



Can High Molecular Weight Cytokeratin 903 Differentiate Benign Usual Breast Ductal Hyperplasia from Atypical Ductal Hyperplasia and Ductal Carcinoma in Situ? Report of a Case and Brief Review of the Literature

Matthew White*, Tejinder Kaur, Fouad berry, Nicole DiTommaso, Christopher Taylor, Amanda Berger, Laura Castillon, Mohamed Aziz

Matthew White, American University of the Caribbean School of Medicine, USA (#1 University Drive at Jordan Road, Cupecoy, Sint Maarten)

Abstract

The diagnosis of pre-cancerous breast lesions is important to evaluate the risk of recurrence and progression to invasive carcinomas. In this report, we present a 35-year-old female with suspicious microcalcification foci consistent with atypical ductal hyperplasia, but short of the qualifying 2 mm size of low-grade ductal carcinoma in situ. We used high molecular weight cytokeratin (HMW-CK- 903) as a marker to further analyze the lesion. A final diagnosis of two small foci of low-grade DCIS in the background of ADH was rendered. In challenging cases, like our case here, the use of such markers can be useful to reach accurate diagnosis and guide management plan. This is especially true when the patient is pregnant, and the lesion is borderline on size for the arbitrary limit of 2 mm, which determines the upgrade of atypical ductal hyperplasia to low-grade ductal carcinoma in situ. After full resection of the lesion, the patient elected not to have post-operative radiation therapy and subsequently had no recurrence at 7 years follow-up. In this manuscript, we review the literature on the difference between, benign ductal hyperplasia, atypical ductal hyperplasia, ductal carcinoma in situ, and invasive carcinoma. We also discuss the importance of a proper diagnosis of pre-cancerous lesions, and how different treatments can be approached during pregnancy.

Keywords: Benign ductal hyperplasia; Atypical ductal hyperplasia; Ductal carcinoma in situ; Benign; Malignant; Prognosis

Abbreviation

HMW-CK: High molecular weight cytokeratin, UDH: Usual ductal hyperplasia ADH: Atypical ductal hyperplasia, DCIS: Ductal carcinoma in situ, LG-DCIS: Ductal carcinoma in situ, IHC: Immunohistochemistry. RT: Radiation therapy

Introduction

Breast cancer is the number 1 most common and the 2nd most deadly cancer in women. Pregnancy-associated breast cancer has an incidence of 1 per 3000 pregnancies. It is defined by breast cancer occurring during or up to 1-year after pregnancy [1].

It is important to distinguish between benign, pre-cancerous, and cancerous lesions of the breast after a lesion is detected on mammography. There is a hierarchy of cancerous growths, with atypical ductal hyperplasia (ADH) being lowest, followed by low-grade and high-grade ductal carcinoma in situ (DCIS). Finally, invasive carcinoma is when the neoplasm has escaped the basement membrane of the duct and invades surrounding tissue. However, this might not always be a sequential progression [2].

There were about 64,000 new ductal carcinomas in situ (DCIS) cases in 2018 with a 10-year survival of 98%. High survival is contributed to treating nearly all cases of DCIS with surgical lumpectomy [3]. With low-grade DCIS and ADH being histologically similar it is important to find a way to distinguish between them, other than the arbitrary 2mm cut-off size required for the diagnosis of low-grade ductal carcinoma in situ [4]. Lumpectomy or adjuvant therapy for ADH is less likely to be implemented than it is for DCIS [5]. Additionally, DCIS is more likely to eventually lead to a diagnosis of invasive cancer than ADH [6,7]. Therefore, it is vitally important to optimize the differentiation of DCIS although this can be challenging due to histological similarities so that the appropriate therapy is implemented.

Here we present a case of a 35-year-old pregnant female with mammographically suspicious microcalcification of the right breast. She had a strong family history for BRCA-1 positive breast carcinoma but no other significant risk factors. In this manuscript, we discuss how the lesion was diagnosed as low-

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***Corresponding author:** Matthew White, American University of the Caribbean School of Medicine, USA (#1 University Drive at Jordan Road, Cupecoy, Sint Maarten), Tel: (403)272-8516; E-mail: matthewwhite@students.aucmed.edu

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grade DCIS (LG-DCIS) as opposed to ADH and demonstrate our use of the marker HMW-CK-90 to help differentiate between usual ductal hyperplasia (UDH), ADH and LG-DCIS.

We also discuss ADH, DCIS, and the risk of developing invasive breast cancer with various management modalities. In addition, we emphasize the modes of treatments, which can be approached during pregnancy.

Clinical Presentation

A 35-year-old female presented with mammographically suspicious microcalcification. She was 4-5 week pregnant and had a strong family history for BRCA-1 positive breast carcinoma. No other significant medical history or other risk factors were reported. Imaging studies was suspicious for malignancy. A 9-gauge core biopsy was performed, and adequate core fragments were obtained for pathologic analysis. In two core fragments, there were scattered atypical foci, each close to the size of 2 mm.

Histomorphologically the foci were consistent with atypical ductal hyperplasia, but short of the qualifying 2 mm required for the diagnosis of low-grade DCIS. Cellular atypia was severe, but pregnancy associated changes were considered in making the diagnostic interpretation. It is known that pregnancy can be associated with significant reactive changes due to hormonal changes. Interpretation of advanced breast lesions should be considered with caution in cases of pregnancy.

Immunohistochemistry (IHC) studies utilizing HMW-CK-903 were performed on the core breast tissue. The internal positive control was very good in the sections in form of strongly positive reaction in usual benign ductal hyperplasia (Figure 1A). There was very weak staining with HMW-CK-903 in the atypical foci, which is in support of ADH (Figure1B). DCIS foci were negative for HMW-CK-903 (Figure 1C). CK 5.6 studies were not very helpful due to mixed staining pattern and lack of good internal

positive control. Smooth muscle myosin heavy chain (SMM-HC) nicely outlined the myoepithelial cell layer indicating absence of invasive breast carcinoma. With the size approaching the qualifying 2 mm, the challenge was; is it enough to call it LG-DCIS, taking in consideration the radiographic atypia and strong family history, or pregnancy changes are strong factor in this atypical change and should be only called ADH.

With the pattern of staining with HMW-CK 903, a final diagnosis of two small foci of LG-DCIS in background of ADH was rendered. The entire suspicious area including the two DCIS foci was excised. The excised breast tissue showed the site of the prior core biopsy with associated reactive reparative changes, but no residual carcinoma was noted in the excised tissue with a safe free margin larger than 2mm. After detailing with the patient the risk and benefit of post-operative radiation therapy (RT), the patient elected not to have post-operative RT. There was no evidence of recurrence or metastasis for 7 years, after which the patient was lost to follow up.

Discussion

Histological identification of ADH is similar to LG-DCIS. However, ADH requires either partial involvement of the ducts or smaller size, and cells are evenly spaced with polarization [5]. Histological morphology is traditionally considered the gold standard of differentiating between UDH, ADH, and LG-DCIS. However, cytokeratin 5, and ER has been shown to improve the differentiation of these lesions. Martinez et al. demonstrated that estrogen (ER) and progesterone receptors (PR) stained negative in UDH and diffusely positively stained in ADH and LG-DCIS. The highest rates of ER and PR staining was found in the latter.

Many markers are used to differentiate tissues when morphology alone is not sufficient. One of these markers include subclasses of cytokeratin (CK). CK is the intermediate filament of

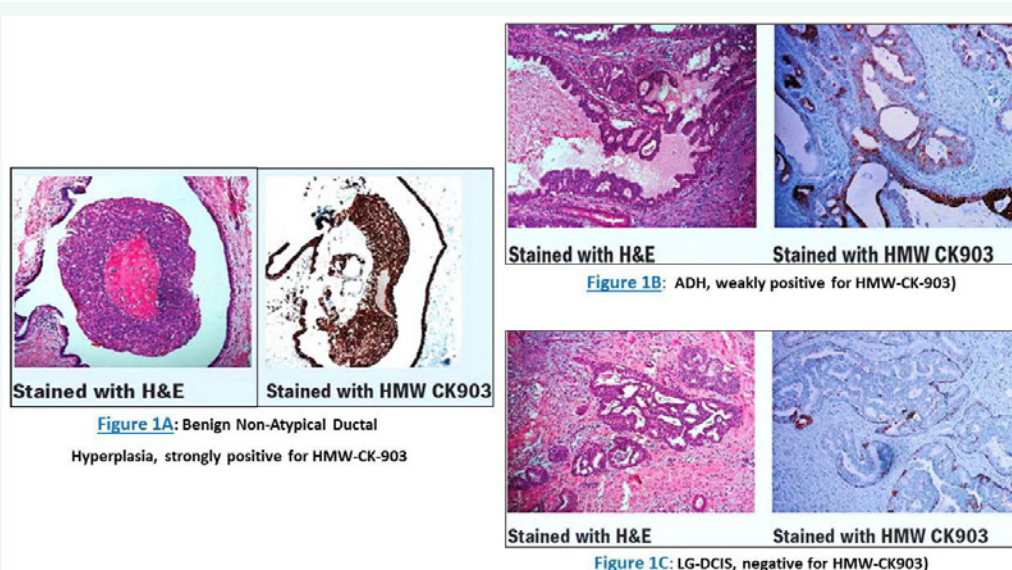


Figure 1 HMW-CK-903 staining pattern in three different breast proliferations. 1A: Benign Usual Ductal Hyperplasia, strongly positive for HMW-CK-903 X40. 1B: ADH, weakly positive for HMW-CK-903 X20; 1C: LG-DCIS, negative for HMW-CK903 X20.



epithelial cells and hair. Types 5/14 are part of the basal cell layer, while 1/10 are supra basilar layer of epidermis [8]. The epidermis switches expression as the cells move up the epidermis. These CKs are associated with squamous cell carcinomas, but little to no association with adenocarcinomas [8].

CK5 was shown to have a diffuse stain in usual ductal hyperplasia, while it was focal in ADH and low-grade DCIS⁹. This indicates the benefit of using multiple markers for a diagnosis of breast cancer when morphology is ambiguous especially due to there being marker overlap in many cases.

We are reporting this case to demonstrate the utility of monoclonal antibody CK-903, specifically for High Molecular Weight Cytokeratin (HMW-CK) 1, 5, 10, 14, in the diagnosis of intraductal breast pathology where morphological criteria failed to clearly define the lesion. Reports of the immune profile of benign breast tissue, ADH, and DCIS from previous studies served as the standard of comparison for the staining pattern in this study [9].

Given the pattern of staining with HMW-CK 903 in our case, a final diagnosis of two small foci of DCIS in the background of ADH was rendered. These two foci were 50% moderately to strongly positive for Estrogen (ER ID5) receptor, and 80% moderately to strongly positive for Progesterone (PG PgR636) receptor.

Additionally, other markers can be used to differentiate invasive from non-invasive breast lesions. Myoepithelial-specific and myoid markers p63 and smooth muscle myosin heavy chain (SMM-HC) are used to show a lack of invasion of surrounding tissue. However, markers to distinguish between different non-invasive lesions require further investigation [5]. A major difference between the diagnosis of ADH and DCIS is a span greater than 2 mm and involvement of 2 or more separate basement membrane-bound spaces of the ducts. However, this appears to be an arbitrary number to differentiate DCIS from ADH [4].

The treatment of different breast lesions varies depending on the lesion characterization, age of the patient, and whether the patient is pregnant. When the diagnosis is ambiguous between ADH and DCIS it is treated as DCIS. This involves local resection, possibly followed by adjunctive RT [4]. The risk of recurrence of invasive cancer occurring after lumpectomy of DCIS alone has been shown to be as high as 39-53%, indicating that further intervention should be considered after surgery. Adjuvant RT has been shown to decrease the recurrence of invasive cancer by half when compared to lumpectomy alone [10].

The highest modifiable risk factor for the recurrence of DCIS is the presence of positive margins in lumpectomy. This is due to neoplastic cells not being removed, leading to a high risk of local recurrence and invasion [10]. Having a negative margin (the distance of excised tissue from the nearest benign tissue surrounding the lesion area) of 2 mm reduces the risk of local recurrence. But, a resection greater than 2 mm has no additional benefit [11]. Van Zee et al. found that the 10-year rate of recurrence was 31% for women with positive margins and 13% for women with negative margins. The statistical difference was

only found in those not receiving RT, those who received RT had no statistical difference [12]. This indicates that in the absence of RT therapy obtaining negative margins are vital, while with RT there was little difference but trended to a decreased risk of recurrence with negative margins. In the case of pregnancy, if negative margins are obtained from DCIS, then radiation can be avoided. However, if positive margins are found, then RT should be considered. In the case of our patient, no positive margins were found, and she elected not to receive post-operative RT. She had no recurrence as of 7 years post-operation.

Treatment of breast cancer during pregnancy is difficult due to the teratogenic effects of many therapies. Surgical treatment of breast and axilla is possible in all three trimesters but has an increased risk of miscarriage in the first trimester. Additionally, methylene blue use in sentinel lymph node biopsy increases the risk of fetal jejunal atresia in the first trimester. Therefore, sentinel lymph node biopsies are performed but usually without dyes [1]. Surgery in the 3rd trimester is ideal with RT ideally reserved for the postpartum period. RT during pregnancy is predicted to have variable effects on the fetus with growth delay likely occurring in all trimesters. Decreased mental capacity can occur if implemented during the first or second trimesters. However, RT to the breast during pregnancy has yet to be reported in the literature as a treatment [1]. Chemotherapy in pregnancy-associated breast cancer poses the highest risk to the fetus in the first trimester. Therefore, the standard 5-fluorouracil, doxorubicin, and cyclophosphamide regimen are best reserved for after the 1st trimester [1].

Those with ER+ tumors saw that tamoxifen was associated with a 42% reduction in any breast event. However, tamoxifen has a high number of adverse events, decreasing compliance [10]. Tamoxifen can decrease the development of breast cancer within 10 years from 21% to 7.5% following a lumpectomy [5]. Tamoxifen and trastuzumab for ER+ and HER2/neu+ tumors respectively are contraindicated during the entirety of pregnancy but can be used postpartum [1].

The risk of breast cancer also includes being positive for BRCA1/2. The patient in our case had a family history of BRCA-1 positive breast cancer. A germline loss in one BRCA gene carries with it a risk of loss of heterozygosity if a somatic cell loses the functional gene [13]. A mutation in BRCA increases the risk of neoplasms most commonly in the breasts, ovaries, and prostate [14]. The loss of BRCA increases the risk of cancer by losing the Homologous repair of double-strand breaks [13]. This can lead to chromosomal abnormalities that risk cancer [5].

If an invasive tumor is the result of the loss of the BRCA gene, treatment with poly ADP-ribose polymerase (PARP) inhibitor (Olaparib) can be used [13]. Olaparib blocks the repair of single-strand breaks in all cells of the patient. With tumor cells having loss of BRCA, this would be lethal for the neoplastic cells while the somatic cells remain safe due to their functional BRCA gene [14]. Olaparib is contraindicated during pregnancy due to potential teratogenicity and should be avoided during breastfeeding due to little knowledge of effects [15].

With many genes involved with cancer the progression to



invasive cancers are of interest. Based on cellular phylogenetic trees, it appears that somatic cells with more single nucleotide variants are the common ancestral cell to both invasive carcinoma and the non-invasive lesions such as ADH rather than a sequential progression from ADH to invasive carcinoma [2]. Chromosomal abnormalities are a potential mechanism of the transition from ADH to IDC, as is the loss of heterozygosity of genes such as TP53, RB1, and BRCA1 [5].

Most lesions that progressed to cancer were not part of one somatic mutation lineage. Therefore, genetic heterogeneity in benign lesions could be relevant for patient management as it is more predictive of progression to invasive cancers. This could indicate that a homogeneous ADH lesion is not likely to progress to invasive cancer, but multiple independent ADH lesions that have heterogeneity are more likely to be a risk for invasive cancer [5]. A stepwise progression from ADH to breast cancer could appear to be happening due to the “field effect” where ADH happens to appear in the tissues prone to invasive cancer but is not directly progressing to cancer [5]. This leading to a newer model low- and high-grade multistep models defined by molecular markers and chromosomal abnormalities [5]. Core needle biopsy at the maximum lesion size could be predictive of upgrades with 78% sensitivity and 80% specificity [5]. Benign breast lesions might not be the direct precursors of invasive lesions but an independent clonal proliferation of common ancestral somatic cells [2].

Khoury et al. showed the risk of diagnosed ADH upgrading to DCIS is 12% or invasive carcinoma is 3% [6]. It is difficult to determine the risk of DCIS progression to ipsilateral breast cancer if left untreated due to 98% of lesions being excised. However, it has been shown that if left untreated, DCIS may progress to invasive breast cancer in 10.5% of cases [7]. While the risk of developing invasive cancer after treatment for DCIS is 5.08% [3].

Black and Asian women having the highest risk ratio of recurrence [3]. It is important to determine if a lesion is ADH or DCIS to accurately predict and minimize the risk when intervention is warranted. Many reports agree with the usefulness of HMW-CK to differentiate benign hyperplastic lesions from atypical proliferation and DCIS. However, when it comes to the differentiation of ADH from DCIS, there is an obvious reservation to agree with the usefulness of this marker. We believe that there is a difference in the pattern of HMW-CK being weak in ADH but absent in DCIS. This can aid in the diagnosis in very challenging cases. Hopefully, a continued investigation will drive further understanding of our observation, with the development of efficacious diagnosis and safe treatments for improving patient outcomes.

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