes Tumors (PT)

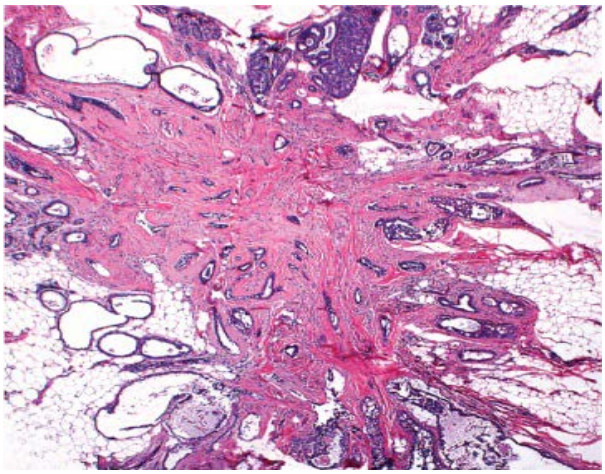
Phyllodes Tumors represent 0.3%-1.0% of all breast tumours and 2%-£% of all fibroepithelial lesions and they are common in womwen aged 35-55 years. PTs canbedivided into three categories: benign, borderline and malignant. To differentiate benign or borderline PTs from fibroadenomas at CNB can be challenging because of small samples; however the European Guidelines for qulity assurance in breast cancer stated that the presence of increased stromal cellularity, little or moderate stromal cellular pleomorphism and infrequent mitotic figures should be reported as B3 lesion. On the contrary, malignant PTs are quite easy to diagnose at CNB due to the presence of marked stromal atypia, increased mitotic activity and stromal overgrowth. To distinguish PTs from fibroadenomas in the preoperative setting is very important because treatment options are different: fibroadenomas are benign lesion and can be treated by observation or local excision; on the contrary, the management of PTs requires radical local excision with a free surgical margin, due to its increased risk of local recurrence.



Borderline phyllodes (left) and mitotic activity (right)

Radial Scar (RS)

This group comprehends breast lesions characterized by a fibroelastic core with branched rays of ducts and lobules surrounded by a continuous myoephitelial cells layer. They often appear in women 30-60years old aged, in association with other alterations such as duct hyperplasia, cysts, sclerosing adenosis; the differential diagnosis with complex sclerosing lesions is the size: RS are smaller than 1 cm. RS are often incidental findings: the frequency in autopsy series range from 14% to 28%. In the past many published data showed an upgrade-to-cancer rate of RS varying from 0 to 43%. Later publications indicate that there is no evidence that RS are premalignant lesions; the early hypothesis that RS evolve into tubular carcinomas can be explained by the coexisting presence of other proliferative lesions including atypia, which can contribute to the overall upgrade rate to cancer. Management of RS is controversial and there is no consensus.



Radial Scar

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DIFFERENT BIOLOGIC BEHAVIORS AND RISK PROFILES AMONG B3 PROLIFERATIVE BREAST LESIONS: A RETROSPECTIVE STUDY

In the current restrospective study, we aimed to assess the histology outcome of lesions recently diagnosed as B3 (2003-2018) in our institution, including a detailed review of the different types of intraductal epithelial atypia with several implications for practice and future research. In addition, more detailed knowledge of the initial clinicopathological implications for optimal use and interpretation of non operative management are needed before a definitive conclusion can be reached concerning the prognostic relevance of specific borderline proliferations. We also aimed to provide robust data on the quality of performance of CNB that can be used to draw up evidence-based revised thresholds for measures of accuracy and an optimal disease-tailored approach, which may reflect an improvement in the radiological evaluation and sampling techniques of breast lesions.

Material and methods

All CNBs reported as B3 in the 15-year period, from January 2003 to December 2018, were studied. The reason for performing CNB was usually the presence of a mammographic or clinical abnormality detected by or reported to the radiographer. The CNBs were performed using either ultrasound-guided core biopsies (14G) or stereotactic vacuum-assisted biopsies (11G). For US guided core biopsy 3-5 specimens were obtained, in case of a vacuum- assisted biopsy 12-16 specimen were achieved.

All patients with known histopathological B3 diagnosis and definitive histology after surgical resection were included (N = 300). Histological concordance between a core biopsy diagnosis and excision specimen was analyzed to determine associated rates of malignancy, also assessing the outcomes of different subtypes.

For the purpose of this study, excision histology findings were categorized as: (i) malignant, including invasive carcinoma, DCIS, and other malignant lesions such as sarcomas and lymphomas; and (ii) benign lesions, including flat epithelial atypia (FEA), atypical ductal hyperplasia (ADH), classical lobular neoplasia (atypical lobular hyperplasia and lobular carcinoma in situ), but also papillary lesions (PL), radial scars (RS) or potential phyllodes tumors (PT). Positive predictive values PPVs for detection of malignancy were calculated for all B3 core cases and for each subcategory as follows: PPV = (number of final malignant diagnoses⁄total number of subjects with B3 diagnosis ×100%).

All cases were additionally divided into 4 major areas (papillary lesions, phyllodes tumors, radial scars and intraepithelial atypical lesions such as flat epithelial atypia, atypical ductal hyperplasia or lobular neoplasia) to provide a complementary standardization of the pathologic diagnosis for the correct risk characterization and management of patients. Quality assurance measures were assessed over the study period and correlations between different morphological variables were evaluated in order to provide further data on the quality of CNB performance of in the assessment of breast lesions. According to subsequent excision biopsy malignant findings, the tumors were classified according to the American Joint Committee on Cancer (8th edition) staging criteria, identifying the invasive BC subtypes based on the hormone receptor status (ER or PR staining), the Ki-67 threshold value (≥20%) and HER2 amplification, to determine whether some variables could be considered independently associated with CNB diagnosis without a requirement for molecular diagnostics (1,2). Patients were also categorized based on the receptor status of their primary tumor as follows: luminal A (ER+ or PR+, and HER2-); luminal B HER2- (ER+, HER2- and at least one of Ki-67 high or PR negative or low); luminal B HER2+ (ER+, HER2-overexpressed or amplified, any Ki-67, any PR); HER2 (ER- or PR-, and HER2+), and basal (ER- or PR-, and HER2-). Tumors were considered HER2-positive only if they were either scored 3+ by IHC (strong, complete membrane-staining in >10% of cancer

cells) or showed HER2 amplification (ratio > 2) using fluorescence in situ hybridization (FISH). Furthermore the pathological characterization of steroid hormone receptor status was classified as Luminal and Non-Luminal profile.

Nuclear grade of the involved invasive BC and axillary LN metastases were also examined by histopathology. Patients who underwent surgical excision were reviewed at a weekly multidisciplinary meeting attended by specialist breast histopathologists, radiologists, surgeons, oncologists, and radiotherapists, in order to retrieve further follow-up informations on the status of all patients who had a B3 with subsequent new primary ipsilateral or contralateral invasive breast cancer occurrence during the study period (median observation time 65.4469 ± 42.61397 months). Data included the primary site, laterality, histology, and extent of any later malignant disease, to define the cumulative BC incidence conferred on a patient having a previous primary uncertain subdiagnosis, and then investigating whether this may represent a true precursor lesion versus a prognostic marker for succeeding tumour development (3,4).

Statistical analysis

The proportion of malignant lesions in the definite histopathology was compared between patients with specific histopathological sub-categories and clinicopathological factors using the chi-square test. P values less than 0.05 were considered statistically significant. Analysis was performed, calculating sensitivity, specificity, and positive (PPV) of the uncertain malignant potential diagnostic categories included in the study (ADH, FEA, LN, PL, RS, PT).

IBM SPSS Statistics 23 (SPSS Statistics, Chicago, IL, USA) was used for statistical analyses.

To address comparisons between little groups in specific sub-categories of malignant/BC after CNB a Mann-Whitney test with exact P values was performed. Moreover, cumulative incidence curves of new primary/relapses of BC after CNB

were estimated with a Kaplan-Meier analysis, and in order to evaluate the association with BC features multivariate models Cox regression was performed.

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Results

B3 core needle histology who had surgical excision comprised 10.1% (300 from 2986) of all CNB results.

The B3 lesions comprised the following histopathological diagnoses: ADH, 35% (105/300); FEA, 16.7% (50/300); LN, 22.7% (68/300); PL, 9% (27/300); PT, 8.6% (26/300) and RS, 8% (24/300).

The lesion type depicted on mammography showed mass or density in 46.3% (n=139) of cases, microcalcifications in 35.7% (n=107) and architectural distortion in 18% (n=54). Ultrasound -guided core biopsies (14G) were performed in 61.3% (n=184) of cases for sonographically visible lesions, and in 38.7% (n=116) of CNB stereotactic vacuum-assisted biopsies (11G) were performed for lesions invisible for US or for the evaluation of microcalcifications. Screen-detected calcifications showed no higher incidence of malignant outcome (15.9%; 17/107) when compared to mass lesions (13.7%, 19/139) or architectural deformities (16.7%, 9/54) (χ^2 analysis, p =0.828), with no association between the radiological finding and the upgrade rate in the final histology. Of these cancer cases (n=45), when radiological abnormality was considered, 37.8% (17/45) were malignant when the feature was calcification, 42.2% (19/45) for mass lesions and 20% (9/45) for architectural distortion.

The clinicopathologic characteristics of the study participants are listed in Table I and II.

When calcification was the main radiological abnormality, the lesion diagnosed on CNB was frequently intraepithelial atypia such as FEA, ADH or LN (92.5% of microcalcifications finding; 44.3% of all atypia) (odds ratio [OR]: 6.886, p<0.001**)**. In contrast, 20.3% (11/54) of architectural distortions were diagnosed as RS (45.8%, 11/24 of all radial scars), and 17.2% of mass lesion was diagnosed as PT (24/139; 92.3%, 24/26 of all phyllodes tumors) or PL (16.5%, 23/139; 85.1%,

24/27 of all papillary lesions). A χ^2 test of this distribution reveals a statistically significant non-casual association ($p < 0.001$).

Malignant lesions included 25 (8.3%) DCIS and 20 (6.7%) invasive cancers, associated with concomitant in situ component in 75% of cases (15/20), giving an overall PPV of 15% (45/300) based on excision histology. Of all these cases, 84.5% (38/45) were diagnosed as epithelial atypia on CNB; 55.2% of those (21/38) were diagnosed as ADH , 15.8% FEA (6/38) and 28.9% LN (11/38).

Lesion specific positive predictive values (PPV) for a subsequent diagnosis of carcinoma were as follows: ADH 20% (21/105), FEA 12% (6/50), LN 16.2% (11/68), papillary lesion 18.5% (5/27), phyllodes tumor 3.8% (1/26) and radial scar 4.1% (1/24). Initial analysis showed that when epithelial atypia was mentioned explicitly in CNB report, 17.0% (38/223) were malignant, whereas when the absence of epithelial atypia was mentioned, only 9.0% (7/77) were malignant on final histology. These results demonstrate a higher but not statistically significant tendency toward lesion upgrade for the 3 major atypical areas (ADH, FEA, LN) (OR: 2.05; 95% confidence interval [CI]: 0.87-4.81; p = 0.092) with a proportionally greatest risk for ADH (OR: 1.78; 95% [CI]: 0.94-3.38; p = 0.075). Furthermore, considering all cases, they were characterized by significantly smaller and more homogeneous lesions compared to other B3 subtypes like PL, PT and RS (15.314±8.06 mm vs 17.532±11.91 mm, p=0.001).

When CNB shows B3 lesion without atypia such as PL, PT, RS (n=77), PPV is 7.1% (1/14) if radiological abnormality is distortion and 12.5% (1/8) or 9% (5/55) if the radiological abnormality is calcification or mass respectively. Contrasting B3 lesions presenting as calcification with atypia such as ADH, FEA, LN (n=223) on CNB demonstrate PPV of 16.1% (16/99), compared to 16.6% (14/84) of mass or 20% (8/40) of architectural findings.

Tables III and IV summarize all NCB histological and radiological categories, excision histology outcomes and category-specific PPV. Among all DCIS (n=25),

40% (10/25) were diagnosed as ADH and 32% (8/25) as LN on CNB, whilst 55% (11/20) of invasive tumors were diagnosed as ADH and 20% (4/20) as FEA.

Of the invasive tumors (n=20), 16 (80%) were ductal, 3 (15%) lobular carcinomas and 1 (5%) malignant phylloides; 35% were grade 1, 55% grade 2 and 10% grade 3. Of the DCIS cases (n=25), 56% were low grade, 32% intermediate grade and 12% were high grade. The median tumor size was 10.9 mm (range 2-50 mm), and a greater proportion of T1 (n=17, 85%) occurred among cases compared with T2 (n=3, 15%). Of the 17 T1 patients, 3 (17.6%) were pT1a, 8 (47.1%) pT1b and 6 (35.3%) pT1c, respectively and 64.7% (n=11) $\leq$ 10 mm.

In this context, the smallest BCs were identified after a previous CNB diagnosis of PL (6.6±4.219, p=0.077) and FEA (14.583±17.7719, p=0.057).

ER≥50% was positive in 39 (86.6%) of 45 tumors and PR≥20% was positive in 34 (75.5%) of 45 tumors. Eight patients (17.7%) had high Ki-67 expression (≥20%) and overexpression of cerbB-2 was found in 2 of 19 invasive tumors (10.5%). Among infiltrating BCs with a well-defined pathological characterization (n=19) the incidence of luminal and non-luminal subtypes was 94.7% (n=18) and 5.3% (n=1), respectively. The majority of ductal and lobular cases (n=19) had luminal A tumors (63.1%, 12/19), followed by luminal B (21.1%, 4/19), HER2-positive luminal B (10.5%, 2/19), basal (5.3%, 1/19), and any non-luminal HER2-positive. Of the invasive cancers, only three (15%) of 20 patients had ipsilateral LN metastases according to the gold standard and were staged as pN1 (two of them with micrometastases, pN1mi). There were 25 patients (55.5%) in stage 0, 16 (35.5%) in stage I, and 4 (9%) in stage II.

At the time of analysis, 19 cases (6.3%, 19/300) developed subsequent primary invasive BCs after previous B3 CNB diagnosis, (10.5% ipsilateral and 89.5% contralateral), of which 6 (13.3%, 6/45) after a preceding surgical excision for malignancy, 3 IDC, 2 DCIS and 1 ILC (16.7% ipsilateral and 83.3% contralateral). New invasive tumors occurred in the study period most often developed following an earlier epithelial atypia CNB finding (n=15/19, 78.9%), with a significantly

strong association with LN (n=8, 42.1%), (OR: 2.679; 95% [CI]: 1.031-6.957; p = 0.037), but without any statistically difference in the time of development for the two subgroups in the observation time (p=0.847). Interestingly, the Chi-Squared test also revealed a new primary growing risk among patients with previous BC diagnosis (OR: 2.864; 95% [CI]: 1.028-7.980; p = 0.037) (Fig.1).

Discussion

Despite the current perspective that CNB has better diagnostic distinction between benign and malignant breast disease than FNAC, borderline (B3) core needle histology occurs in a similar or higher proportion of cases as does atypical or borderline cytology (1,2). Uncertain (B3) core needle histology, however, has more significant implications in that most cases progress to surgical intervention, and debate continues on whether selected cases may be spared excision (3). Therefore, accurate radiological-pathological correlation is the prerequisite for accurate treatment applying histological criteria and diagnostic terminology to guide risk stratification and appropriate patient management.

In our study there was **no association between radiological finding and outcome**, but **calcification was more likely to be associated with epithelial atypia** compared to mass lesions or architectural distortions, particularly ADH and LN, while it was rare in papillary lesions and radial scars. In agreement with previous results, we showed that the most frequent B3 lesion identified was atypical proliferations, and the PPV for detection of malignancy for this diagnosis was 17.0% (4-5-6-7). However, the PPV also varied among the different subtypes of epithelial atypia, with the highest rate detected for ADH whilst the lower rates were associated with lobular neoplasia and FEA.

ADH and low nuclear grade DCIS show not only morphological similarities, including cytological and architectural features, but also immuno-phenotypical overlap (both are estrogen receptor (ER)- and progesterone receptor (PR)- positive and HER2-negative) with especially genomic alterations (30).

This supports the standard clinical practice of performing excision biopsy of all lesions with a diagnosis of epithelial atypia, even if some parameters may be useful to take into consideration before resection, such as the number of cores, type of needle used, lesion biology and diameter (31).

Furthermore, the PPV for FEA was 12%, which was lower than the ADH, but **5 out of 6 malignant lesion identified after FEA presented with calcification** (OR: 12.5; 95% [CI]: 1.28-120.85; p = 0.01). Recent studies suggest that some cases of FEA are associated with ADH and several types of low grade invasive carcinoma, particularly tubular and invasive lobular carcinoma, suggesting a linked pathogenesis, but also presenting management dilemmas for pathologist and clinicians, as participation in breast screening programs continues to expand (32-33-34). We expect future studies of FEA in CNBs will have larger numbers of cases as recognition and classification of the spectrum of CCLs (columnar cell lesions) by surgical pathologists improves, in order to define the BC risk and clinical outcomes associated with this diagnosis (35).

The proportions of papillary lesions that later proved to be malignant was 18.5%, which is in the range of 7-26% found in most other cohorts, and then suggesting that histologic underestimation occurred at a frequency similar to that in the other atypical finding undergoing CNB (8-9-10). There were no statistically significant associations between lesions manifesting as a mass, calcifications, or distortion and subsequent DCIS or invasive carcinoma revealed by the excisional biopsy, and controversy remains about whether needle core biopsy is sufficiently accurate in the diagnosis of benign pathology. However, in addition to the predominantly papillary pattern, the associated solid or cribriform architecture, generally characterized by a slow-growing potential and good prognosis, was more likely present in CNB specimens from malignant lesions, but as in all evaluations of the breast specimens, discordant imaging and histologic findings should prompt a repeat biopsy and a join review of cases is recommended (36-37).

In this context the radial scars were more represented among the architectural distortions (45.8%, n=11/24), with no cases of BC upgrade inside the following radiologic abnormality, as suggested by earlier reports of low malignancy rates, and thus delineating a potential spectrum of lesions to be monitored through the use of sub categorization criteria and managed safely by regular mammographic

surveillance (11-12). It has been suggested that the association between RS and breast cancer could result from an unrepresentative core biopsy, rather than be the result of an evolution from pre-malignant to malignant disease over time. Thus, extensive samples obtained on percutaneous biopsy have been identified as factors that may spare a patient from undergoing surgical excision, as they can be more representative of the lesion (38-39).

In this series, carcinomas detected after a B3 diagnosis often showed favorable histopathological features: 55.5% were DCIS whilst only 10% of invasive were high grade, the majority were of small size (64%≤10mm) with prevailing hormone receptor positivity (71.1%) or low Ki-67 labelling index (82.3%), and only 15% of invasive cancers showed lymph node metastasis. Although limited, the available molecular data support the concept that overlapping morphologic and immunohistochemical features provide evidence for these uncertain malignant lesions being a candidate precursor for the progression to low-grade DCIS and invasive carcinoma mostly characterized by luminal phenotype and HER2 negativity, with specific consequences for the clinical outcome (13-14-15-16-17). In this context, the molecular genetic profiles closely reflect the degree of proliferation and atypia in B3 categories, indicating some of these proliferations represent both a non-obligate, intermediary step in the development of some forms of malignancies as detailed by comparative genomic hybridization data and divergent chromosomal alterations (18).

Such genetic features may thus be influential in stratifying the molecular evolution of well versus poorly differentiated tumors, thereby implicating a potential evolutionary relationship between the level of genetic instability and the morphologic complexity, in order to characterize their biologic behavior and ultimately define better management in the more advanced lesions (19-20). On this regard, also the extended association of a concomitant in situ component with invasive carcinoma detected in our study seems to correlate with lower disease aggressiveness and metastatic potential according to distinct prognostic factors

such as lower ki67 index and fewer involved nodes, with a trend towards superior overall survival (21). Hence, given that fast-replicating (high Ki-67) cancer disease might be expected to contain a higher burden of dysfunctional tumor suppressor genes, our study results support the possibility that pure IDC tumors arise as a result of more drastic suppressor gene defects, eventually favoring the therapeutically challenging basaloid phenothype in the BC progression pathways (22). Similarly, if multiple neoplastic or atypical lesions are detected by high resolution imaging, more recently generation sequencing approaches of carcinoma and concurrent lesions of uncertain malignant potential might change the direction of the patients' management by treating or removing not only cancerous lesions, but also the reservoir of genetically diverse neoplasias to prevent recurrence (42). In the same time, these borderline histological findings and other benign proliferations could be the result of a "field effect" where non-related tumors are co-located within a cancer prone tissue and additional micro-environmental risk factors, such as alcohol consumption, smoking or obesity (43).

Our results show a significant reduction in the PPV for B3 diagnoses as a group (14.2%), since previous studies have reported PPVs from 20% to 35%, worthy of further investigation (23-24-25). The performance of CNB had improved over time since its introduction suggesting this increase is likely to be the result of detection of more subtle lesions following the introduction of digital mammography, advanced ultrasound resolution and also increasing use of vacuum-assisted biopsy yielding more tissue for diagnosis (26). Furthermore it is important to emphasize that the B3 category is not only used for identifying lesions with an increased rate of epithelial malignancy but even to recognize predisposing risk factors for the subsequent development of cancer disease in either breast, potentially leading to several clinical recommendations (27). The minimum cumulative risk of new primary invasive BC occurrence during the study period was 6.3%, with greater incidence in the contralateral breast especially after previous epithelial atypia on CNB, figuring to be a prognostic marker as well as a true precursor lesion for

which close follow-up may be considered adequate (28-29). About that, LN has generally been considered a risk indicator, conferring an increased rate of development of invasive carcinoma of about 1-2% per year, with a lifetime risk of 30-40% and equal chance for both breasts (40-41). Particularly, studying the association of atypia with these distinct low and high grade multistep models of BC progression, could give an insight into identifying patients with a high risk of recurrence, for an intensified degree of subsequent surveillance and management in problematic and contentious cases (44-45). However, non-consistency in specific molecular events attributed to progression may reflect inter-tumoral heterogeneity or that the number and combinations of drivers are equally important for the cancer phenotype independently of gene amplification (46). Therefore, limitations of our study that had to be discussed are on one hand the relatively rare diagnosis of B3 lesions as well as the retrospective design of our study. Furthermore one has to consider the different use of diagnostic terms for identical pathological lesions that may hinder a clear comparison of pathological diagnosis over the recent years.

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Conclusion

Breast cancer is a heterogeneous disease, and much research has been directed towards identifying subtypes to aid risk stratification. The paradigm of early breast cancer management is thus shifting towards 'personalizing' therapy as a function of morphological, biological, and molecular disease variables. This study raises the possibility that the association of B3 lesions with a malignant category could be factored into future adjuvant therapeutic decision-making. If verified, this distinction would in turn suggest an as-yet-undetermined molecular basis for the divergent clinical behavior of these uncertain potential breast lesion subtypes, holding the strategy to define subgroups, in which surgical intervention might not be appropriate, but also focusing the value of surgical treatment for distinct entities.

In view of the existing literature our data clearly support the heterogeneous biology of B3 lesions diagnosed by CNB and their risk for associated malignancies. Radiological findings provide useful information regarding the nature and outcome of these screen-detected lesions. For example, calcification with suspicious or malignant characteristics and histological evidence of epithelial atypia were the most frequent abnormalities observed in B3 NCBs associated with cancer disease. Epithelial atypia detected on CNB has a relatively greater PPV for malignancy requiring to consider surgical excision. Pure ADH showed a higher PPV than LN and FEA in this category group but future work is needed to test the observations noted here, and to explore molecular explanations for the putative differences in natural history.

Understanding the genetics of B3 lesions might lead to effective strategies to prevent development and progression of associated BC and shed light on the malignant progression model, in particular the relationship with non-low grade as well as ER- carcinoma. With the improvement of next generation sequencing

technologies, a careful selection of a larger cohort of uncertain histological findings than studied to date (with and without carcinoma of different grades), reviewed by an experienced pathologist, would give an insight into early diagnosis and preventive therapeutic strategies, potentially reducing the burden of related overtreatment.

TABLES

Table I. Details of patients characteristics.

Variables	Number	(%)
Proportion of B3 lesions	**300**	**100**
Benign	255	85
Malignant	45	15
Radiological findings		
Calcification	107	35.7
Mass	139	46.3
Architectural distortion	54	18.0
Diagnostic B3 Category		
ADH	105	35.0
FEA	50	16.7
LN	68	22.7
PL	27	9.0
PT	26	8.6
RS	24	8.0
Histological findings		
Atypical proliferations	223	74.3
No atypical proliferation	77	25.7
Core biopsy procedure		
Ulltrasound	184	61.3
Stereotaxic	116	38.7

Table II. Association between radiologic abnormality and diagnosis on CNB and on final excision.

Radiological Abnormality	CNB B3 diagnosis							Final excision diagnosis		
	ADH	FEA	LN	PL	PT	RS	P[a]	Benign (%)	Malignant (%)	P[a]
Calcification	36	27	36	3	0	5	>0.001	90 (84.2)	17 (15.8)	0.001
Mass	46	16	22	23	24	8		120 (86.4)	19 (13.6)	
Architectural distortion	23	7	10	1	2	11		45 (83.4)	9 (16.6)	
Total	105	50	68	27	26	24		255 (85)	45 (15)	

Table III. Lesion of uncertain malignant potential (B3) on CNB with excision histology outcome for different B3 lesions and associated PPV for breast malignancy.

Category of B3 with excision histology (number of cases)	Final excision diagnosis			
	Benign	Malignant in situ no. (%)	Malignant invasive no. (%)	PPV(%) excision histology
ADH (105)	84	10 (9.5)	11 (10.5)	20.0 (21/105)
FEA (50)	44	2 (4.0)	4 (8.0)	12.0 (6/50)
LN (68)	57	8 (11.8)	3 (4.4)	16.2 (11/68)
PL (27)	22	4 (14.8)	1 (3.7)	18.5 (5/27)
PT (26)	25	0 (0)	1 (3.8)	3.8 (1/26)
RS (24)	23	1 (4.1)	0 (0)	4.1 (1/24)
Total (300)	255	25 (8.3)	20 (6.7)	15 (45/300)

Table IV. Details of the clinicopathologic tumor characteristics.

Variables	Number	(%)
Proportion of malignant lesions	**45**	**100**
DCIS	25	55.6
Invasive	5	11.1
Invasive + DCIS	15	33.3
Tumour classification		
Tis	25	55.6
T1a	3	6.7
T1b	8	17.7
T1c	6	13.3
T2	3	6.7
Nuclear grade		
1	21	46.7
2	19	42.2
3	5	11.1
DCIS nuclear grade	**25**	**100**
1	14	56.0
2	8	32.0
3	3	12.0
Invasive nuclear grade	**20**	**100**
1	7	35.0
2	11	55.0
3	2	10.0
Invasive Histology	**20**	**100**

IDC	16	80
ILC	3	15
Other	1	1
Nodal metastasis	**20**	**100**
negative	17	85
positive	3	15
Lymph node classification	**20**	**100**
N0	17	85
N1	3	15
Pathological stage	45	100
0	25	55.5
I	16	35.5
II	4	9
Estrogen receptor (ER)	45	100
50>	39	86.6
50≤	6	13.4
Progesterone receptor (PR)	45	100
20>	34	75.5
20≤	11	24.5
Ki-67 index	45	100
20>	8	17.7
20≤	37	82.3
c-erbB-2 (HER2)	**19**	**100**
0	7	36.9
1+	5	26.3

2+	5	26.3
3+	2	10.5
Breast cancer Subtype	19	100
Lum A	12	63.1
Lum B-	4	21.1
Lum B+	2	10.5
HER2	0	0
Basal	1	5.3
Breast cancer Profile	**19**	**100**
Lum	18	94.7
Not Lum	1	5.3
Subsequent primary after B3	19	100
Ipsilateral	2	10.5
Contralateral	17	89.5

Figure 1. Kaplan–Meier analysis of new primary BC incidence among the two patients subgroups adjusted for presence of atypia: ADH, FEA, LN vs others (PL, PT, RS) in the observation time.