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Review

"Borderline" epithelial lesions of the breast: what have we learned in the past three decades?

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Summary
Atypical ductal hyperplasia (ADH), atypical lobular hyperplasia (ALH) and flat epithelial atypia (FEA) are common lesions mainly detected during mammographic screening. They are considered lesions at risk for the development of breast cancer, and they have been documented as non-obligate precursors of low grade *in situ* carcinomas. In a monumental work in 1991 Rosai gathered them as "borderline epithelial lesions", and he described and demonstrated the subjectivity in their microscopic interpretation. Such subjectivity persists nowadays and limits considerably the diagnostic consistency. With his incredible ability to see, analyze and rationalize, Rosai introduced the concept of "*mammary intraepithelial neoplasia (MIN) of either ductal or lobular type, followed by a grading system*" which would have better represented the biological continuum between these lesions and benign and malignant lesions.

Key words: atypical hyperplasia, carcinoma in situ, non-obligate precursor, border-line in situ lesions

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Conflict of interest
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The diagnostic problem and the risk categories

In 1991, a deep-thinking paper entitled "*Borderline epithelial lesions of the breast*" with several practical considerations was published by Juan Rosai in the American Journal of Surgical Pathology [1]. He was strongly convinced that this issue merited a focus because in the breast, the concept of borderline epithelial lesions is intimately linked with that of atypical ductal (ADH) and atypical lobular hyperplasia (ALH), "*proliferative processes placed somewhere between the usual type of hyperplasia and carcinoma in situ (CIS), both in terms of morphologic features and propensity for the development of invasive carcinoma*". Second, in agreement with Azzopardi, one of the masters of breast pathology, Rosai thought that "*atypical or borderline lesions of the breast are practically non-existent, and only our inadequate grasp of the subject explains and partially justifies the interim use of those terms*".
At that time, the most used morphological criteria to define atypical hyperplastic lesions were those described by Page et al. [2,3] "*a lesion in which either cytologic or pattern criteria of ductal CIS (DCIS) are met, but both are not present in full flower, as well as the lesion in which criteria for DCIS are present, but not uniformly so throughout at least two spaces*" for ADH and "*a lesion with cytologic appearances identical to those of LCIS found in lobular units, but in which less than one-half of the acini in a unit are filled, distorted and distended with a uniform*

population of characteristic cells" for ALH. However, the same authors admitted " …*the seeming lack of clarity or firmness in the definition of atypical hyperplasia*", but optimistically concluded that despite this fact " …*experienced surgical pathologists and histopathologists frequently recognize such a category*" [4]. In 1990, by applying the same morphological criteria of Page, Tavassoli and Norris suggested to change the extent of the lesion to ≤ 2 mm in contiguous ducts, instead of 2 ducts, to diagnose ADH [5]. The 2019 edition of blue book on breast tumors accepted both cut-offs [6]. UDH and ADH may be represented both morphologically and dimensionally in Figure 1A and 1D, respectively. Figure 2 is a graphical representation of the dimensional criteria used to differentiate ADH from low grade DCIS.
Undoubtedly dimensional criteria are clear cut and may be more easily reproduced. However, in small lesions, from a pragmatic standpoint we believe that it is important to compare histology with radiological findings, to perform levels on the block(s) where the lesions have been identified and to define the three-dimensional organization of the lesion in order to assess the real ex-

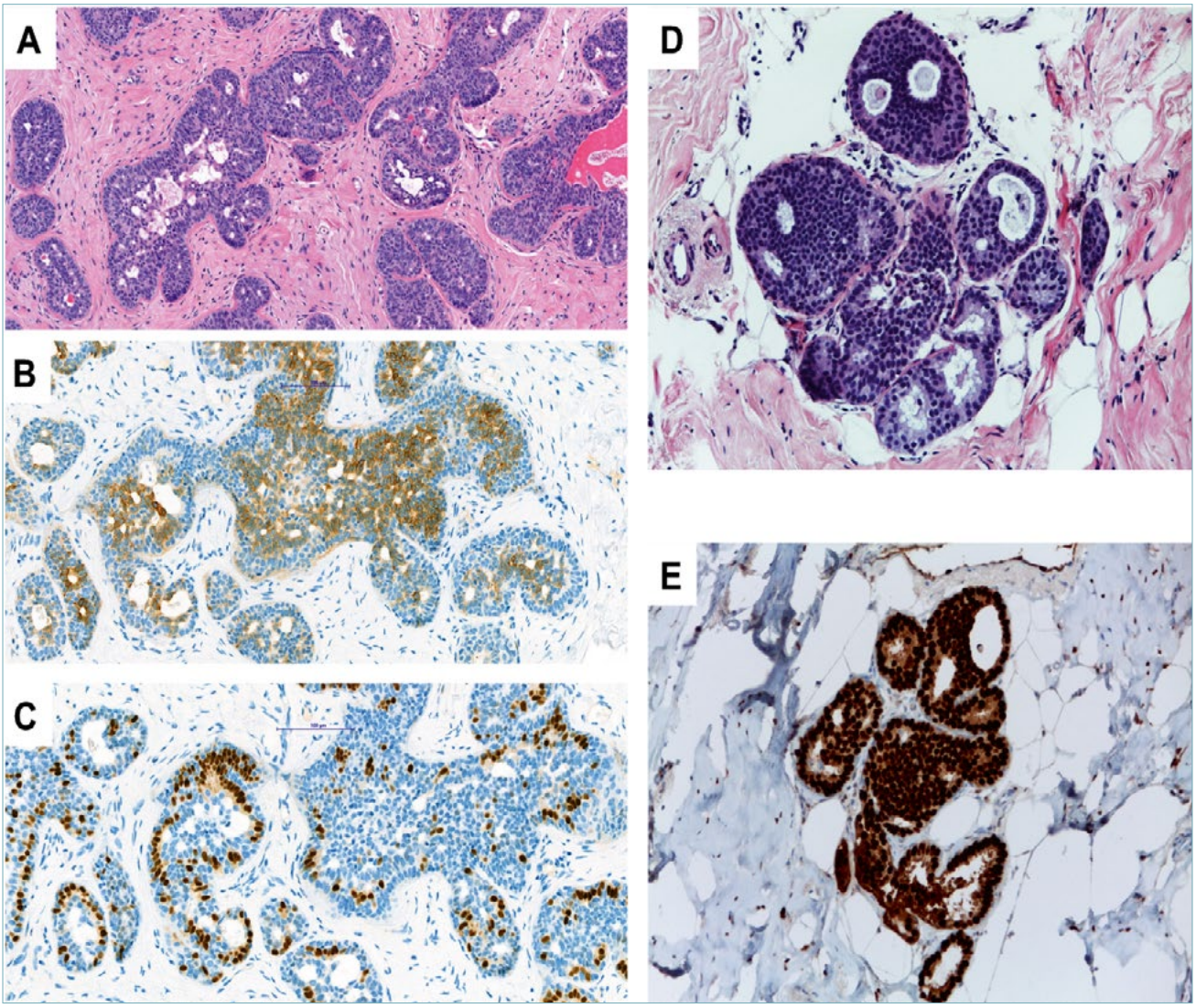

Figure 1. Representative micrographs of usual ductal hyperplasia (UDH) and atypical ductal hyperplasia (ADH). UDH shows a proliferation of cells with a streaming pattern and haphazard orientation with respect to one another (A), heterogeneous expression of basal cytokeratins (B) and estrogen receptor (C). ADH features a monomorphic proliferation of clonal proliferation featuring monomorphic cells with uniform-sized nuclei growing in arcades, cribriform, or solid patterns (D) with homogeneous expression of estrogen receptor (E).

tension of it. These issues may be exemplified looking at Figure 3 of the paper by Allison et al. [7] which represents diagnostic areas from the two cases of 72 with the highest agreement with the diagnosis of ADH. Figure 3A is representing a single "ductal" structure isolated in the interlobular stroma, it is less than 2 mm (and < 2 basement membrane bound spaces), visible at low power and it shows obvious cytologic monotony and a cribriform architectural pattern (typical of DCIS) at higher magnification. This structure could be anatomically referred to a "subsegmental duct" that continues with terminal duct and acini, structures which are known to be primarily involved by low grade DCIS. Thus, we think that cases like this one merit to be compared with radiological findings (*e.g.* calcification extension) to be sure that we are not missing a DCIS.

Apart from pure morphological criteria, Page and Dupont [2,3] determined the corresponding risks for the development of invasive carcinoma of the "atypical" category. Rosai considered these as the best-designed and carried out studies with this aim until that moment: "*The atypical hyperplasia group was found to be at a risk which was almost exactly in between that of moderate or florid hyperplasia without atypia on one hand and that of CIS on the other*".

In 1988, the consensus meeting of the Cancer Committee of the College of American Pathologists [8], approved the three "risk categories" of breast cancer, with a moderate increase of risk (x5) for ADH and ALH (category III). Later on, Dupont and Page [9] described ductal involvement by "*an insinuated characteristic population of cells between attenuated luminal cells and basement membrane*" and specified that this pattern slightly increases the risk of cancer in ALH.

Rosai's comment, regarding the use by pathologists of these risk categories, was as follows: "*the widespread adoption of this practice presupposes the existence of a reasonable degree of intraobserver and interobserver concordance in the placement of the lesions in the various categories that, to the best of my knowledge, has never been tested*". He thus decided to circulate slides among world known breast pathologists (David Page among them) asking them to select among hyperplasia, ADH, ALH, carcinoma *in situ*, and normal tissue for the lesions in the circled area on each slide. Disagreement spanned from hyperplasia (without atypia) to carcinoma *in situ*. Notably, not a single case reached 100% interobserver agreement [1]. With this study, Rosai highlighted the very subjective judgement of "atypicality" and the very subjectively understood definitions of ADH and ALH and he concluded: "*A further, inescapable conclusion derived from this admittedly small survey is that we are far from having reached uniform diagnostic criteria in this field*" [1].

One year later, in 1992, Page and Rogers proposed to use combined histologic and cytologic criteria for the diagnosis of ADH [10]. In 2000, the members of the European Commission Working Group on Breast Screening Pathology using these criteria reached an agreement of K 0.35 for ADH diagnosis [11]!

In addition, Schnitt and Vincent-Salomon described in 2003 the so called "columnar cell lesions of the breast", which "*represent a spectrum of lesions which have in common the presence of columnar epithelial cells lining variably dilated terminal duct lobular units, ranging from those that show little or no cytologic or architectural atypia to those that show sufficient cytologic and architectural features to warrant a diagnosis of atypical ductal hyperplasia or ductal carcinoma in situ*[12], which were then universally recognized with the term "Flat Epithelial Atypia -FEA".

In Rosai's paper these lesions were part of the set of slides sent for evaluation (see his Figs. 2, 8, 9) [1]. They were classified either as benign or ADH. Schnitt in a review [13] concluded "*clinical significance at this time, the appropriate management of patients whose breast biopsies show flat epithelial atypia in the absence of diagnostic areas of ADH or DCIS is unknown and requires evaluation in further clinical outcome studies*". Numerous studies have considered the issue related to up-grading of pre-operative diagnoses of FEA, ADH to DCIS, or infiltrating carcinomas and different criteria have been proposed but, the problem remains to be solved.

Another three decades have passed since Rosai's paper and many studies have been published on FEA, ADH and ALH definition and diagnostic (dis-)agreements. With the advance of screening programs, we are encountering these "atypical proliferative lesions" more and more frequently and make diagnoses leading at the excision of microscopic "atypicality" because of the "risk" of cancer and with the hope to reduce this risk.

What other methods may solve the diagnostic problem? Past and present

Rosai pointed out that different ancillary techniques were proposed to obtain a sharper and more reproducible separation among the various diagnostic categories. Some of them, like estimation of DNA content, by cytophotometry or flow cytometry and electron microscopy, are nowadays obsolete [1].

In 1991, immunohistochemical (IHC) tests were limited by the low availability of antibodies and Rosai sceptically considered IHC as a solving method [1]. Currently, pathologists are successfully using monoclonal

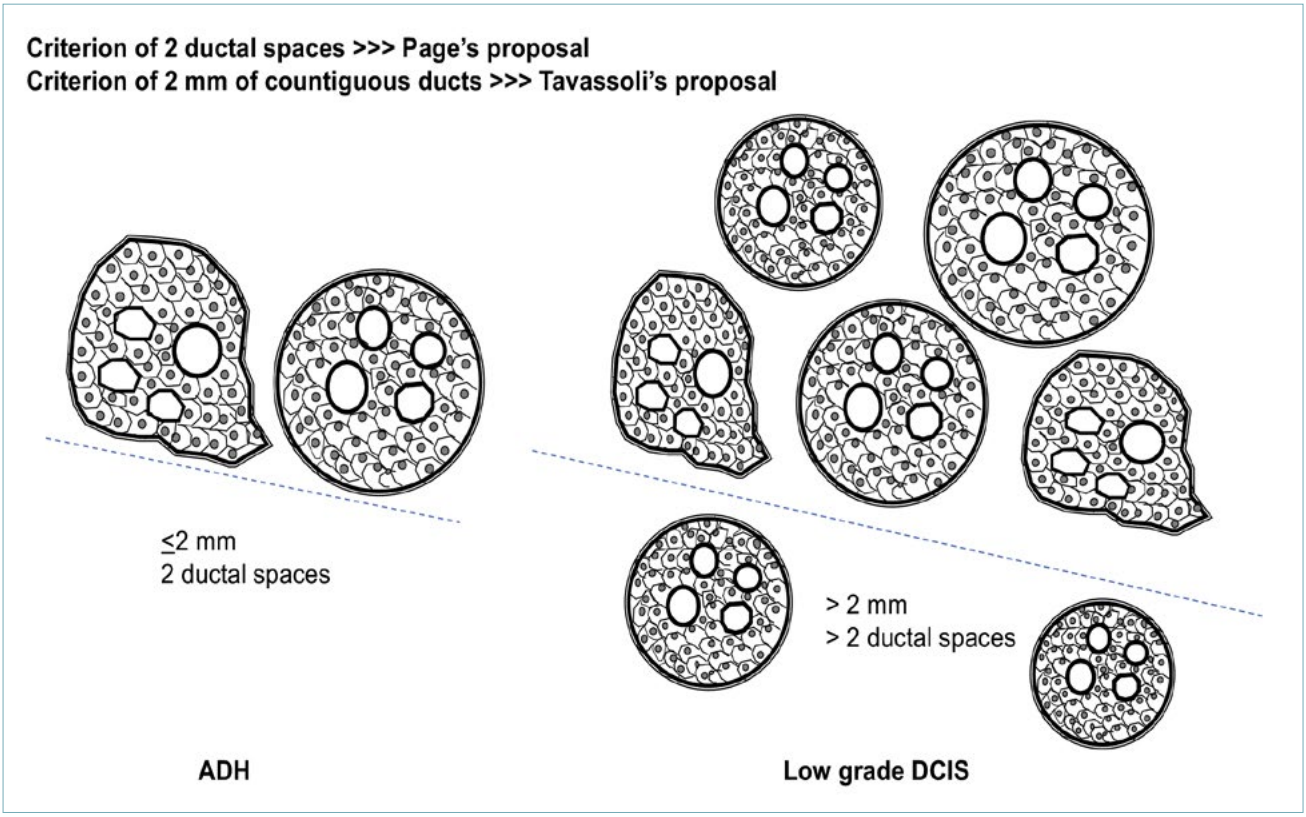

Figure 2. Graphical representation of the difference between ADH and low-grade DCIS. These two lesions are morphologically indistinguishable and the distinction is based on a dimensional criterion, whose cut-off is set at 2 mm (proposed by Tavassoli and colleagues) or at 2 contiguous duct spaces (proposed by Page and colleagues).

antibodies against cytokeratins (CK14 and CK5/6) for diagnostic differentiation of usual hyperplasia from ADH [14] (Fig. 1B), but so far, no specific antibodies are available to differentiate ADH from DCIS.

Rosai reported that morphometry of nuclear area was used, to separate ductal hyperplasia without atypia from DCIS [1,15]. In 2000, Guski et al. applied image analysis of argyrophilic nucleolar organizer regions (AgNORs) to differentiate ADH from DCIS [16]. Digital image analysis of Ki67 IHC expression, with a cut-off of 2% of cell proliferation, has been used to stratify risk in women with atypical hyperplasia. High Ki67 expression increased the risk of breast cancer by fourfold within 10 years after the first excisional breast biopsies, whereas patients with low Ki67 lesions had a risk compared to the general population [17].

Finally, Rosai reported one of the first papers linked to the use of oncogene (RAS) alterations or enhanced levels of expression of their proteins in borderline lesions [1,18]. Danforth [19] and Kader et al. [20] in 2018 both published comprehensive literature reviews on molecular alterations of atypical hyperplasia of the breast. ADH and ALH show gains or losses of whole chromosomes and loss of heterozygosity/allelic imbalance changes, which involve all informative markers on specific chromosome arms, specifically on 16q and 17p. This is consistent with the pattern found in low grade DCIS and well differentiated breast cancers, while only single markers of allelic imbalance involved normal breast tissue. Gene expression profile show that atypical breast hyperplasia molecularly pertains to the "luminal category" with overexpression of estrogen-related genes (*ESR1*, *EZH2*). We know that Estrogen Receptor-alpha (ER-α) are intensely and uniformly expressed in luminal cells of FEA and ADH (Fig. 1D, 1E), while a decrease of ER-beta expression has been reported [21]. At difference with atypical lesions and low grade DCIS, ER expression is heterogeneous in UDH (Fig. 1C). To our knowledge, no specific somatic mutations are related to atypical breast hyperplasia, although a high prevalence of premalignant lesions has been observed in prophylactically removed breasts from women at hereditary risk for breast cancer with germline mutations [22].

How Rosai proposed to solve the risk problem

Rosai agreed with Harvey and Fechner[1,23] statement, " …*the difference between the phrases* "*atypical hyperplasia*" *and* "*carcinoma in situ*" *gives the morphologic spectrum a semantic dividing point, which is far sharper in words than in the histologic images*". He was fascinated by the proposal of Buckely et al. for uterine cervix "*of dropping the dysplasia/carcinoma in situ dichotomy at this site and its replacement for a single term-cervical intraepithelial neoplas*ia, *or CIN-coupled with a grading system that would indicate increasing degrees of severity* [1,24]. Rosai, thus, proposed the concept of "*mammary intraepithelial neoplasia (MIN) of either ductal or lobular types*". He suggested two grading options: one would be to have *three grades, corresponding to hyperplasia, atypical hyperplasia, and carcinoma in situ*, the other, a "*four or five grading system could be devised to allow for the separation between mild and moderate/florid hyperplasia, or between cribriform/papillary/micropapillary/solid ductal CIS and comedocarcinoma*".

Tavassoli, in 1997, proposed again the pathological concept of "mammary intraepithelial neoplasia" as a solution to the problem of differential diagnosis [25]. Then the terminology was changed to "ductal intraepithelial neoplasia" DIN to explain the progression of intraductal proliferative lesions from usual epithelial hyperplasia to DCIS as a sequential lesion [26]. DINs were classified into three categories: DIN1 includes usual hyperplasia, ADH, and low-grade DCIS; DIN2, and DIN3 correspond to intermediate- and high nuclear grade DCIS, respectively.

DIN classification was adopted by the WHO breast tumor blue book in 2003 [27] and dismissed in the next edition of the blue book [28]. In the last WHO edition [29] ADH is defined as "an epithelial proliferative lesion with cytological and architectural features similar to those of low-grade ductal carcinoma in situ (DCIS) but less developed in architecture, degree of terminal duct lobular unit involvement, and contiguous extent". Thus, we are back again to uncertainty and as stated by the authors of the ADH WHO chapter [29] "*Variability in diagnosis is frequently related to subtle differences in professional opinion and diagnostic thresholds and may be reduced when additional consensus or second reviews are sought with the assistance of immunohistochemistry*".

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Author's contributions

A.S. and J.K.: conception and writing; C.M.: writing and production of graphical images.

Ethical consideration

No ethical issue was raised by this work.

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