Glycogen-rich Clear Cell Carcinoma of Breast: A Case with an Unusual Macroscopic Presentation and A Good Clinical Outcome

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Abstract

**Background:** Glycogen-rich clear cell breast carcinoma is a rare histological subtype of breast carcinoma with intermediate-high grade histology, mostly detected as a breast lump with unfavourable prognosis and an aggressive clinical course. Herein is described a case detected on a screening program as two groups of microcalcifications within a non-palpable area of parenchymal distortion in the upper-quadrant of the right breast. Fine needle aspiration cytology confirmed malignancy. Distant metastases were ruled out. The patient underwent a quadrantectomy with sentinel lymph node biopsy followed by a modified radical mastectomy. She was alive and disease free four years after conventional chemotherapy.

**Materials and methods:** Quadrantectomy and mastectomy specimens were processed for routine histology. Three sentinel lymph nodes were analysed in intraoperative consultation and completely submitted for histology. Immunohistochemistry and in situ hybridization were applied on representative samples of tumor.

**Results:** Quadrantectomy showed multiple scattered foci of white tissue, ranging from <1 mm to 5 mm, in an adipose area of 36 mm in diameter. Both groups of microcalcifications were indistinct on gross examination. Microscopically multiple foci of invasive glycogen-rich clear cell carcinoma grade 2, Nottingham score 7, were observed. High grade intraductal clear cell carcinoma was also present. One sentinel lymph node showed micrometastases. Rare eosinophilic cells were a minor component identified in tumor as well as in micrometastases. Both cell types expressed the same tumor profile. Mastectomy contained residual high grade intraductal carcinoma without axillary metastases.

**Conclusions:** The aim of this report is to illustrate a clinically silent tumor with an unusual multifocal invasive growth pattern not previously described in this tumor type, with lymph node micrometastases as an early event revealing an aggressive biology. In this case, early detection and treatment favored a good clinical outcome and provided an insight into the development of this histological subtype.

**Keywords:** Glycogen-rich; Clear cell carcinoma; Micrometastases; Her2/neu; Sentinel lymph node; Breast cancer; Mammogram

Introduction

Glycogen-rich clear cell carcinoma (GRCCC) is a rare histological subtype of breast carcinoma. Its estimated incidence is 1-3% of breast carcinoma cases [1,2]. Fewer than a hundred cases have been reported since the first description in 1981. All cases have been diagnosed in females within the age of 41-78. Clinical presentation is similar to ductal not otherwise specified (NOS) carcinoma, mostly discovered as a breast lump ranging from 1 to 10 cm in size [1,3]. Grossly, GRCCC has either circumscribed or irregular borders. Papillary intraductal / intracycstic features are also on record [4]. Microscopically, more than 90% of tumour cells have polygonal clear cytoplasm containing glycogen, with irregular hyperchromatic nuclei, a predominant solid growth pattern featuring histological grade 2-3. In some cases cells with eosinophilic cytoplasm were also noticed. Intraductal component with clear cell features, either in the pure form or associated with invasive tumor, is usually of solid, papillary and/or comedo pattern [3,5]. Immunophenotype is not uniform [6]. GRCCC express consistently cytokeratin CK 8-18, CK7, and AE3 [4]. Hormone receptor expression as well as Her2/neu status revealed no differences with ductal NOS carcinoma [6]. Flow cytometry of six GRCCC revealed a nondiploid content of DNA and all had a high S-phase fraction [7]. Lymphatic dissemination was a frequent feature [8,9]. Most reports describe an aggressive clinical course with poor prognosis, although when matched stage by stage with usual breast carcinoma, differences do not appear significant [1].

Case Presentation

50 year-old female post-menopausal, without hormonal therapy, no family history of breast cancer, in treatment for arterial hypertension with enalapril. A screening mammogram showed two groups of microcalcifications in the upper-quadrant of the right breast, within a non-palpable area of parenchymal distortion, BI-RADS 4, suspicious for malignancy (Figure 1). Axillary lymph nodes were radiological and clinically negative. Fine needle aspiration cytology yielded a poor cellular material with a few malignant cells. Full staging investigations ruled out distant metastases. The patient underwent a...
quadrantectomy with sentinel lymph node biopsy followed by radical modified right mastectomy.

Figure 1: A-Right breast mammogram showing two groups of microcalcifications within an area of parenchymal distortion. B-Detail of the suspicious area.

Materials and Methods

Quadrantectomy specimen was fixed in buffered formalin, sliced at 5 mm intervals and submitted for histology. Three sentinel lymph nodes were analyzed in intraoperative consultation. Complete submission of lymph nodes for routine histology and immunohistochemistry was performed according to standard protocols. Modified radical mastectomy and axillary lymph nodes were processed for routine light microscopy. All sections were stained with hematoxylin and eosin (H&E). Periodic acid stain (PAS) with and without prior diastase digestion, Mucicarmine and Alcian blue stains were performed on representative histological sections. Immunoperoxidase labelling using streptavidin-biotin-peroxidase complex and 3'3'-diaminobenzidine as the chromagen, was performed with the automated Bend-Max staining system (Vision BioSystem – U.K.), using antibodies (Novocastra, UK) for Estrogen receptor (ER) (clone 6F11), Progesterone receptor (PR) (clone 16 and SAN27), Her2/neu (clone CB11), CK8-18 (clone 5D3 and LP34), E-Cadherin (clone 36 B5), Smooth Muscle Actin (SMA) (clone asm1), S-100 (clone polyclonal from cow brain), p63 (clone 7JUL), GCDFP-15 (clone 23A3), Ki-67 (clone Mib-1). For each marker the evaluation of the immunoreaction was performed on representative slides with tumor and adjacent normal breast tissue as an internal control. Evaluation of Her2/neu status was made according to the international scoring system, using an external positive control. Chromogenic in situ hybridization (CISH), with a double probe (ZytoDot 2C SPEC Her2/ CEN T 17 probe kit-ZYTOVISION, Gmbh, Germany) was applied to the same paraffin block used for immunohistochemistry, with positive and negative controls provided by the manufacturer.

Results

Quadrantectomy measured 60 × 30 × 15 mm. On cut sections it was identified a non-palpable area of 36 mm in maximum diameter, predominantly adipose, with randomly scattered foci of white tissue, ranging from <1 mm to 5 mm in diameter. Both groups of microcalcifications were indistinct on gross examination. This pathologic area was completely submitted for histology. Microscopically revealed an invasive carcinoma grade 2, Nottingham score 7 with more than 90% of clear cells with PAS+diastase labile cytoplasm, growing in solid nests and sheets supported in a delicate capillary framework (Figure 2 ). Cells with mildly eosinophilic cytoplasm and higher nuclear pleomorphism were also seen. High grade intraductal carcinoma was identified showing solid, papillary and comedo patterns with microcalcifications. Ductal apocrine metaplasia was also found. Lymphatic invasion was noticed. Surgical margins were positive for carcinoma. One out of three sentinel lymph nodes showed micrometastases containing both clear and eosinophilic malignant cells (Figure 2). The immunohistochemical profile is shown in Table 1, and confirmed the diagnosis of carcinoma over other primary tumors with clear cells. GCDFP-15 was negative in both clear and eosinophilic cells, in the tumour as well as in metastases. Alcian blue and Mucicarmine stains were both negative. Tumor markers predictive of therapeutic response are shown in Table 2, confirming a luminal immunophenotype.

Figure 2: A) Sheets of clear cells with intense PAS positive cytoplasm (PAS stain 20X). B) Clear cells after diastase digestion. (PAS-Diastase stains 20X). C) Solid nests of tumour cells infiltrating adipose tissue (H&E 10X). D) Microcalcification within a duct with clear cell carcinoma (H&E 20X). E) Micrometastases in sentinel lymph node. Note eosinophilic cytoplasm in this group (H&E 20X). F) Group of metastatic clear cells with strong reactivity for CK8-18 (40X).

Mastectomy specimen showed residual intraductal carcinoma that measured 10 mm in diameter. Twelve axillary lymph nodes were dissected, all without metastases. Chemotherapy with 5’ Fluorouracil, Adriamicine and Cyclophosphamide was started postoperatively. The patient was disease-free 4 years after treatment.
GRCCC is a rare histological subtype of breast carcinoma with an incidence of 1–3% of breast carcinomas. The fact that fewer than a hundred cases were reported since its first description, suggests that this tumour type may be under-diagnosed. Literature is rather confusing when concerned with diagnostic criteria, presence of GRCCC with mucus secretion [10], GRCCC with invasive lobular growth pattern, and GRCCC with focal neuroendocrine [4] or apocrine features [3-5]. Since glycogen can be detected in non-clear cell carcinomas and clear cell change may be caused by substances other than glycogen, diagnosis of GRCCC requires: 1) Malignancy criteria. 2) Clear cell morphology in more than 90% of tumour cells and 3) Demonstration of glycogen in clear cells. The differential diagnosis of GRCCC of the breast includes primary benign tumors (adenomyoepithelioma and clear cell hidradenoma), as well as primary malignant tumors (signet-ring cell carcinoma, histiocytoid lobular carcinoma, lipid-rich carcinoma, secretory carcinoma apocrine carcinoma) and metastatic tumors with clear cell features (clear cell carcinoma of the kidney, adrenal gland, lung, and other organs). Cytchemistry and immnnohistochemistry are useful in differentiating these neoplasms.

In this case, the PAS+ diastase labile intracytoplasmic material is consistent with glycogen. Homogeneous expression of CK 8-18 and negativity for myoepithelial markers exclude a myoepithelial neoplasm. The presence of intraductal carcinoma and the absence of suspicious lesions in other organs favour a primary breast neoplasm. The invasive growth pattern and lymphatic invasion with nodal micrometastases confirmed its malignant nature. The clinical presentation of this invasive tumor as a non-palpable area of parenchymal distortion correlated well with the macroscopic appearance in quadrantectomy. The small, scattered foci of tumor and the absence of significant desmoplasia contributed to the non-palpable quality and difficult gross visualization of its extent.

On histology and electron microscopy GRCCC resembles the breast bud epithelial cells of the 13 week-old human embryo [11], indicating that could be considered as a dedifferentiated carcinoma rather than an otherwise common breast carcinoma with glycogen storage as a metaplastic change. Moreover, the presence of glycogen in other types of breast carcinoma could be an expression of dedifferentiation and tumor heterogeneity. On the other hand, Fisher et al. first described the presence of clear and eosinophilic cells in metastases [3] and suggested that the later have apocrine features. Along the same lines, Hayes et al. [5] hypothesized that GRCCC might be a variant of apocrine carcinoma. The tumor here reported contained such eosinophilic cell population but the apocrine marker GCDFP-15 was not expressed either in clear or in eosinophilic cells, a finding also reported recently [6]. In my opinion, eosinophilic cells are malignant clear cells that could have lost their cytoplasmic glycogen and this morphological change could be related to a genetic and/or metabolic switch in a subset of malignant clear cells, revealing the tumor heterogeneity.

Expression of tumor markers is not uniform on literature review. Hormone receptor status does not appear to be different from ductal NOS carcinomas [1,6,9]. The incidence of Her2/Neu overexpression was quite similar to that found in common breast carcinomas. In this case, immunohistochemical determination of Her2/Neu status scored 2+ and study of amplification by CISH showed polosity of chromosome 17. This finding correlates well with the nondiploid DNA content in GRCCC, described by Toikkanen and Joensuu [7].

Opinions differ among researchers concerning prognosis. On clinical ground, when GRCCC is matched stage by stage with usual breast carcinoma, differences do not appear significant. Most reported cases of GRCCC presented with a breast lump, axillary metastases and run an aggressive clinical course explained, at least in part, by the advanced stage of disease. Despite the practice of screening programs worldwide, very few clinically silent cases were reported, probably because this tumor type is easily missed in biopsy and fine needle aspiration cytology. Early stage disease provides valuable insights into tumor biology. The case presented herein showed that multifocal stromal invasion and lymphatic metastases were early events in tumor development indicating an aggressive biology. Sato et al described recently a case with carcinomatous lymphangiosis and an extremely aggressive behaviour [8]. The good clinical outcome of the patient here reported is related to early detection and treatment. Nevertheless it is worth recognizing that cases presenting with a lump without axillary metastases are also on record. In such cases histological tumor grade is a relevant factor. If a high grade GRCCC has no metastases then at least two biologically distinct high grade subtypes might be postulated, and probably they could be recognized by molecular signatures.

Conclusions

As expected, breast cancer screening programs are providing valuable material to perform insights into early developmental stages of infrequent breast tumors.

Nevertheless large series and molecular investigations are needed to better understand these rare cancer subtypes to provide significant information useful for future personalized therapy.
Consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Competing Interests

The author declares that has no competing interests.

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References