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Morphopathological and immunohistochemical features of a pure mucinous breast carcinoma – Case report

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ABSTRACT
Pure mucinous carcinoma is a rare special type of breast carcinoma with a 2% incidence and it is usualy asociated with a good prognosis. It must distingished from the mixed subtype of mucinos breast carcinoma, which has an invasive non-mucinous component in more than 10% of the tumor and change the favourable outcome of the first subtype. In this report we present a case of a premenopausal woman with a lump in right breast wich histopathologically proved to be a pure mucinous carcinoma associated with high grade ductal carcinoma in situ. Immunohistochemical and ancillary studies demonstrate a great heterogeneity of the neoplastic cells, with different molecular profile for each component of the tumor. The presence of ductal carcinoma in situ with a different imunophenotype from pure mucinous carcinoma rise the ipothesis of a different tumor cell biology which may change clincal evolution.

Keywords: Pure mucinous breast carcinoma, ductal carcinoma in situ, immunohistochemistry, CISH

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Introduction

Despite numerous prevention and screening programs for early detection of breast carcinoma (BC), it still ranks second worldwide incidence and the leading cause of mortality among women, especially in the developing countries [1, 2]. It is responsible for 1.7 milion of all new cases of BC and for more than a half of milion of deaths according to the latest data [2].

Mucinous carcinoma is a rare special type of BC with 2% from all breast cancer and is defined as pools of extracelular mucin in wich cluster of malignant cells are floating [3]. When more than 90% of the tumor is represented by a mucinous morphology, than it is considered as pure subtype of mucinous breast carcinoma, wich is associated with an excellent prognosis as demonstrated by previous studies [4, 5]. The second subtype of mucinous BC is the mixed-type and it is considered as a diagnose when the non-mucinous component is present in more than 10% of the tumor [6]. The most frequent type of the non-mucinous component is invasive ductal carcinoma - no special type (IDC-NST) [7]. The mucinous carcinoma prognosis worsens when it

is associated with a ductal invasive carcinoma, being similar to that of the later component [8].

In this report we present a case of a premenopausal woman with a lump in right breast wich histopathologically proved to be a pure mucinous carcinoma associated with solid and comedo-type ductal carcinoma in situ (DCIS). Immunohistochemical and ancillary studies demonstrate a great heterogeneity of the tumor cells which sugest a different molecular pathways of progression.

Materials and mehods

Case report. A 47 years old woman was admitted in the surgical department of The Hospital County Constanta for a tumor mass in her upper-outer quadrant of the right breast. The lesion was detected by the patient 7 months ago and her clinical history did not notice anything significant. Mammography confirmed the presence of a well-circumscribed lobulated nodule with radiologic features highly sugestive for malignancy. Computer tomography or clinical examination revealed no other abnormalities. The sectorectomy was performed and frosen sections proved the clinical suspicions of invasive carcinoma.

Methods. Tumor samples were selected and 4 μm thick sections of formalin fixed, paraffin-embedded tissue block of the tumor were stained with hematoxylin and eosin. Microscopic examination estabieshed the histopathological type of BC using the criteria recommended by the World Health Organization [3] and grade according to the Scarff-Bloom-Richardson scale modified by Elston and Ellis [9]. After epitope retrieval, immunostain was performed on the best representative slides using a pannel of four antibodies from DakoCytomation–Denmark (Tabel 1). 3,3'diaminobenzidine (DAB) was used as chromogen, resulting a brown stain of the antigen and the final stage was Mayer's haematoxylin as a counterstain. The semiquantitative scoring method was applied for immunohistchemical expression of hormonal estrogen and progesteron receptors and the cut-off value for positive result was 1% nuclear stain [10]. Recomandations of Dowsett et al. were used for evaluation of Ki67 immunostain, where the cut-off value for low or high expression was 14% nuclei stained [11]. Newest ASCO/CAP guidelines were applied for assesment of HER2 status (over-expression or absence of the HER2/neu protein)[12]. For those cases with an equivocal result at immunohistochemistry, molecular study was performed in order to establised the cerbB2 gene amplification in tumor cells. Chromogenic in situ hybridisation (CISH) was the method used for this purpose with ZytoDot SPEC HER2 Probe Kit from ZytoVision and the results were evaluated according to manufacturer's criteria.

Table I The pannel of antibody used for immunohistochemical examination (source, clone, dilution)

Antibody*	Clona	Dilution	Antigen retrieval
ER	Rabbit monoclonal antibody1D5	Ready to use	EnVision ™ FLEX Target Retrievel Solution, high PH
PR	Mouse monoclonal antibody PgR 636	Ready to use	EnVision ™ FLEX Target Retrievel Solution, high PH
HER2neu	Rabbit Imunoglobulin HercepTest	Ready to use	Epitope Retrieval Solution supplied by HercepTest™ kit
Ki-67	Mouse monoclonal antibody MIB-1	Ready to use	EnVision ™ FLEX Target Retrievel Solution, high PH

*Vendor - Dako, Denmark

Results

Macroscopic evaluation of the surgical specimen revealed a solid mass measuring 2.5x1.8 cm, well-defined, firm and white-tan color with translucid areas (Figure1).

Morphopathological examination observed clusters of malignant cells floating in lakes of mucus, with low nuclear grade (Figure 2A) characteristic for subtype A (hypocellular) of pure mucinous BC. An invasive component of moderate differentiated IDC -NST was identified, but because it didn't exceed 10% of the tumor volume, then pure mucinous carcinoma was the correct diagnose (Figure 2B). It was also observed an intratumoral component represented by

solid and comedo-type DCIS with high nuclear grade (Figure 2C).

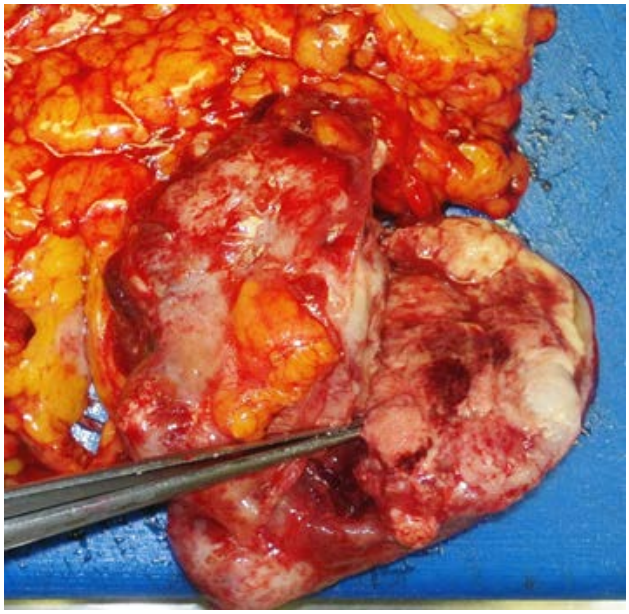

Figure 1 Macroscopic aspect of pure mucinous carcinoma: a well – circumbscribed solid tumor with a lobulated contour, white-tan colour and translucid areas

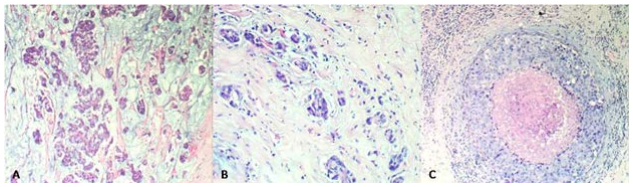

Figure 2 Microscopic features of the tumor: A) mucinous BC with pools of mucin in which clusters of tumor cells are floating (HE, 4x); B) IDC-NST component (HE, 4x); C) comedo-type DCIS (HE, 10x)

Immunohistochemical examination showed different patterns of expression for each biomarkers and also for the different component of the tumor. It was noticed a high level of expression for hormonal receptors (ER and PR) in the mucinous compartment in contrast with moderate expression for IDC-NST and none for DCIS (Figure 3.A,B,C).

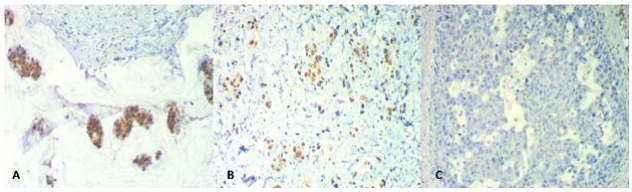

Figure 3 ER immunostain: A) intens nuclear stain in the tumor cells of the mucinous BC (IHC, 10x); B) moderate nuclear imunostain of IDC-NST (IHC, 10x); C) absence of ER expresion in the tumor cells of DCIS (IHC, 10x)

It was also obtained different IHC scores when overexpression of HER2neu oncoprotein was evaluated by immunohistochemistry: IHC 0 - negative for mucinous BC (incomplete and faint membrane stain in less than ≤ 10% of malignant cells); IHC 2- equivocal in IDC-NST (incomplete and weak to moderate membrane stain within > 10% of neoplastic cell); IHC +3 – positive for DCIS (intense and complete membrane stain in more than 10% of malignant cell) (Figure 4.A,B,C). The rate of nuclear proliferation proved to be low because the Ki67 index is less than 14% within all tumor cells.

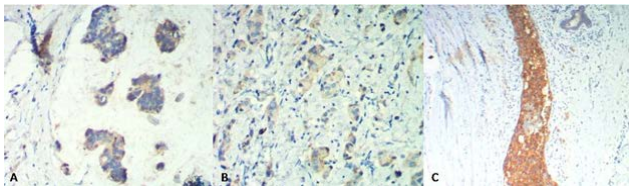

Figure 4 Immunohistochemical HER2neu oncoprotein status: A) IHC score 0 –negative in mucinous BC (IHC, 10x); B) IHC score 2 – equivocal in the DCI-NST (IHC, 10x); C) IHC score 3 – positive in DCIS (IHC, 4x)

Molecular study was further performed to evaluate HER2neu gene amplification. The result for CISH exam varied depending on tumor components: mucinous BC and IDC-NST with no amplification, both had shown a normal nuclei each with two HER2 signals (Figure 5 A,B); DCIS with a high level of amplification, characterised by a mixture of numerous small clusters and rare big clusters, with more than 7 signals HER2 present per nucleus (Figure 5C).

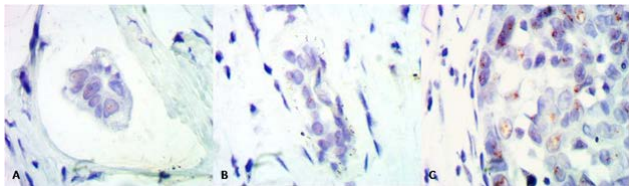

Figure 5 HER2neu gene status by CISH: no amplification for mucinous BC component (A) and for IDC-NST component (B) (20x); high level of HER2neu gene amplification in DCIS component (C) (20x)

Discussion

Mucinous breast carcinoma with its synonyms (colloid BC, mucoid carcinoma; mucinous adenocarcinoma) is one of the rarest special subtypes of BC with an incidence range between 1% to 2% [3,8]. Most cases are diagnosed after the fifth decade of life, frequently of white ethniticity [13]. The mean age 68.3yo obtained by a large retrospectiv study proved that it is statistical significantly higher than that observed in cases with IDC-NST (61 yo) [3, 13]. Even if our patient is younger than the mean age observed in large data base studies, it still fall between the values recorded of 21 to 94 yo [14]. As in the present case, most of the tumors are located in the upper outer quadrant (44%) and also the tumor diameter is close to the 2.2cm mean value observed in different study, but larger tumors up to 25 cm were also described [13,15]. Morphopathological features of the tumor consist with subtype A of pure mucinous BC (hypocellular type), the most frequent type but there are also described forms with hipercellularity (subtype B) or with intermediate features (subtype AB), with no impact on prognosis [8]. The only differences between subtype A and B are the age of the patient at the time of diagnose (older patient for subtype B) and the presence of neuroendocrine differentiation in subtype B [16]. The mucinos component of our case is well differentiated which is consisted with previous reports, where well differentiated tumors had been found in up to 65% of subtype A mucinous BC [17]. The presence of an IDC-NST in less then 10% of the tumor have imposed exclusion of a mixt mucinous BC diagnose [3]. This has a profound impact on prognosis, because the survival rate for pure mucinous BC is better than the mixt mucinous BC wich has a similar prognosis to that of IDC–NST [5]. In a large study with 1221 pacients with mucinous BC, the overall survival was 80% versus 77% for IDC-NST [18].

DCIS is frequently found in the pure mucinous BC, usually at the periphery of the tumor and it has been discribed all morphological subtypes [19]. In the study of Gadre SA et al, solid DCIS was identified in almost half of cases, comedo-type DCIS up to 30 % cases of mucinous BC, most of the well and moderate differentiated and only 11% of cases with high nuclear grade as in our report [20].

Numerous immunohistochemical studies on mucinous BC show that almost all cases are positive for hormonal receptors, especially for ER. The ER expression varied from 73.4% [4] to 100% [21] positivity, which are higher compared with those for PR: 65.4% [4] to 85.7% [22]. It was also proved positive immunostain for androgen receptor (AR) but with a high range of values: 1.7% [23] –21.7% [24]. This hormonal profile argue the good prognosis of this specific subtype of BC. In present case, it was also obtained a positive results for hormonal receptor both in the mucinous and in the IDC-NST component consisted with literature reports. The absence of ER and PR expression in the in situ component wasn't surprising because it had been demonstrated that high-grade DCIS, especialy comedo-type has a negative ER and PR imunostain [25, 26, 27]. Both biomarkers have a predictive role since patients with a positive imunostain receive treatment with trastuzumab [28]. More than that, it has been proved that high-grade DCIS with absence of ER expression is associated with a high risk of recurrence [29].

The proliferation rate of tumor cells was assesed using Ki67 imunostain and it was observed a low Ki67 index for all components of the tumor. These values are in concordance with previous reports which consistently showed low rate of Ki67 expression for numerous cases of pure mucinous BC, up to 91.4% in one study [22]. On the other hand, in

IBC and DCIS proliferation rate evaluated by Ki67 imunostain recorded different values from low to high rates and it is usualy correlated with tumor grade [30]. Recently, a high KI67 index proved to be an independed prognostic factor, those patients having a high risk for recurrence as IBC [31]

HER2neu oncoprotein is one of the four memberes of HER family and plays and important role in cellular growth [32]. If for IBC, the negative impact on prognosis of HER2neu overexpresion is well establised, its role is still debate in DCIS [33]. The study of Borgquist S. et al. proved that its positivity is associated with lower risk of recurrent invasive BC [34]. Recently, it was demonstrated that HER2neu amplification implies a high risk for DCIS recurrence [35]. Also it has been demonstrated that HER2neu amplification is aproximatively three time higher in DCIS than IBC-NST [36]. In adition, HER2neu overexpresion in high-grade DCIS is frequently associated with extensive comedo-type necrosis, a high rate of nuclear proliferation and with a negative hormonal status [36]. In contrast, mucinous BC is usualy negative to HER2neu, only 5% of cases being reported as positive [18]. In present case, we observed a heterogenity of HER2 neu immunostain from negative, in mucinous component, to an equivoc result for the small IBC-NST component and positive in DCIS component. CISH examination demonstrated the absence of HER2neu amplification in IDC-NST and in mucinous componet, in contrast with high level of amplification for tumor cell of in situ component.

So, it was noticed a different molecular profile for different areas of the tumor: ER+/PR+/ HER2neu - for mucinous BC and IBC-NST versus ER- /PR- / HER2neu + for DCIS. These results rise the ipothesis of a different tumor cell biology for this type of breast lesions, which may have a unfavourable impact on prognosis.

Conclusions

Pure mucinous breast carcinoma in a premenopausal women is a very rare special subtype of BC and usualy are assoicated with a favourable prognosis. Even if it is associated with an excelent prognosis, the presence of DCIS with a different imunophenotype may change clincal evolution of this tumor. Because its consecutively high risk for an in situ carcinoma recurrence, the patient mast be consistently evaluated.

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