

Ovarian metastasis from breast cancer in three Chinese females

Three case reports

Jiang Wang, MM^{a,b}, Wei Tian, MD^a, Yunxiang Zhou, MM^a, Xiaowei Zhang, BS^b, Yongchuan Deng, PhD^{a,*}

Abstract

Rationale: The biological behavior and clinical features of ovarian metastasis from breast cancer remain unclear; diagnosis and treatment of this condition are challenging.

Patient concerns: We reported three cases of ovarian metastasis from breast cancer in Chinese women aged 44, 46, and 30 years. The prognosis was different in all three patients; however, no symptoms of ovarian metastasis were observed.

Diagnosis: All three premenopausal patients were diagnosed with hormone receptor-positive breast cancer and two of them had axillary lymph node metastasis. All three women had multiple extra-ovarian metastases when ovarian metastasis was detected.

Interventions: All patients received systemic antitumor therapy and underwent bilateral ovariectomy.

Outcomes: Patient 1 had stable bone metastasis; patient 2 had stable lung metastasis and died of heart disease, and patient 3 had multiple brain metastases, which suggested poor outcomes.

Lessons: It is important to screen for ovarian metastasis from breast cancer when evaluating suspicious ovarian masses detected via transvaginal ultrasound in patients with a breast cancer history. Therefore, we recommend simple laparoscopic bilateral oophorectomy not only for pathological diagnosis but also for metastatic tumor removal and therapeutic castration. In such cases, systemic therapy is essential because ovarian metastasis is often a component of systemic metastatic disease.

Abbreviations: CA = cancer antigen, CT = computed tomography, ER = estrogen receptor, GATA3 = GATA-binding protein 3, GCDFP15 = gross cystic disease fluid protein 15, HER2 = human epidermal growth factor receptor 2, IDC = invasive ductal carcinoma, MRI = magnetic resonance imaging, OFS = ovarian function suppression, POC = primary ovarian cancer, PR = progesterone receptor.

Keywords: bilateral oophorectomy, breast cancer, hormone-receptor, ovarian metastases, systemic therapy

1. Introduction

Metastatic breast cancer to the ovaries is rare. A previous study has reported that 75 of 10,955 new cases of ovarian cancer were

Editor: N/A.

JW and WT contributed equally to this work.

This study was approved by the Ethics Committee of Dongyang People's Hospital and the second affiliated hospital of Zhejiang university. Informed written consent was obtained from the patients for publication of this case report and accompanying images.

Wei Tian received a grant from the National Natural Science Foundation of China (grant no. 81502598).

The authors have no conflicts of interest to disclose.

^a Department of Surgical Oncology, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, ^b Department of Surgical Oncology, Dongyang People's Hospital, Dongyang, Zhejiang, People's Republic of China.

* Correspondence: Yongchuan Deng, Department of Surgical Oncology, The Second Affiliated Hospital, School of Medicine, Zhejiang University, 88 Jiefang Road, Hangzhou 310009, Zhejiang, People's Republic of China (e-mail: dyc001@zju.edu.cn).

Copyright © 2019 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Medicine (2019) 98:17(e15395)

Received: 22 November 2018 / Received in final form: 26 March 2019 /

Accepted: 2 April 2019

<http://dx.doi.org/10.1097/MD.00000000000015395>

diagnosed as metastatic breast cancer, accounting for 0.68% of ovarian tumors neoplasms.^[1] It is often diagnosed accidentally during ovarian surgery, autopsy, and castration treatment,^[1–9] implying that the number of ovarian metastasis cases is often underestimated. There is no consensus on the prognosis, monitoring, and treatment of ovarian metastasis of breast cancer. We investigated the clinical characteristics of ovarian metastases of breast cancer with the aim of exploring optimal diagnosis and treatment strategies. Here, we present cases of ovarian metastasis from breast cancer in three Chinese women.

2. Case 1

A 44-year-old woman presented to our department with a complaint of a lump in the left breast for 1 month in April 2007. Physical examination detected an irregularly shaped hard mass measuring approximately 4 cm. Axillary examination found nothing positive. Sonography detected a 37 × 15 mm amorphous and inhomogeneous hypoechoic mass in the left breast with dendritic blood flow signals. Mammogram indicated an irregular mass with a diameter of 2.7 cm in the left breast. An ultrasound-guided core needle biopsy was performed, and pathological diagnosis of the biopsy sample revealed an invasive ductal carcinoma (IDC). Consequently, she underwent modified radical mastectomy. Postoperative pathological diagnosis revealed IDC (approximately 1.5 cm as the largest contiguous dimension) with in situ components, 4 in totally 21 dissected lymph nodes with metastasis. Immunohistochemistry test indicated estrogen receptor (ER)- and progesterone receptor (PR)-positive (+) and human

epidermal growth factor receptor 2 (HER2)/neu-negative (–) breast cancer. Adjuvant chemotherapy with FEC-T (5-fluorouracil, epirubicin, and cyclophosphamide for three cycles, followed by docetaxel for three cycles), followed by adjuvant radiotherapy and endocrinal therapy with tamoxifen, was suggested. However, due to poor patient compliance, chemotherapy was not completed after three cycles of FEC, and neither radiotherapy nor adjuvant endocrinal therapy was administered.

Sixty-five months after surgery, the patient complained of chest tightness. A computed tomography (CT) scan of the chest showed massive pleural effusion on the left chest. Pleural fluid cytology revealed malignant cells characteristic of breast cancer metastasis. Subsequent bone scan indicated multiple bone metastases, which were confirmed by CT scan. Brain magnetic resonance imaging (MRI), abdominal and pelvic CT, and ultrasound examination of lymph nodes yielded no significant findings. Cisplatin pectoral perfusion was performed as local treatment. Gemcitabine combined with paclitaxel was administered for six cycles as systemic treatment, and zoledronic acid was also used. After chemotherapy, all pleural effusion disappeared and bone metastasis was stable as detected by CT scans. No new lesion was found on image examinations. Thereafter, tamoxifen was used as maintenance therapy.

Eight months later (78 months after the initial surgery), vaginal ultrasound revealed solid cystic hypoechoic adnexal masses on the left side with visible blood flow signals, and a small amount of ascites (Fig. 1A); serum cancer antigen (CA) 125 level was in the normal range while serum CA153 level was slightly elevated to 35 U/mL (normal: 0–30) at routine follow-up. Pelvic MRI scans revealed an irregular mixed mass (Fig. 1B) and multiple metastases in the pelvic bones and bilateral upper femur, but no new bone lesions were found compared with the initial bone CT. Other organs and lesions were stable on image examinations. Bilateral adnexectomy was performed. Postoperative pathological diagnosis revealed metastasis in the left ovary that was phenotypically similar to breast cancer, whereas the right ovary and both fallopian tubes showed absence of metastasis (ER+, PR+, HER2–, GATA-binding protein 3-positive [GATA3+], and gross cystic disease fluid protein 15-positive [GCDFP15 Local Stove+] on immunohistochemistry) (Fig. 1C and D). Subsequently, we changed her endocrine therapy to letrozole, an aromatase inhibitor. In October 2018, 55 months since she received letrozole, routine examination revealed no emerging visceral metastasis and bone CT scans have suggested slow progression of bone metastasis.

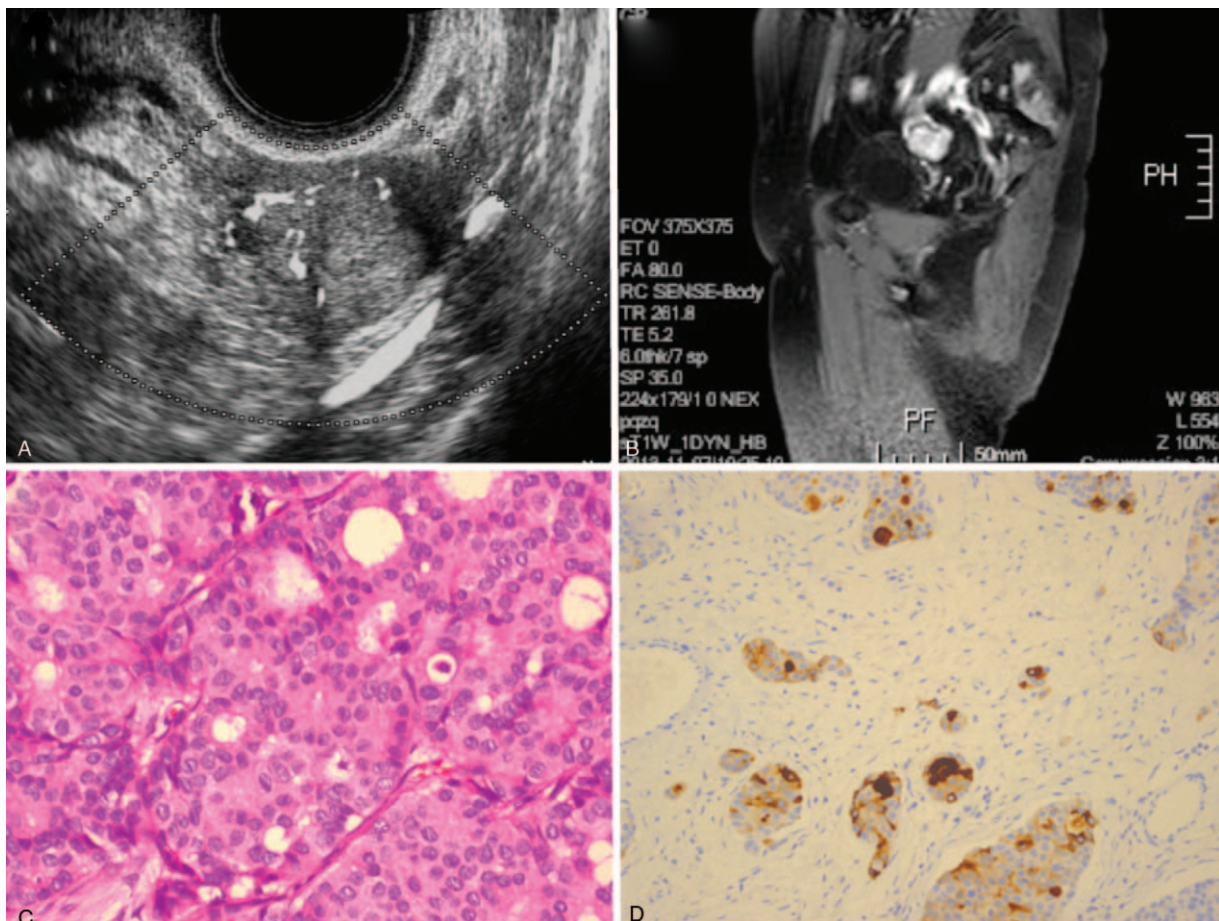


Figure 1. (A) Solid cystic hypoechoic adnexal mass. (B) Sagittal enhanced T1-weighted image showing an irregular lesion of approximately 2.5 × 2.7 cm in the left accessory area. (C) Ovarian biopsy; hematoxylin and eosin stain, 40× magnification. (D) Ovarian biopsy; gross cystic disease fluid protein 15 stain, 20× magnification.

3. Case 2

A 46-year-old woman presented with a complaint of a lump in her right breast for 2 months in October 2010. On examination, a lump approximately 1 cm in diameter was found in the upper inner quadrant of the right breast. Axillary examination found nothing positive. She received three cycles of FEC as neoadjuvant chemotherapy after being diagnosed with IDC by core needle biopsy, and then underwent right breast-conserving surgery plus axillary lymph node dissection. Pathological results identified the tumor as IDC (ER+, PR+, and HER2- on immunohistochemistry) and revealed metastasis in four out of nine lymph nodes. The patient was started on tamoxifen after three cycles of postoperative docetaxel chemotherapy and adjuvant radiotherapy. Sixteen months after the operation, new multiple asymptomatic nodules were detected in her right lung, which were considered as metastatic carcinoma on CT. Brain MRI, abdominal and pelvic CT, bone emission CT, and ultrasound examination of lymph nodes found nothing positive. As there was no visceral crisis, she was treated with ovarian function suppression (OFS, using goserelin) and tamoxifen.

After 2 years of slow disease progression, new lesions emerged in the left lung on CT scan. Since asymptomatic visceral metastasis remained, the treatment regimen was changed to OFS (goserelin) plus aromatase inhibitor. After 22 months of slow progression, without new lesions in the lung or other organs, vaginal ultrasound indicated a solid mass (2.57 × 2.05 cm in size)

in the right attachment area (Fig. 2A). Serum CA125 level was in the normal range. Intervention was recommended, but the patient refused and requested only for observation. The adnexal mass gradually enlarged on sonography; simultaneously, both CA125 and CA153 levels gradually increased in the next 8 months. Thereafter, ultrasound indicated that the tumor size increased to 3.3 × 2.3 cm (Fig. 2B), while associated imaging examinations found no new lesions in the lung and other organs. Bilateral adnexectomy was performed, and the peritoneal flushing fluid showed cancer cells. Postoperative pathology suggested metastatic adenocarcinoma in the right ovary and fallopian tube umbrellas. Immunohistochemistry results were as follows: ER-, PR-, GATA3+, and GCDFP15 Local Stove+. Fulvestrant was suggested as maintenance therapy. However, she died of a heart attack in the same year (April 2017).

4. Case 3

A 30-year-old woman with a child aged <1 year presented with a lump in the left breast that gradually enlarged for 4 months. Pathological results of the biopsy of the lump revealed mucinous carcinoma in June 2014. Preoperative abdominal and pelvic ultrasound, ultrasound of superficial lymph nodes, and chest CT scan found nothing positive. She then underwent breast-conserving surgery with sentinel lymph node biopsy. Histopathology showed a tumor measuring approximately 5 cm in the largest dimension

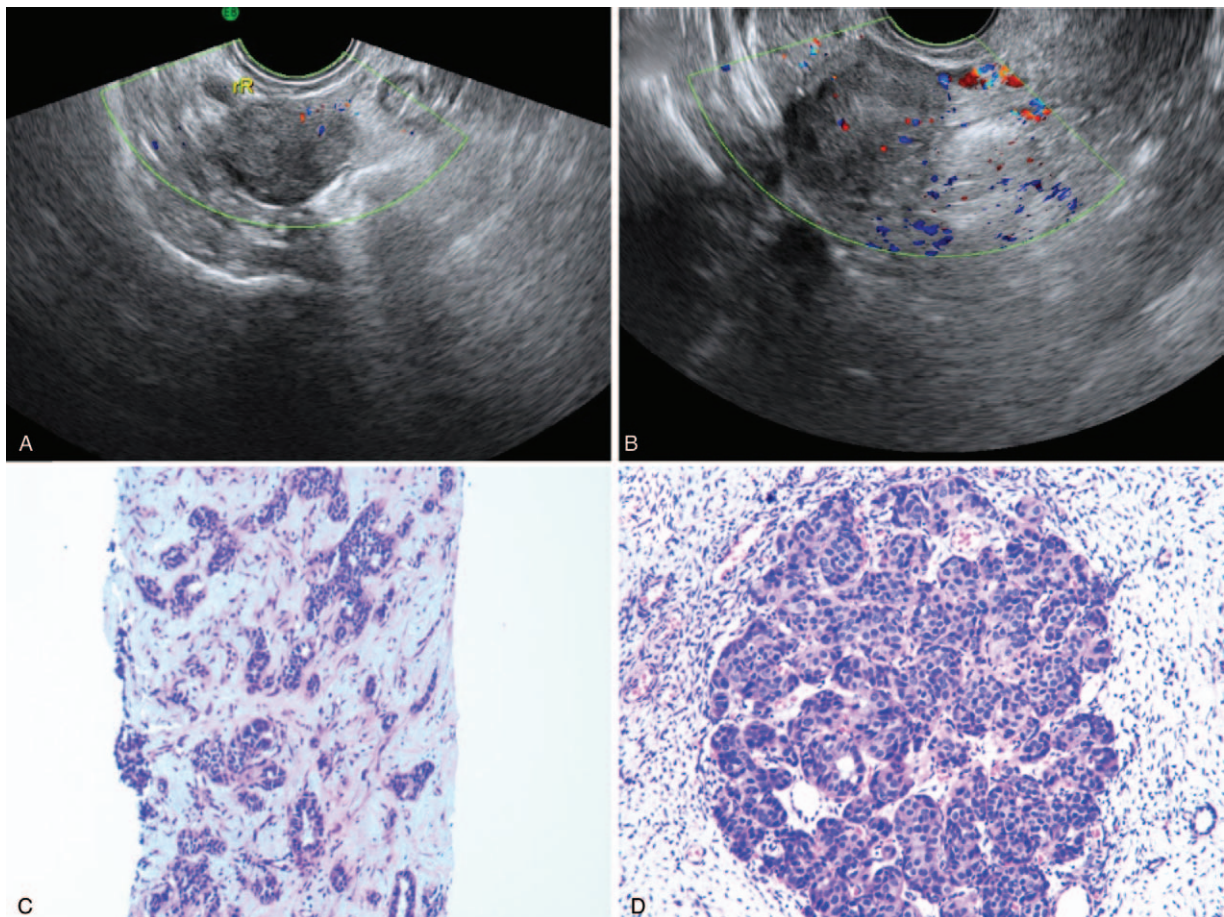


Figure 2. (A) Solid adnexal mass detected by ultrasound for the first time. (B) Enlarged mass detected by ultrasound after 8 months. (C) Liver biopsy; hematoxylin and eosin stain, 5× magnification. (D) Ovarian biopsy; hematoxylin and eosin stain, 10× magnification.

and no metastasis in the five sentinel lymph nodes as well as ER+ (100%), PR+ (80%), and HER2/neu- results. Adjuvant chemotherapy was suggested, but the patient refused. She received tamoxifen with postoperative radiotherapy. On regular follow-up at 6 months after operation, CA153 level increased to 218.4 U/mL. Subsequent liver MRI suggested multiple areas of metastases within the liver, while bone emission CT scans indicated multiple vertebral metastases. Histopathology of liver biopsy suggested poorly differentiated carcinoma, and the morphological consideration was IDC. Immunohistochemical results indicated ER+ (3+, 90%), PR+ (1+, 1%), and HER2/neu- (Fig. 2C). She was administered two cycles of epirubicin and cyclophosphamide. When liver MRI detected progression of liver lesions, she was administered four cycles of paclitaxel and gemcitabine. Due to the stability of liver lesions, she underwent hepatic radiofrequency ablation followed by another two cycles of chemotherapy (paclitaxel and gemcitabine). Thereafter, OFS (goserelin) plus exemestane was suggested as maintenance therapy.

After 3 months, liver ultrasound revealed new lesions, and the treatment was changed to OFS plus capecitabine. After 5 months, the liver lesions progressed again. The second liver biopsy revealed the same pathological type of lesions as before, and the radiofrequency treatment was administered again. Therapeutic ovarian castration was then performed. Pathological results showed a small number of tumor nests on one side of the ovarian tissue. Based on partial immunohistochemistry results (ER+ [3+], PR+ [3+], and HER2-), metastatic breast cancer was considered (Fig. 2D). Systemic therapy was then changed to fulvestrant plus everolimus. After 3 months, her liver lesions continued to progress. Subsequently, albumin-bound paclitaxel was used twice until rapid progression of liver lesions and multiple metastatic lesions throughout the whole brain was detected on MRI. Due to severe bone marrow suppression, she received palliative whole-brain radiotherapy followed by capecitabine monotherapy (September 2018).

5. Discussion

Breast cancer is the leading cause of cancer among women and ranks fifth among all cancer deaths in China.^[10–12] Metastasis of breast cancer to distant organs is the most common cause of death in breast cancer cases. The most frequent metastatic sites of breast cancer include the liver, lung, bone, and brain. Metastatic breast cancer to the ovaries is relative rare. While gastrointestinal tract represents the first original site of non-genital tract metastatic ovarian neoplasms, breast cancer represents the second original site. The percentage of ovarian metastasis in breast cancer patients was reported to be 1.5–25%.^[2,3] Here, we reported three additional cases of ovarian metastasis from breast cancer in Chinese females.

Several clinicopathologic characteristics of primary breast cancer are thought to be associated with increased risk of ovarian metastasis from breast cancer. The literature reports that ovarian metastasis more likely occurs in women who were initially diagnosed with breast cancer at premenopausal stages (median age, around 50 years).^[4–7] All three patients in our study were premenopausal women, although their ages were slightly younger than as previously reported. The risks of ovarian metastasis are different among IDC, invasive lobular carcinoma, and other morphological subtypes. It has been reported that invasive lobular carcinoma has three times greater metastatic tendency to occur in the ovaries, peritoneum, and gastrointestinal

tract. However, due to a higher proportion of IDCs among all morphological subtypes of breast carcinomas, more IDCs occur in ovarian metastases from breast cancer. This finding is consistent with our observations. Two of our three patients were primarily diagnosed with IDC. Furthermore, ovarian metastasis from breast cancer is more common in patients with hormone receptor-positive breast cancer and breast cancer with axillary lymph node metastasis,^[2,5–8] and all three of our patients showed this characteristic.

Ovarian metastases from breast cancer are frequently asymptomatic and are commonly diagnosed incidentally with follow-up image examination of breast cancer or therapeutic oophorectomy. None of our patients had any symptoms. The diagnosis of ovarian metastases from breast cancer is thus very challenging. Medical history, clinical manifestations, imaging examinations, serological examinations, and pathological tests (including immunohistochemistry) should therefore be considered to improve diagnostic accuracy, especially during differential diagnosis from primary ovarian cancer (POC).

Adnexal masses in breast cancer patients are mostly benign lesions, including ovarian mucinous cystadenoma, and simple cysts.^[9,13] However, when asymptomatic ovarian masses are detected during follow-up image examination of breast cancer, the existence of ovarian metastases should be taken into consideration. Ovarian metastases from breast cancer should be particularly distinguished from POC, which has an elevated risk especially in patients with BRCA (breast cancer susceptibility gene) mutations. Medical history is the most fundamental element for diagnosis, especially for the time interval between the primary diagnosis of breast cancer and the manifestation of ovarian masses. The intervals of ovarian metastasis from breast cancers are frequently longer than those of POCs. Two of our three patients were diagnosed with ovarian metastasis several years after the primary diagnosis of breast cancer, consistent with the findings of previous reports. A large case series reported that 109 patients with ovarian metastasis from breast cancer had a nearly 3-year interval between initial diagnosis of breast cancer and diagnosis of ovarian metastasis, and seven patients had an even longer interval of >10 years.^[4] Other studies also found that the median time intervals are approximately 5 years.^[2,6–8]

The long incubation period from the primary diagnosis of breast cancer to the diagnosis of ovarian metastasis can be explained by the theory of tumor cell dormancy. This dormancy can occur years after the initial cancer treatment as residual cancer cells can enter dormancy and evade therapies.^[14] In most patients, the diagnosis of ovarian metastasis is accompanied by the diagnoses of other organ metastases, and ovarian metastasis is often a component of multiple metastatic disease. This characteristic was observed in all three of our patients, who had extraovarian metastases. Transvaginal ultrasound can be primarily useful for detecting and diagnosing ovarian mass. Although the condition lacks specific clinical manifestations, evaluation of features such as bilateral, solid, small, and highly vascularized ovarian masses coupled with breast cancer history can strongly suggest the possibility of ovarian metastases, as shown in Cases 1 and 2. Many studies suggest that up to 50% of ovarian metastases are bilateral.^[15,6,15,16] Moreover, MRI has high accuracy for differentiating benign and malignant ovarian tumors.^[17] Regarding serum markers, patients with metastases showed lower levels of serum CA125 (<80 U/mL), higher levels of serum CA153 (>100 U/mL), and lower BRCA mutation probability (<20%) than patients with primary ovarian

tumors.^[18] However, regular screening of serum markers such as CA125 or CA153 is not recommended as they lack sensitivity.^[19]

For breast cancer patients with suspicious ovarian mass, we recommend a simple laparoscopic oophorectomy to provide the pathological diagnosis. Bilateral, solid, small-sized, and highly vascularized masses are characteristic features of ovarian metastases on macroscopic examination.^[20] During macroscopic examination, metastatic lesions are mainly located in the ovarian medulla and/or cortex and manifest as isolated nodules or multiple discrete nodules dispersed in normal ovarian tissue.^[21] Immunohistochemical tests are a useful method to distinguish breast cancer ovarian metastases from POCs. Positive staining for GCDFP15, mammaglobin, and GATA3 can suggest a diagnosis of breast origin metastasis.^[22] Besides these, ER, PR, and HER2 also need to be tested^[23] to guide further possible endocrine therapy or anti-HER2 therapy. In our study, we also found a negative conversion of ER and PR in the ovarian tissue of one of the patients.

Regarding the management of ovarian metastasis from breast cancer, surgery can be a primary and extremely important step. Surgery provides both diagnosis and treatment effects. Although some gynecologists recommend complete cytoreductive surgery,^[24] the exact treatment effect remains controversial. As mentioned previously, ovarian metastasis from breast cancer is often a component of multiple metastatic disease. Traditional gynecological cytoreductive surgery cannot exactly achieve “cytoreductive” effects. Thus, we suggest that a simple laparoscopic bilateral oophorectomy or salpingo-oophorectomy is acceptable. This procedure can provide not only pathological diagnosis but also removal of metastatic tumor and definite castration, which is an important procedure of endocrine therapy for premenopausal hormone receptor-positive breast cancer. Systemic therapy (such as chemotherapy, endocrine therapy, and anti-HER2 therapy) is also essential to address the coexistence of other organ metastases. Furthermore, oncologists need to choose the appropriate treatment regimen based on patients’ tumor burden, molecular subtypes of cancer, and patients’ physical performance, according to guidelines such as the 4th ESO-ESMO International Consensus Guidelines for Advanced Breast Cancer (ABC 4). In our study, all three patients underwent systemic therapies, including chemotherapy, and endocrine therapy, which prolonged their survival.

6. Conclusion

In this report, we presented three cases of ovarian metastasis from breast cancer in premenopausal Chinese females. All three were young age when diagnosed with primary breast cancer and they all presented with asymptomatic ovarian metastases along with multiple extraovarian metastases. Based on the findings, we recommend a simple laparoscopic bilateral oophorectomy or salpingo-oophorectomy^[24] for breast cancer patients with suspicious ovarian masses. The procedure provides not only pathological diagnosis but also removal of the metastatic tumor and definite castration. Moreover, systemic therapy (such as chemotherapy, endocrine therapy, and anti-HER2 therapy) is essential as ovarian metastasis is often a component of systemic metastatic disease.

Author contributions

Conceptualization: Wei Tian.

Investigation: Xiaowei Zhang.

Supervision: Yongchuan Deng.

Writing – original draft: Jiang Wang, Yunxiang Zhou.

Writing – review & editing: Wei Tian.

References

- [1] Skirnisdottir I, Garmo H, Holmberg L. Non-genital tract metastases to the ovaries presented as ovarian tumors in Sweden 1990-2003: occurrence, origin and survival compared to ovarian cancer. *Gynecol Oncol* 2007;105:166–71.
- [2] Curtin JP, Barakat RR, Hoskins WJ. Ovarian disease in women with breast cancer. *Obstet Gynecol* 1994;84:449–52.
- [3] Simpkins F, Zahurak M, Armstrong D, et al. Ovarian malignancy in breast cancer patients with an adnexal mass. *Obstet Gynecol* 2005; 105:507–13.
- [4] Webb M, Decker DG, Mussey E. Cancer metastatic to the ovary: factors influencing survival. *Obstet Gynecol* 1975;45:391–6.
- [5] Ayhan A, Guvenal T, Salman MC, et al. The role of cytoreductive surgery in nongenital cancers metastatic to the ovaries. *Gynecol Oncol* 2005; 98:235–41.
- [6] Bigorie V, Morice P, Duvillard P, et al. Ovarian metastases from breast cancer: report of 29 cases. *Cancer* 2010;116:799–804.
- [7] Pimentel C, Becquet M, Lavoue V, et al. Ovarian metastases from breast cancer: a series of 28 cases. *Anticancer Res* 2016;36:4195–200.
- [8] Eitan R, Gemignani ML, Venkatraman ES, et al. Breast cancer metastatic to abdomen and pelvis: role of surgical resection. *Gynecol Oncol* 2003;90:397–401.
- [9] Tuncer ZS, Boyraz G, Selcuk I, et al. Adnexal masses in women with breast cancer. *Aust NZ J Obstet Gynaecol* 2012;52:266–9.
- [10] DeSantis CE, Ma J, Goding Sauer A, et al. Breast cancer statistics, 2017, racial disparity in mortality by state. *CA Cancer J Clin* 2017;67:439–48.
- [11] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin* 2017;67:7–30.
- [12] Chen W, Zheng R, Zhang S, et al. Cancer incidence and mortality in China in 2013: an analysis based on urbanization level. *Chin J Cancer Res* 2017;29:1–0.
- [13] Hann LE, Lui DM, Shi W, et al. Adnexal masses in women with breast cancer: US findings with clinical and histopathologic correlation. *Radiology* 2000;216:242–7.
- [14] Sosa MS, Bragado P, Aguirre-Ghiso JA. Mechanisms of disseminated cancer cell dormancy: an awakening field. *Nat Rev Cancer* 2014;14:611–22.
- [15] Peters IT, van Zwet EW, Smit VT, et al. Prevalence and risk factors of ovarian metastases in breast cancer patients <41 years of age in the Netherlands: a nationwide retrospective cohort study. *PLoS One* 2017;12:e0168277.
- [16] Gagnon YTB. Ovarian metastases of breast carcinoma. A clinicopathologic study of 59 cases. *Cancer* 1989;64:892–8.
- [17] Shaha PR, Khetawat R, Sahoo K, et al. Pelvic mass lesions in females: tissue characterization capability of MRI. *J Clin Diagn Res* 2017;11: TC01–5.
- [18] Tserkezoglou A, Kontou S, Hadjileftheriou G, et al. Primary and metastatic ovarian cancer in patients with prior breast carcinoma. Pre-operative markers and treatment results. *Anticancer Res* 2006;26(3B):2339–44.
- [19] Perrotin F, Marret H, Bouquin R, et al. diagnostic et pronostic des métastases ovariennes du cancer du sein. [Incidence, diagnosis and prognosis of ovarian metastasis in breast carcinoma]. *Gynecol Obstet Fertil* 2001;29:308–15.
- [20] Testa AC, Ferrandina G, Timmerman D, et al. Imaging in gynecological disease (1): ultrasound features of metastases in the ovaries differ depending on the origin of the primary tumor. *Ultrasound Obstet Gynecol* 2007;29:505–11.
- [21] Peters IT, van der Steen MA, Huisman BW, et al. Morphological and phenotypical features of ovarian metastases in breast cancer patients. *BMC Cancer* 2017;17:206.
- [22] Rabban JT, Barnes M, Chen LM, et al. Ovarian pathology in risk-reducing salpingo-oophorectomies from women with BRCA mutations, emphasizing the differential diagnosis of occult primary and metastatic carcinoma. *Am J Surg Pathol* 2009;33:1125–36.
- [23] Cardoso F, Senkus E, Costa A, et al. 4th ESO-ESMO International Consensus Guidelines for Advanced Breast Cancer (ABC 4). *Ann Oncol* 2018;29:1634–57.
- [24] Sal V, Demirkiran F, Topuz S, et al. Surgical treatment of metastatic ovarian tumors from extragenital primary sites. *Int J Gynecol Cancer* 2016;26:688–96.