Immunohistochemical Expression in Male Breast Cancer: Two Case Reports

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Male breast cancer is rare, and the incidence is less than 1% of all breast malignancies in both men and women. It is possible that, because male patients are unaware of male breast cancer, there is a delay of diagnosis and, consequently, more advanced stages are commonly encountered in these patients. Some studies have engaged in molecular studies of male breast cancers because of the possibly different characteristics, prognosis, and treatment between male and female malignancies. However, a dearth of studies still exists, most likely because of the rarity of the disease and lack of a large patient base for study. Among the molecular markers of breast cancer, p53, Ki-67, HER-2/neu, and Bcl-2 are the most frequently studied. Here we present two rare cases and a review of the literature concerning the relationship between immunohistochemical markers and their impact in order to provide surgeons with more information about the disease and further techniques for treatment of these patients.

Key Words: male breast cancer, p53, Ki-67, HER-2/neu, Bcl-2
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Male breast malignancy is extremely rare, and the incidence is approximately 1% of all breast cancers [1]. Because of the rarity of the disease, contemporary natural history, diagnostic approach, treatment regimen, and prognosis about male breast cancer are not fully understood compared with its female counterpart [2]. Consequently, the lack of awareness among both surgeons and patients about male breast cancer often leads to delayed diagnosis [1]. Despite a histology that is similar to the female counterpart, male infiltrating ductal carcinomas seem to have different immunohistochemical characteristics [3]. Because early diagnosis can achieve better prognosis, early awareness of the disease is important. In this study, all male breast cancer patients treated surgically from 2000 to December 2005 were retrospectively reviewed from the archives of the Department of Pathology, Kaohsiung Municipal Hsiao-Kang Hospital. Only one male primary breast cancer (Case 1) was found. The incidence was approximately 0.7% of all breast cancers. Three male breast cancer cases were retrieved from the archives of the Department of Pathology, Kaohsiung Medical University Chung-Ho Memorial Hospital between 1988 and January 2005. Two patients were lost to follow-up. Only one clinical file was available (Case 2). The incidence was also 0.7%, similar to that of Kaohsiung Municipal Hsiao-Kang Hospital. Here we present two rare cases from the Kaohsiung Municipal Hsiao-Kang Hospital and Kaohsiung Medical University Chung-Ho Memorial Hospital (the Table compares various characteristics of these two patients). We hope that better understanding of this malignancy will be achieved after more reports are published.

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Table. Comparison between two male patients with breast cancer

Parameter	Patient 1	Patient 2
Age of diagnosis	70 years old	76 years old
Site	Left	Right
Tumor size	1.1 cm (greatest diameter)	2 cm (greatest diameter)
Histologic grade	II	II
Estrogen receptor	+ (75%)	+ (20%)
Progesterone receptor	+ (80%)	+ (100%)
HER-2/neu	–	–
Ki-67	–	–
p53	–	+ (60%)
E-cadherin	+ (75%)	+ (80%)
Bcl-2	+ (90%)	+ (70%)

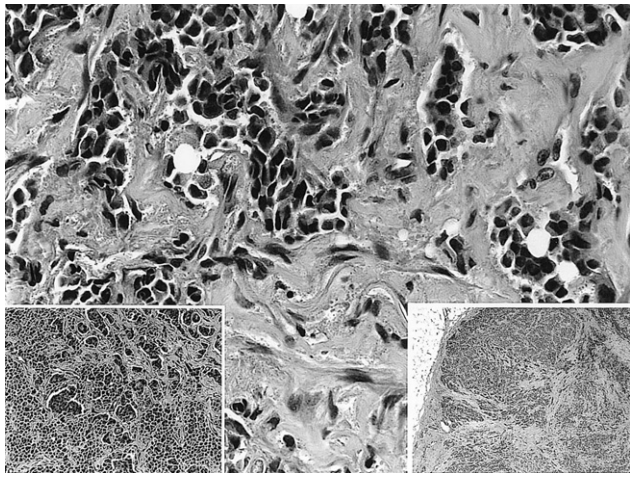

Figure 1. *Microscopic appearance of tumor tissue (Case 1). Cords of hyperchromatic cancer cells with occasionally focal luminal formation (H&E, original magnification ×400). Left lower smaller insert: infiltrating ductal carcinoma of no special type with cords and sheets of carcinoma cells (H&E, original magnification × 200). Right lower smaller insert: sheets of closely packed carcinoma cells invade adjacent adipose tissue (H&E, original magnification × 40).*

Case Presentations

Case 1

A 70-year-old male patient presented with a history of painless enlargement of a mass in the left breast near the nipple. His family history was unremarkable. He had regular follow-up for control of hypertension. He drank alcohol occasionally and was a nonsmoker. The remaining past medical history was unremarkable. Physical examination showed a hard, firm, fixed, and palpable lump without nipple inversion and discharge in his left breast. The size was 1.2 cm in its greatest diameter. No axillary or supraclavicular lymphadenopathy was found. An ultrasonogram of the left breast demonstrated a 1.2-cm nodule in the inner region near the nipple with indeterminate sonographic diagnosis. The shape was irregular, and the margin was jagged. No retrotumoral acoustic effect was noted. The nodule revealed hypoechoic layering and heterogeneity. The specimen submitted for frozen section consisted of one tissue fragment measuring 2 × 2.2 × 2 cm. On serial section, a grayish white and ill-circumscribed tumor measuring 1.1 × 0.9 × 1.2 cm was revealed. The intraoperative diagnosis was malignancy. The patient underwent modified radical mastectomy and axillary lymph node dissections. Specimen samples later sent to pathology consisted of the breast and axillary content. An elliptical piece of skin that was included measured 7.9 × 1.4 cm. A 1.2-cm surgical section line was found from the margin of the areola. The nipple and areola were unremarkable. There was no evidence of edema or inflammation in the skin. Microscopic examination showed grade II infiltrating ductal carcinoma with a minor component of carcinoma *in situ*. All of the margins were free of disease (Figure 1). A metastatic lesion was noted in 1 of 11 lymph nodes that were dissected, measuring 1.9 cm in its greatest diameter. The lesion was estrogen-receptor (75%) and progesterone-receptor (80%) positive. Immunohistochemical tests for HER-2/neu, p53, and Ki-67 were all negative. The tumor revealed a partial positive result for E-cadherin (~75%) and Bcl-2 (90%) (Figure 2). Partial loss of expression was also found in the staining of cytokeratin 7. The patient had chemotherapy and returned regularly to the clinic for follow-up. He was still disease-free nearly 4 months after surgery.

Case 2

A 76-year-old male presented with a 2-year history of painless enlargement of a lump in the right nipple and areola. His family history was unremarkable. He had regular follow-up for control of hypertension and diabetes mellitus. He had had one episode of cerebrovascular accident 7 years earlier, but without any complications. The remaining medical history

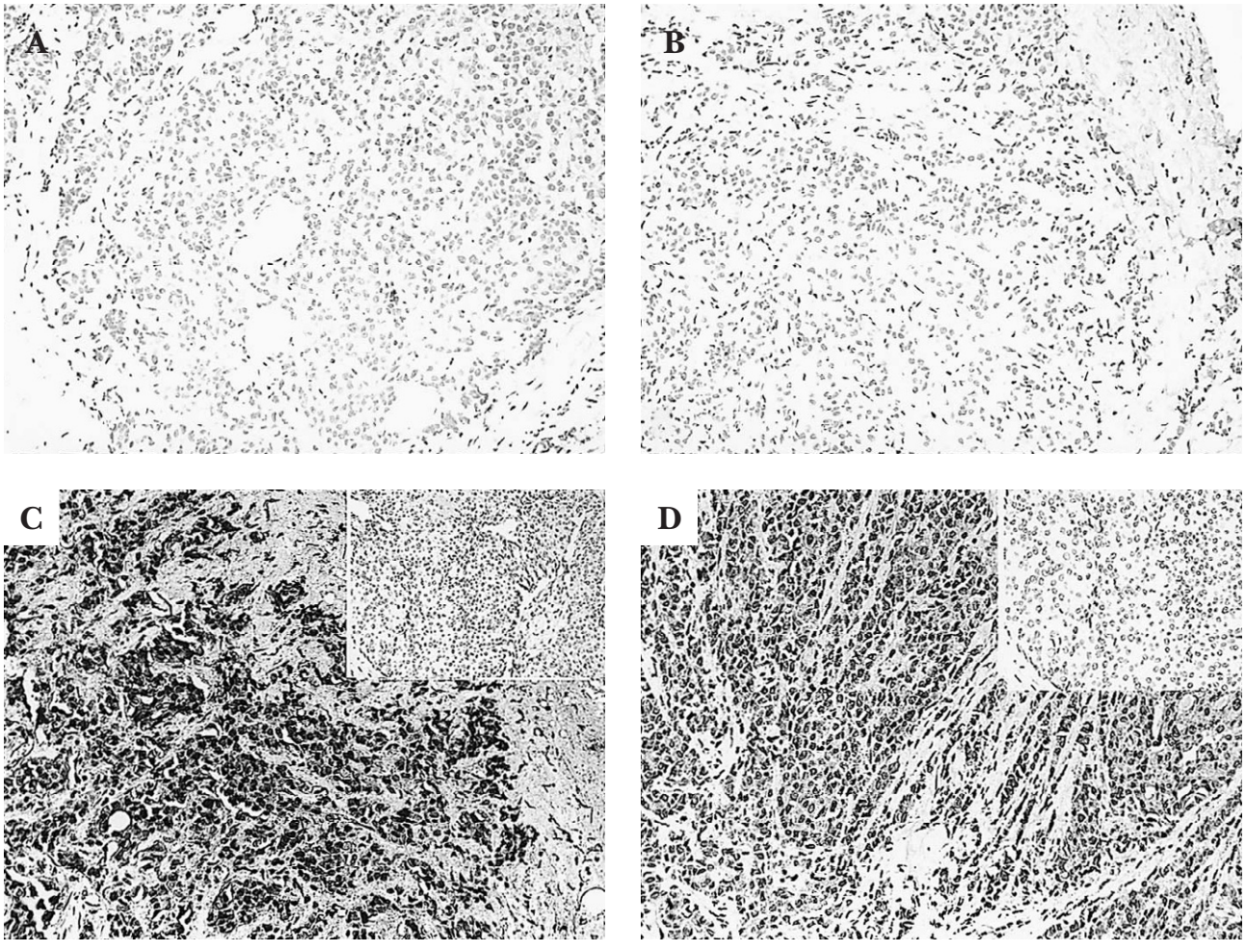

Figure 2. *Immunohistochemical features of tumor tissue (Case 1). (A) Totally negative Ki-67; (B) negative p53 (C); positive E-cadherin with partial loss of expression in the smaller insert. (D) Positive Bcl-2 with partial loss of expression in the smaller insert (all original magnification × 200).*

was unremarkable. Physical examination showed a hard, firm, fixed, and palpable lump without nipple inversion and discharge in his right breast. The size was approximately 2 cm in its greatest diameter. No axillary or supraclavicular lymphadenopathy was found. The ultrasonogram of the right breast demonstrated a 2-cm nodule in the nipple and areola. The impression was malignancy. The shape was irregular and the margin was jagged. No retrotumoral acoustic effect was noted. The nodule revealed a hypoechoic region. The specimen submitted for frozen section consisted of one tissue fragment measuring 2.1 × 1.4 × 0.2 cm. On serial section, there was a grayish white and ill-circumscribed tumor measuring 2.1 cm in its greatest diameter. The intraoperative diagnosis was malignancy. The patient underwent modified radical mastectomy and axillary lymph node dissections. Specimen samples later sent to pathology consisted of the breast and axillary content. An elliptical piece of skin that was included measured 16.3 × 4.5 cm. A 3.5-cm surgical section line was found from the margin of the areola. A residual tumor measured 3.5 × 2.7 × 3.5 cm in size. The nipple and areola were unremarkable. There was no evidence of edema or inflammation in the skin. Microscopic examination showed grade II infiltrating ductal carcinoma with direct invasion in overlying skin, areola, and nipple. All of the margins were free of the disease (Figure 3). A metastatic lesion was noted in one of the two sentinel lymph nodes dissected out, measuring 0.5 cm in its greatest diameter. The lesion was positive for estrogen receptor (20%) and progesterone receptor (100%). Immunohistochemical testing was negative for HER-2/neu and Ki-67. The tumor tested partially positive for E-cadherin (~80%), p53 (60%), and Bcl-2 (70%) (Figure 4). The patient did not have chemotherapy. He was regularly followed up in our clinic and was disease-free nearly 3 years after surgery.

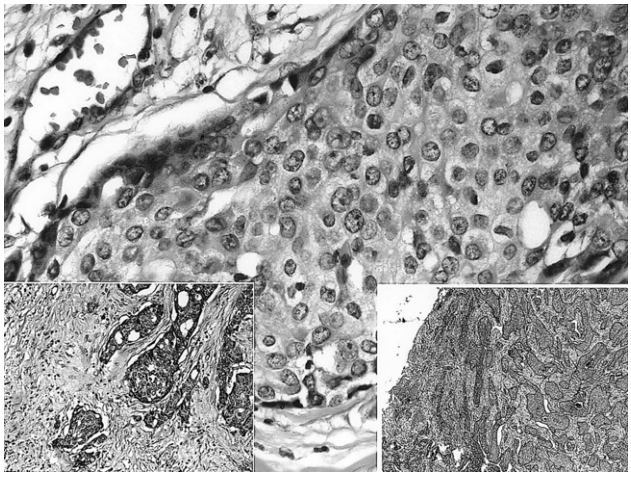

Figure 3. *Microscopic appearance of tumor tissue (Case 2). Organoid hyperchromatic cancer cells with distinct nucleoli (H&E, original magnification × 400). Left lower smaller insert: there is a prominent cribriform pattern (H&E, original magnification × 200). Right lower smaller insert: carcinoma of no special type with organoid arrangement (H&E, original magnification × 40).*

Discussion

Benign or malignant male breast disease is relatively rare, being diagnosed in only 0.5–1 in 100,000 men per year [4]. The etiology and pathogenesis are not fully understood, it accounts for less than 1% of all breast malignancies, and is responsible for only 0.1% of male cancer deaths [4–6].

The most common symptom and sign of male breast cancer is a painless, asymmetric, or centrally located mass with a slight predilection for the left side of the breast [5]. The mass may range from 0.5 to 12.5 cm [5]. The diagnostic features of male breast cancer are similar to its female counterpart, such as bloody discharge in 75% of patients, changes in the areola in as many as 20% of cases, and Paget's disease in 5% of patients [5].

The average age at diagnosis is around 60 years. The disease is rare in young men and occurs in men 10 years older than equivalent female patients [5]. Several

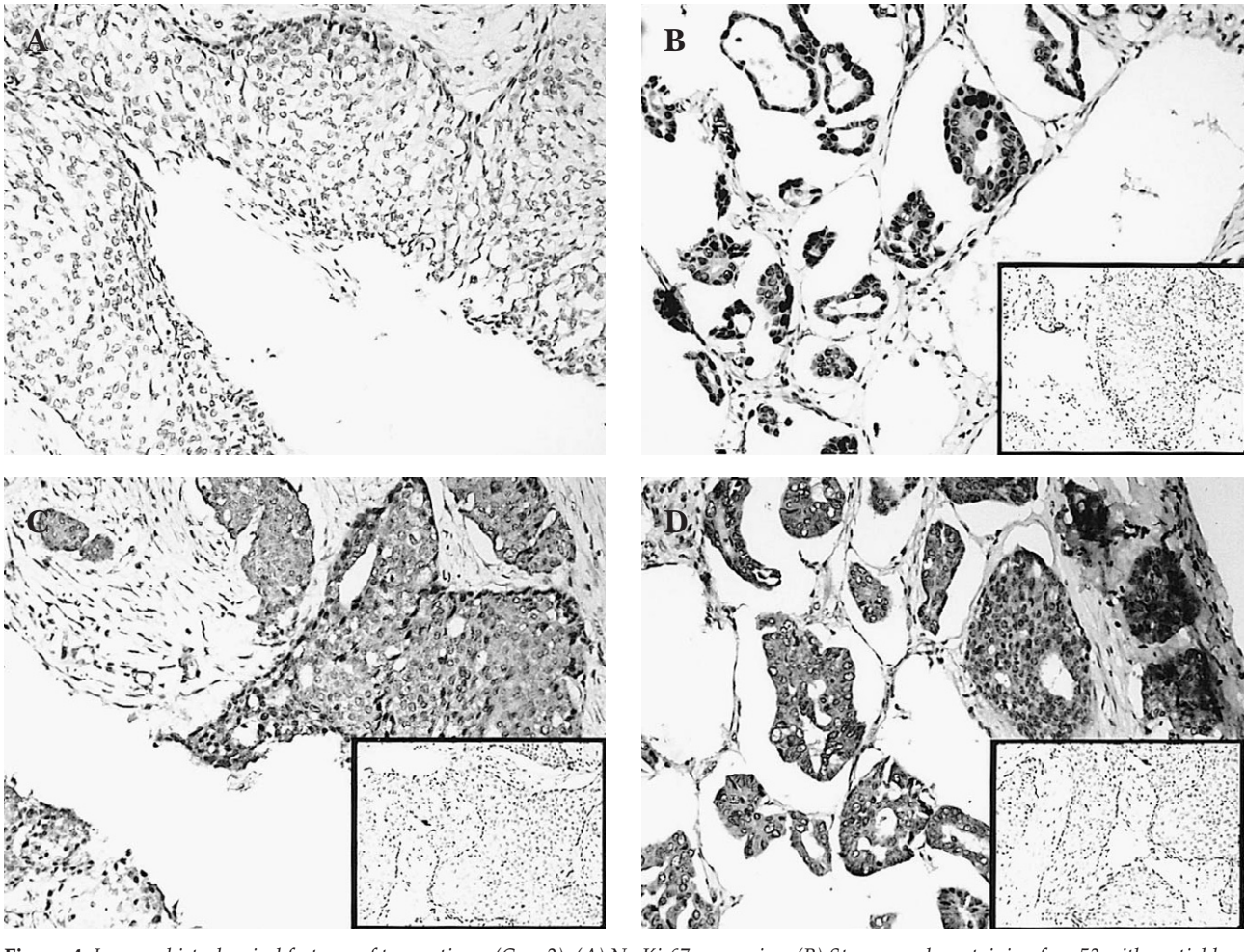

Figure 4. *Immunohistochemical features of tumor tissue (Case 2). (A) No Ki-67 expression. (B) Strong nuclear staining for p53 with partial loss of expression in the smaller insert. (C) Positive E-cadherin with partial loss of expression in the smaller insert. (D) Positive Bcl-2 with partial loss of expression in the smaller insert (all original magnification × 200).*

predisposing factors have been extensively discussed [6]. Among these, family history is thought to be a major risk factor [4]. Predisposing breast cancer genes have been searched for, and *BRCA1* and *BRCA2* are the most notable [4]. *BRCA2* plays an important role in male breast malignancies, whereas the definite role of *BRCA1* in pathogenesis of male breast cancer remains to be elucidated [4,6]. Males with the *BRCA2* mutation are reported to be younger and associated with a poorer prognosis than female patients [7]. Other factors have been proposed, including excess exogenous estrogen, androgen deficiency, imbalanced estrogen–testosterone, hormonal changes due to hypogonadism, mumps orchitis, undescended testes, testicular injury, hepatic disease, Klinefelter's syndrome, obesity, benign breast disease, gynecomastia, a history of breast malignancies, and environmental factors, such as radiation or certain occupations [5,6]. Despite the extensiveness of this investigated list, discordance still exists; for example, it is unclear whether gynecomastia is truly a risk factor [7], and although alcohol use, liver disease, electromagnetic field radiation, and diet have been proposed as possible etiologies, findings are inconsistent across studies [5–7]. In our cases, no obvious risk factor was found.

Infiltrating ductal carcinoma composes about 70% of male breast cancers, the majority of which are of high histologic grade [5]. Lobular carcinoma was once thought to be nonexistent because male breasts lack lobules and acini, but a few case reports have been found [5]. Different characteristics, prognosis, and sensitivity to hormonal treatment seem to exist between male and female breast cancers [3].

Higher percentages of estrogen and progesterone receptor reactivity are associated with male breast cancers and may be a result of low estrogen levels [3,5,7]. The higher percentage of reactivity probably explains the good hormonal response in men [5]. HER-2/neu proto-oncogene overexpression is seen in 20–30% of female breast cancers, whereas, in contrast, only 5% positivity is seen in male breast malignancies [7,8]. The HER-2/neu is a transmembrane receptor protein with tyrosine kinase activity and is associated with poor outcome in female breast cancers [8]. HER-2/neu overexpression seems not to correlate with pathologic grade, tumor state, hormonal status, and lymph node status in male breast malignancies [9]. HER-2/neu gene amplification does not correlate completely with overexpression [8]. Despite the lack of standardized immunostaining protocol and standardized interpretive positive criteria, immunohistochemistry remains the most commonly used method to assess the HER-2/neu status [3, 8]. Our cases showed positive estrogen and progesterone receptors and negative HER-2/neu. The role of HER-2/neu in male breast cancers has still to be elucidated, but probably does not play an important role in predicting clinical behavior [5,8,9].

First discovered in 1979, p53 was thought to be the first tumor suppressor gene that can repress abnormal cell activity [10]. Its overexpression is found in a majority of tumors but occurs at a significantly lower frequency in female breast cancers [3,5,10]. Some studies have found that p53 was low in male carcinomas and high in female malignancies [3]. Others have found a similar incidence in both sexes [11]. In female breast cancers, p53 has been proved to be significant in prognosis and is associated with recurrence, more aggressive disease, and a poorer survival rate [5,10,11]. Other studies, however, have found no such correlation [11]. The proliferation marker, Ki-67, is found in 38% of male patients. Decreased survival was significantly associated with positive results for Ki-67 [11]. Case 1 had neither p53 nor Ki-67 activity. Case 2 had 60% of p53 expression with 0% of Ki-67.

Male breast cancers also differ from female malignancies in their high expression of Bcl-2 in 94% of cases [3,11]. Notably, the Bcl-2 family genes are associated with apoptosis, as markers of cells resistant to apoptosis [11,12]. Bcl-2 proteins are involved in the mitochondrial apoptosis pathway, and their overexpression is observed in many tumors, including solid tumors as well as hematologic malignancies [13]. Some investigators have found inverse immunostaining between p53 and Bcl-2 in both male and female malignancies [3]. Bcl-2 proteins inhibit apoptosis, and, consequently, we would not expect an inverse relationship between these two [3,12]. No explanation was found for this intriguing phenomenon—either in male breast cancers or in female counterparts [3]. The role of Bcl-2 proteins in cancer pathogenesis is confusing. Some studies have suggested that their expression might be a predictor of response to endocrine therapy and favorable prognosis [12], whereas others have proposed that their overexpression might confer resistance to chemotherapy [13]. In our patients, Bcl-2 was positive in 90% (Case 1) and 70% (Case 2) of tumor cells. In our literature search, we failed to find studies concerned with the meaning of heterogeneous loss of Bcl-2 expression.

E-cadherin is a transmembrane glycoprotein and a calcium-dependent cell–cell adhesion protein [14,15]. It is expressed on the cytoplasmic membrane and plays a role in epithelial tumorigenesis [16]. The gene is located on

chromosome 16q22.1 [15]. Because of its potential role as an invasion/tumor suppressor and unproved role in predisposition to breast cancer, it has been widely studied [15,16]. Normal mammary ductal epithelial cells strongly express E-cadherin [14]. Some studies have proposed that only lobular tumors showed E-cadherin mutation [17]. E-cadherin is frequently found in infiltrating ductal carcinomas and is responsible for complete loss of immunoreactivity in invasive lobular carcinomas [14,15]. Its expression is also a good diagnostic aid in differential diagnosis between invasive ductal and lobular carcinomas [15]. Heterogeneous loss of expression is seldom described in the literature and probably correlates with loss of differentiation, acquisition of invasiveness, increased tumor grade, metastatic behavior, and poor prognoses [15]. The association between E-cadherin, estrogen and progesterone receptors, and HER-2/neu is controversial, as is its role in breast cancers [14–16]. Probably because of the rarity of male breast cancers and lack of large numbers of patients, studies of these potential markers are difficult. In our cases, we also observed 25% (Case 1) and 20% (Case 2) loss of E-cadherin expression.

Traditionally, mammography has an important role in modality of investigation. High-frequency linear transducers also play an increasingly important role in the field of biopsy and imaging [18]. The ultrasonographic features of male breast carcinomas are the same as those seen in females, having hypoechoic and irregular margins [18]. Our patients showed imaging features similar to those in the literature [18].

Although some studies are involved in the molecular aspects of male breast cancers, because of the possibly different characteristics, prognosis, and treatment between male and female malignancies, discrepancies still exist [3,14–16,19]. The most important prognostic factors are axillary nodal status, tumor grade, tumor size, and lymphatic or vascular involvement [20]. When nodal status and tumor stage are compared, the outcome seems equal for both male and female cases [21]. The standard treatment for male breast cancer is similar to female malignancies, including modified radical mastectomy combined with sentinel node biopsy and axillary nodes dissection [22,23]. Adjuvant therapy includes hormonal therapy (tamoxifen), radiotherapy, chemotherapy, and orchiectomy [22–24]. Possibly as a result of the rare occurrence and unawareness of male breast cancer, delayed diagnosis and advanced stages of malignancies are frequently encountered [1,25]. Male breast cancer must be considered when a male patient presents at the clinic with a lump in his breast [25].

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男性乳癌的免疫化學表現：病例報告和文獻回顧

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男性乳癌很少見，佔男性和女性乳癌總數不到一個百分比。或許忽略到這項疾病，男性乳癌常有延遲診斷和比女性較高的腫瘤分期，可能男性和女性乳癌在特質、預後和治療方面有所不同，所以一些人投入研究男性乳癌的領域，但是研究的成果差異卻存在，這些研究的標記中，**p53**、**Ki-67**、**HER-2/neu**、和 **Bcl-2** 是最常被研究的，研究差異性的原因可能出在男性乳癌案例較少和缺乏大規模的研究。在此我們提出二個罕見的病例，並且回顧一下文獻，探討免疫化學標記和它們的影響，讓外科醫師多了解一下這個疾病和治療的方法。

關鍵詞：男性乳癌，p53，Ki-67，HER-2/neu，Bcl-2

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