

INTRODUCTION

Breast Cancer (BC) represents less than 1% of all pediatric malignancies and less than 0.1% of all breast tumors [1-4]. It is even more uncommon in young boys [4]. Secretory Breast Cancer (SBC), representing the majority of BC in the pediatric population [1-3,5], was originally termed 'Juvenile BC' [7]. Afterwards, Tavassoli and Norris [8] reported 19 cases with an age between 9 to 69 [9]. Of the approximately 120 cases currently published, almost two-thirds are in adults with a large range of age at diagnosis [1,6]. Consequently, in 1980s, the more descriptive term 'SBC' replaced the previous definition [10,11]. Indeed, this tumor has peculiar features, being morphologically characterized by abundant eosinophilic secretions in intracellular vacuoles and intercellular spaces [2,12-16]. More than 90% of SBC present a specific genetic-molecular marker consisting in a balanced translocation t(12;15) that results in the ETV6-NTRK3 fusion gene [5,17-27]. Its biological consequence is a chimeric tyrosine-kinase which activates a pathway with a consequent transforming activity for fibroblasts and breast ductal epithelium. Remarkably, this translocation has never been detected in other types of BC but is associated with some non-epithelial pediatric mesenchymal cancers [13,19]. SBC has generally been reported to be negative for oestrogen receptor (ER), progesterone receptor (PgR) and HER-2 and to express cytokeratine 5/6, 14 and 17, c-Kit (CD117), epidermal growth factor receptor (EGFR) and vimentin [11,12,14,20]. Nevertheless, SBC and male SBC particularly has an indolent clinical behaviour, being slow-growing with a low malignant potential [6], highlighting the heterogeneity of histological types and prognosis within basal-like carcinomas.

Herein, we report a case of SBC in a 5½ year-old boy who not only had the typical balanced translocation t(12;15) but also had a peculiar gene duplication with the potentiality to be involved in the process of breast carcinogenesis. Furthermore we reviewed the literature to analyze the present state of knowledge on SBC.

CASE REPORT

The parents of a 5½ years-old boy noted a lump in the left retroareolar region. Ultrasonography revealed a 10 mm nodule, with regular margins and poor vascularisation like a benign lesion, without enlarged lymph nodes. The boy was referred to a Pediatric Hospital since his paternal grandfather had been diagnosed with a BC. Clinical examination was negative for nipple discharge or retraction. The family history revealed that the boy's paternal grandfather had been diagnosed with colon cancer at 53 years, melanoma at 65 years and tubular BC at 79 years. The paternal grandfather's brother had colon cancer, the nephew had BC at 40 years. The paternal grandmother's sister had BC at 79 years and his grandmother's brother had colon cancer too [Figure 1]. Given the breast lesion and the family history, a wide excision without a preoperative core-biopsy was performed.

Macroscopically, the specimen was a 2x1x0.6 cm of fatty tissue with a well-circumscribed yellowish-brown mass of 0.8 cm. Microscopically, the sample revealed clear margins and a tumor composed of solid areas and glandular/tubular structures with intraluminal eosinophilic secretory material, surrounded by hyalinised fibrous tissue. The epithelial-tumor cells were bland-looking with round nuclei and eosinophilic vacuolated cytoplasm, positive for periodic acid-Schiff (PAS) and Alcian blue. Atypia was minimal and the mitotic-activity was low. It was positive for cytokeratine 7, protein S-100, epithelial membrane antigen (EMA), E-caderin, vimentin, and EGFR but negative for ER, PgR, GATA-3, cytokeratine 5/6, CD117, calponin, and HER2. The Mib-1 index was between 1- 6% in different areas. [Figure 2-3-4A,B-5A,B] Polymerase Chain Reaction (PCR) detected translocation t(12;15), demonstrating the presence of the ETV6-NTRK3 fusion gene. Based on morphologic, immunohistochemical and molecular features, the boy was diagnosed with SBC and was sent to a specialized Breast Center: the clinical exam was negative (except for the recent surgery) and the blood tests were normal. The ultrasonography of the breast and lymph nodes was negative. Therefore, a sentinel lymph node biopsy (SLNB) without further breast surgery, radiotherapy (RT) or adjuvant systemic therapy was planned. The pre-operative lymphoscintigraphy revealed a regular migration of the Tc99-tracer with the evidence of one sentinel node. The SLNB was performed successfully and the lymph node resulted negative.

After the operation, the sequence analysis of BRCA1-2, integrated with deletion/duplication analysis of BRCA1, was performed. The results were negative in our proband and, surprisingly, in his paternal grandfather. Furthermore, in consideration of the multiple cancers in the relatives of the paternal branch, TP53-gene was analysed in grandfather's DNA without any pathogenic variant. To investigate the presence of microdeletions/microduplications in genes involved in neoplastic pathogenesis, array-CGH-analysis on the boy's DNA was executed (Agilent-180K-Platform) revealing the presence of a 3q28 duplication: arr3q28x3 (GRCh37), confirmed by a second analysis method (Real-time-PCR). The same copy number variant was identified in father (healthy) and paternal grandfather's DNA samples. This locus includes only one gene, FGF12 (OMIM *601513), a member of the fibroblast growth factor (FGF) family with mitogenic and cell survival activities.

At the last follow-up, 48 months after the operation, no local or systemic recurrences have been diagnosed.

REVIEW OF THE LITERATURE AND DISCUSSION

Because of its rarity, the published experience with SBC has largely been limited to case reports and small case series [1,3,6,10,11,18,19,21,28-71]. There are also two papers, based on data from National Data Base: Horowitz et al [9] identified 109 cases using the Survival, Epidemiology and End Result database, 83 of which had complete data and

were included in the analysis; Jacob et al [6] reported the National Cancer Data Base (NCDB) experience with 246 female patients. A noteworthy limitation was their inability to re-review the histological samples to confirm the SBC-diagnosis, the ER and PgR status and the tumor grade. For the purposes of this article, from now on, we will report both data emerging from the various case reports and the two aforementioned retrospective studies, highlighting the sometimes discordant results. For our review, we used <https://www.ncbi.nlm.nih.gov/pubmed>, searching for “secretory AND breast AND cancer”, with an update done on 1st February 2018.

According to literature, SBC affects both children and adults with an age at presentation ranging from 3 to 89 years [1,6,17] with a median age of 25 years[17].

This tumor occurs in both sexes, tending to appear earlier in males. While the prior literature showed a male-to-female ratio of 1:6 [19,21,28,29], the recent NCDB review [6] demonstrated a ratio of 1:31. According to the WHO classification, SBC accounts for 0.2% of male BC [2,3] and so far, to the best of our knowledge, only 32 cases of SBC in male patients have been published in literature [Table 1]. Among these, the age-range goes from 3 to 79 years with a median age of 19 years. Eleven cases are boys less than 14 years-old, 4 are adolescents (age 15-18) and 17 are adults [30].

Given this paucity of data there is no consensus regarding how SBC should be managed and therefore it is imperative to report any new case observed in order to establish the most suitable therapeutic approach. The case reported herein is extremely unusual because concerns a 5 ½ years-old boy and this age of onset is one of the earliest reported: only two other male patients were younger [31,32].

The typical clinical presentation is an asymptomatic, slow-growing, painless, mobile, well-circumscribed, palpable mass occurring anywhere in the breast [1]. Similarly to IDC, the most common location appears to be the upper-outer-quadrant [1,33]. Prepuberal patients and any-age male patients tend to present with a subareolar mass frequently associated with nipple discharge [12].

According to the reported single cases and series, tumour size ranges from 0.5-16 cm but is more often between 1.5-3 cm [6,11,21] and tends to be larger in adults [12]. The 16 cm case concerns a man who had the breast lesion since his childhood but was operated only at the age of 40-years. This corroborates the fact that SBC has a peculiar slow growth and a very indolent behaviour.

Typically, SBC is a unicentric lesion [12,34,72] that sonographically appears as a round/oval, well-circumscribed, hypoechoic/isoechoic, usually homogeneous mass [11,12,29,36,73,74]. At mammography, in contrast to IDC, SBC has variable non specific features ranging from a benign-looking discrete, lobulated, solitary mass with smooth or irregular borders to suspicious asymmetric densities with spiculated margins and rarely microcalcifications [12,71,84]. In children or male patients a retroareolar dense mass is the most common finding [12,35]. As a consequence, SBC can be interpreted as compatible with an area of gynecomastia.

Therefore, to establish the diagnosis of SBC, a surgical biopsy is mandatory.

Macroscopically, SBC often appears as a solitary mass. Margins are usually well delineated but can be infiltrative. [2,12-15]. Morphologic features are those of grade 1-2 tumors [20,75]. Indeed, according to the NCDB review [6] SBC is more likely to be well differentiated (32% vs. 18%) and less likely to be poorly differentiated (11% vs. 36%) compared to IDC. An intraductal component is frequently present and there are three possible histological patterns: tubular, compact and honeycomb (micro cystic) in varying combination [1,10,12,17,37]. Besides typical grown-patterns, SBC could also present with a papillary-predominant architecture [28,62,64] to be carefully differentiated from sclerotic intraductal papilloma, intraductal or invasive papillary carcinoma and mammary analogue secretory carcinoma (MASC). This last one is a new salivary gland tumor entity [76-79] with morphologic (well-circumscribed nodules with secretion, micro cystic, solid and tubular growth patterns), immunohistochemical (positivity for S-100 protein) and genetic characteristics (ETV6-NTRK3 fusion gene) similar to those of SBC. Interestingly a papillary-cystic growth pattern is characteristic in MASC [64,76-79] but a papillary predominant architecture has seldom been reported in SBC [12,28,62,64].

Atypia is minimal/absent and mitotic activity is usually low [1,3]. The two distinctive pathological characteristics are intra/extracellular secretions and granular eosinophilic cytoplasm [3,8,38]. In fact, the intercellular spaces and the cells themselves contain an abundant secretion of PAS and alcian-blue positive material consisting in sulphated mucopolysaccharides and mucin [10,11,13,20,21,29,39]. Nevertheless, many BC demonstrate features of secretion, but this feature alone is not sufficient to diagnose SBC. Immunohistochemically, it frequently shows triple-negative features and basal-like differentiation [14,18]. It is normally ER, PgR and HER-2/neu negative [3,40] with a low proliferation index, ranging from 1% to 34% [12,14,29,37,39]. However some studies have reported tumors weakly ER/PgR positive [11,15,22,28,30,36,40]. The NCDB [6] found a significant percentage of SBC ER (64%) and PgR (44%) positive even if less positive compared to IDC. Concerning this aspect, there are currently conflicting data. In 2002 Li et al [3] reported on 15 cases all of them negative; de Bree et al [16] described a cohort of male patients with SBC where about 50% were hormone-receptor-positive. It is noteworthy that reporting of ER and PgR status in the NCDB only begun in 2004 thus limiting their data to only 137 cases where hormonal status was reviewed. Nevertheless, these last results underline that SBC is not exclusively receptor negative, as previously thought. In contrast to BC of non special type (NST), the predictive and prognostic significance of a positive hormone receptor status in SBC remains unclear [30,39]. Also the case reported herein was ER and PgR negative with a MIB-1 index of 1-6%.

The correlation between known risk factors for BC and SBC is not yet fully understood. Although the characteristic hypersecretion may suggest a hormonal pathogenesis, no evidence of hormonal abnormalities has been documented [12]. Furthermore, since the presence of hormone-receptors is extremely variable, the pathogenesis of SBC is probably not linked to sex hormones [80]. Coexistence of juvenile papillomatosis or gynecomastia have been described; however no precancerous lesions or conditions have been proven to be associated with SBC [12].

In 2002, Tognon et al [22] discovered that SBC is characterized by a specific genetic and molecular marker, consisting in a balanced translocation t(12;15) resulting in the expression of a chimeric tyrosine-kinase encoded by the ETV6-NTRK3 fusion gene [30]. The ETV6 gene is located on chromosome 12 and encodes an E26-transformation-specific transcription factor resulting in the ETV-protein which is expressed in healthy breast epithelial cells. The NTRK3 gene is located on chromosome 15 and encodes a membrane-receptor-tyrosine-kinase. The biological consequence of this translocation is the fusion of the dimerization domain of the transcriptional regulator ETV6 with the neurotrophic membrane receptor tyrosine kinase 3 (NTRK3). The resulting chimeric tyrosine-kinase activates the Ras-Mek1 and PI3K-Akt pathways which are important for breast cell proliferation and survival having a potent transforming activity for fibroblasts and breast ductal epithelium. The chimeric fusion protein may also contribute to the expression of mammary growth factor [80]. The ETV6-NTRK3 fusion gene has been confirmed in up to 92% of SBC [5,17,18,20,21,23-27,30] and has not been detected in any other types of BC [21,32,35]. Interestingly, this translocation is also associated with other non-epithelial pediatric mesenchymal cancers including congenital fibrosarcoma, mesoblastic nephroma and acute myeloid leukaemia [1] and with secretory carcinoma of the skin [24,64,76-79].

The finding of a fusion transcript in SBC and the demonstration that ETV6-NTRK3 could transform murine-mammary-epithelial-cell lines has challenged widely accepted beliefs on breast carcinogenesis and might be the target of future treatment for this unique type of BC [5,30,80].

The case reported herein was positive for translocation t(12;15). Furthermore, to investigate the presence of microdeletions/microduplications in genes involved in neoplastic pathogenesis, array-CGH analysis on the DNA of the boy revealed the presence of a 3q28 duplication confirmed by a second analysis method. The same copy number variant was identified in father's (healthy) and grandfather's DNA samples. This locus includes only one gene, FGF12, a member of the FGF family with mitogenic and cell survival activities. This family of growth factors is involved in various biological processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion. At the time of the evaluation, this gene was not related to any human disease. Recently FGF12-gene was linked to EIEE47 (Epileptic Encephalopathy, Early Infantile, 47) (OMIM #617166) [81] and potentially related to Idiopathic Ventricular Tachycardia [82]. Overall, the evidences based on current literature do not provide evidence that the identified duplication could have played a role in the pathogenesis of the child's disease; moreover, the genomic imbalance is present also in the healthy father. On the other hand it is not possible to exclude a reduced penetrance or a variable expressivity of the copy number variation. In addition, we should consider that FGF12 has a broad mitogen and cell-survival activity, thus it could be a potential cancer-predisposing gene, along with unidentified modifier genes or environmental factors. Further investigations are mandatory.

SBC and particularly male SBC has an indolent clinical behaviour, being slow-growing and low aggressive. Li et al reported an exceptional case of a 41 years-old man with a breast mass present from his childhood, finally resulted a SBC [30]. Axillary lymph node involvement is uncommon, especially in tumors less than 2 cm [12,28,33,34,83]. Studies have shown that lymph-node metastasis are particularly rare in children and adolescents [46,47,84]. Even in this case the NCDB review [6] shows a different data, stating that there is no difference in node positivity between SBC and IDC (32% versus 34,2% respectively). In our patient the clinical presentation was paradigmatic, with a 1 cm retroareolar nodule associated to transient nipple-discharge and no lymph node involvement.

Practically no distant metastases are observed [10,21,39]: to date, only 6 cases of distant metastases have been reported in association with large tumour size and multiple lymph node involvement [11]. SBC has late local recurrences and prolonged survival even when lymph node involvement is present [3,9,3083].

Death from this type of carcinoma is rare [11,41]: five and ten-year disease-free survival (DFS) is 94% and 91% respectively [9,20]. Therefore SBC has an exceptionally good prognosis [6,8,10,13,20,28,39]. It seems to be less aggressive in children than in adults and, according with de Bree et al [16], it tends to be more aggressive in men. A good prognosis is associated with age under 20 years, tumor size of less than 2 cm, circumscribed margins and absence of peripheral stromal invasion [8,37]. Indeed rarely there may be foci of invasion in the surrounding tissue and associated ductal carcinoma in situ (DCIS), which can be responsible for local recurrence after incomplete excision [11,37,39,40]. A family history could mean a poorer prognosis [8].

Laè et al [5] have demonstrated that SBC belongs to the phenotypic spectrum of basal-like BC expressing basal-cell markers like cytokeratine 5/6, 14 and 17, CD117, EGFR, vimentin, S-100 protein and alpha-lactalbumin [12,75]. The tumor cells are also positive for e-Caderin, as expected in any ductal BC [12]. No reactivity is observed for gross cystic disease fluid protein (GCDFP-15 or BRST-2) or monoclonal carcinoembryonic antigen [12]. It is noteworthy that the case reported herein is not a 'pure' basal-like tumor. Indeed, tumor cells were positive for S-100 protein and negative for cytokeratine 5-6 which are both basal-cell markers. Furthermore they were positive for cytokeratine 7, which in contrast, is a luminal-cell marker.

All basal-like tumors were considered to have a poor prognosis but we currently know that they comprehend various histological types some of which (the adenoid cystic or apocrine carcinoma) with better prognosis. In this context, SBC

is the perfect example of a triple negative cancer that, differently from ductal cancers, has a low proliferation index and low-grade features. Therefore, SBC with its excellent prognosis and long-term outcome, in contrast to the majority of triple negative or basal BC, highlights the heterogeneity of basal tumours and the need for comprehensive histopathologic evaluation [5,14,20].

Therapy is controversial [41-43] and given the extremely good prognosis associated with SBC, several authors suggest less aggressive treatments compared to those needed for IDC [39]. However, because SBC is so rare, there are no consensus guidelines [6,10,34,36,40].

Based on expert opinion and published case series, the management of SBC is considered to be first and foremost surgical [10,34,39] without consensus regarding the optimal extent of surgery. Some authors suggest mastectomy as the most appropriate treatment in children [28] considering that local recurrence is believed to be more likely the result of incomplete excision rather than of inherent aggressiveness [37,39,44]. On the other hand, most of the authors suggest breast preservation in prepuberal children [28] in order to ensure proper breast development and to avoid relevant body image impairment. Therefore, whenever possible, children should be treated initially by wide local excision with clear margin and sentinel lymph node biopsy. Breast tissue peripheral to the tumor should be sampled and examined for intraductal foci, since these can indicate an increased risk of local recurrence [37]. Mastectomy may be necessary in case of large tumors or to control local recurrence [37]. Given that nodal metastases in children tend to be small and undetectable at clinical examination [37], sentinel node biopsy is in any case the standard.

Nowadays there is insufficient evidence to recommend post-operative radio-therapy and/or chemotherapy [21,39]. Radiotherapy is used in adults after quadrantectomy but is not advisable for children [18]. This option can be discussed with a careful assessment of the risk/benefits ratio in view of the impairment of costal growth that can follow radiation therapy [10] and of the possible late side-effects such as lung fibrosis or asymmetry of the chest [2,45].

There are no convincing data on hormonal manipulation and no recommendations are available for children. Based on the published case series that documented a high percentage of hormone receptor negative SBC, a hormonal treatment should be avoided [10]. Nevertheless, in the NCDB review [6] SBC was found to be frequently hormone receptor positive and the use of hormonal therapy resulted similar for SBC and IDC patients.

Because of the indolent nature, the good prognosis and the low metastatic potential, even in patients with axillary node metastases there are still no reliable data to recommend adjuvant chemotherapy [42]. Furthermore, SBC appears to be nonresponsive to various chemotherapy protocols [37]. There are several reported cases of patients with SBC with distant metastases treated with either single agent or combination chemotherapy without success. Among the drugs reported there are 5-FU, adriamycin, epirubicin, cyclophosphamide, carboplatin and even newer agents such as docetaxel [21]. All the patients treated with chemotherapy had disease progression while on treatment, highlighting clearly that this neoplasm is not chemo-sensitive. [21]. These observations are in contrast with reports on the high chemosensitivity to common agents typical of congenital fibrosarcomas and mesoblastic nephromas, two other neoplasms associated with the ETV6-NTRK3 translocation [21]. This suggests that SBC, due perhaps to its slow growing behaviour, progressively acquires additional genetic alterations than ultimately confer chemoresistance [21]. In the case reported the patient did not receive any adjuvant treatment and at the last follow-up, 48 months after the operation, he is disease free.

CONCLUSION

SBC is a low-grade triple-negative (ER-/PgR-/HER2-) carcinoma that expresses basal-cell markers. It is extremely rare, especially in male patients. Nevertheless, despite the low frequency, it elicits a lot of interest both for the peculiar morphology, the characteristic genetic and molecular expression and the excellent prognosis, differently to other basal-like tumors. It can pose a diagnostic and therapeutic challenge because of the paucity of data published in literature and at present there are no consensus guidelines regarding the optimal strategy. A multidisciplinary approach and a patient-tailored treatment is the most suitable option in this rare cancer type.

DISCLOSURE

No conflict of interest are declared.