Case study

DETECTION OF METASTATIC BREAST CARCINOMA CELLS IN BONE MARROW BY FLOW CYTOMETRY

ABSTRACT

Breast cancer is the most common cause of cancer death in women worldwide. Cytological, histological, and immunohistochemical techniques are routine laboratory tests for determining tumor subtypes. Over the past few years, laboratory diagnostic tests for breast cancer have become more complex, sophisticated, and specialized. This report describes the case of a young patient with metastatic breast cancer whose diagnosis was based on flow cytometric analysis of bone marrow aspirate. Flow cytometry showed to be an important tool in cancer diagnosis. Its application as a routine laboratory test for the diagnosis of solid tumors, such as breast cancer, can help provide fast results while increasing diagnostic coverage.

1 INTRODUCTION

Breast cancer is the most common cause of cancer death in women worldwide.[1] Despite advances in detection strategies and multi-professional approaches, many women are still diagnosed with advanced-stage breast cancer, which decreases their chances of cure, especially in cases of metastasis.[2] Time of detection and histological type are important prognostic factors. Cytological, histological, and immunohistochemical techniques are routine laboratory tests widely used for determining tumor subtypes.[2] Over the years, laboratory diagnostic tests for this cancer have become more complex, sophisticated, and specialized, resulting in faster results and more personalized treatments for each tumor subtype.[3] This report describes the case of a young patient with a history of chronic bone pain. Diagnosis of metastatic breast cancer was based on analysis of bone marrow aspirate by flow cytometry.

2 PRESENTATION OF CASE

The patient is a 28-year-old woman, adopted, mother of three children, with a history of postpartum depression, undergoing treatment for lactation mastitis in the right breast. The patient presented with five months of worsening bilateral lumbar pain radiating to the thorax

and lower limbs. Two months later, she returned to the hospital with epistaxis, alopecia, lymphadenopathy, exertional dyspnea, petechiae in the lower limbs, and weight loss of 20 kg. Laboratory examination revealed bicytopenia. The patient was admitted to the hospital with fever and night sweats. Clinical findings and patient history favored initial hypothesis of lymphoproliferative neoplasm. Bone marrow aspirate and biopsy were collected for immunophenotypic, histological, and immunohistochemical examination.

A standardized panel of monoclonal antibodies for samples suspected of hematological neoplasms (anti-CD45-V500, anti-CD34-PerCP-Cy5.5, anti-CD3-APC, anti-CD19-PE-Cy7, anti-CD56-PE, anti-CD38-APC, anti-CD20-PB, and anti-CD8-FITC) was used for flow cytometry. Samples were also labeled with an anti-HER2-PE antibody using a protocol standardized for samples from female patients with suspected non-hematologic malignancies of unknown primary. Sample acquisition was performed on a FACSCanto II (BD Biosciences, San Jose, CA, USA), and data were analyzed using Infinicyt® version 1.7 (Cytognos, Spain).

Cytology of MGG-stained bone marrow aspirate revealed non-hematological cells with atypical, enlarged nuclei (**Error! Reference source not found.**A to C). Morphological and flow cytometric analysis of bone marrow aspirate negated the initial hypothesis of lymphoproliferative neoplasm.

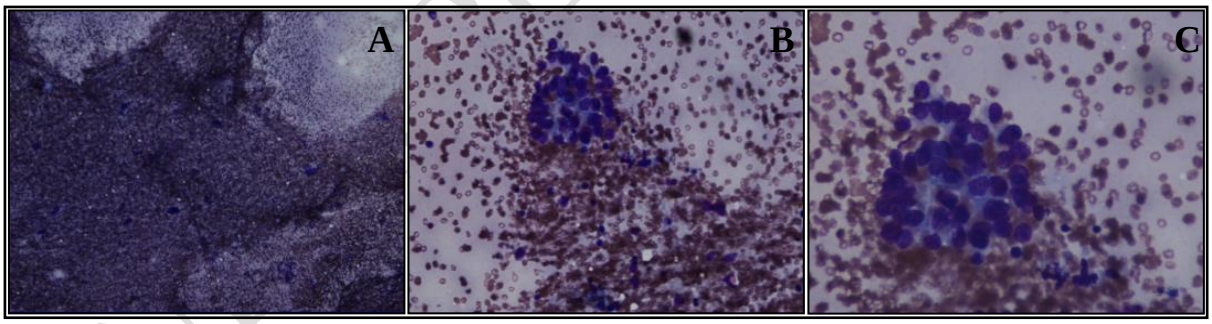

Figure 1 - (A–C) Myelogram of bone marrow aspirate stained with May–Grünwald–Giemsa at 4× (A), 10× (B), and 40× magnification (C).

In the following step, diagnosis of malignant breast neoplasm was confirmed by detection of $HER2^{+}$ cells by flow cytometry (Figure 2A to F). Flow cytometry results, therefore, helped define the antibody panel for immunohistochemistry analysis of bone marrow biopsy.

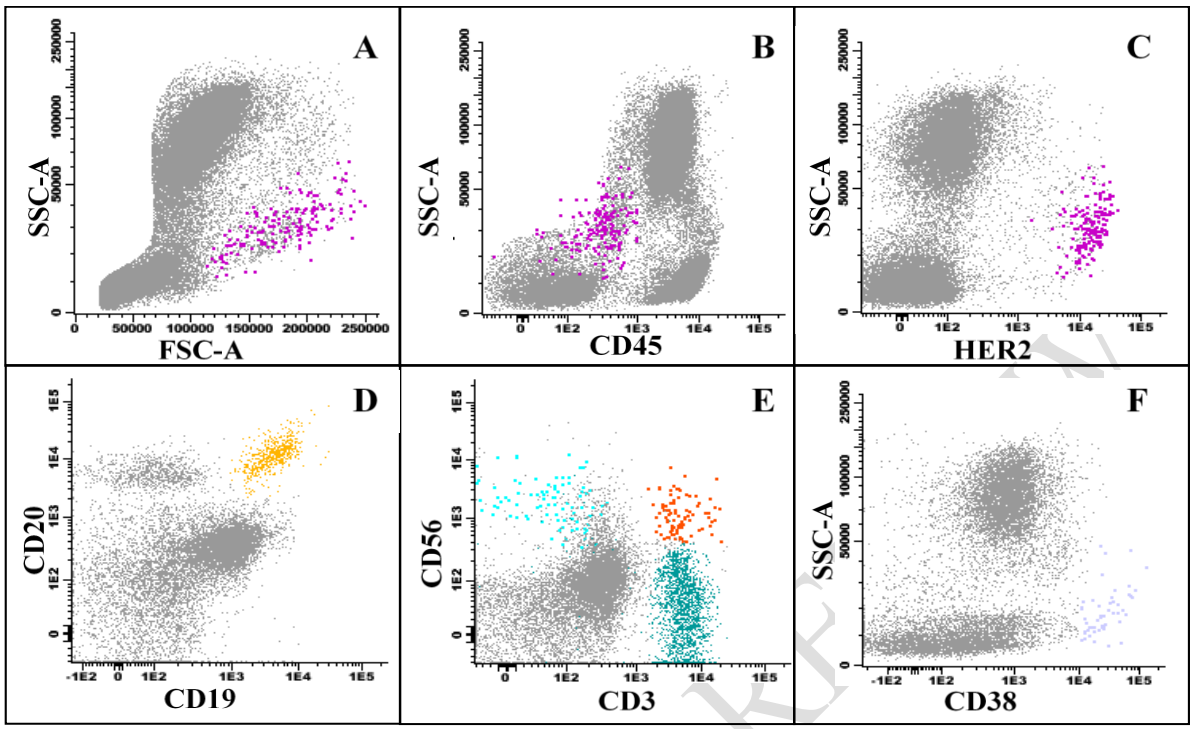

Figure 1 - Immunophenotypic profile of bone marrow aspirate cells by flow cytometry. (A–C) Size (FSC) and granularity (SSC) of non-hematopoietic cells ($CD45^{-}/HER2^{+}$), shown in pink. (D) B lymphocytes ($CD19^{+}/CD20^{+}$) highlighted in yellow. (E) T lymphocytes ($CD3^{+}$) highlighted in green, NK cells ($CD56^{+}$) in blue, and NKT cells ($CD56^{+}/CD3^{+}$) in orange. (F) Plasma cells ($CD38^{++}$) highlighted in lilac.

Immunohistochemistry results (Figure 3A to 3F) showed the presence of epithelial cells in bone marrow biopsy with positive expression of ER, HER2, GCDFP-15, mammaglobin, the pool of CK, CK7, E-cadherin, and PR. These phenotypic characteristics were compatible with metastatic breast carcinoma in bone marrow.

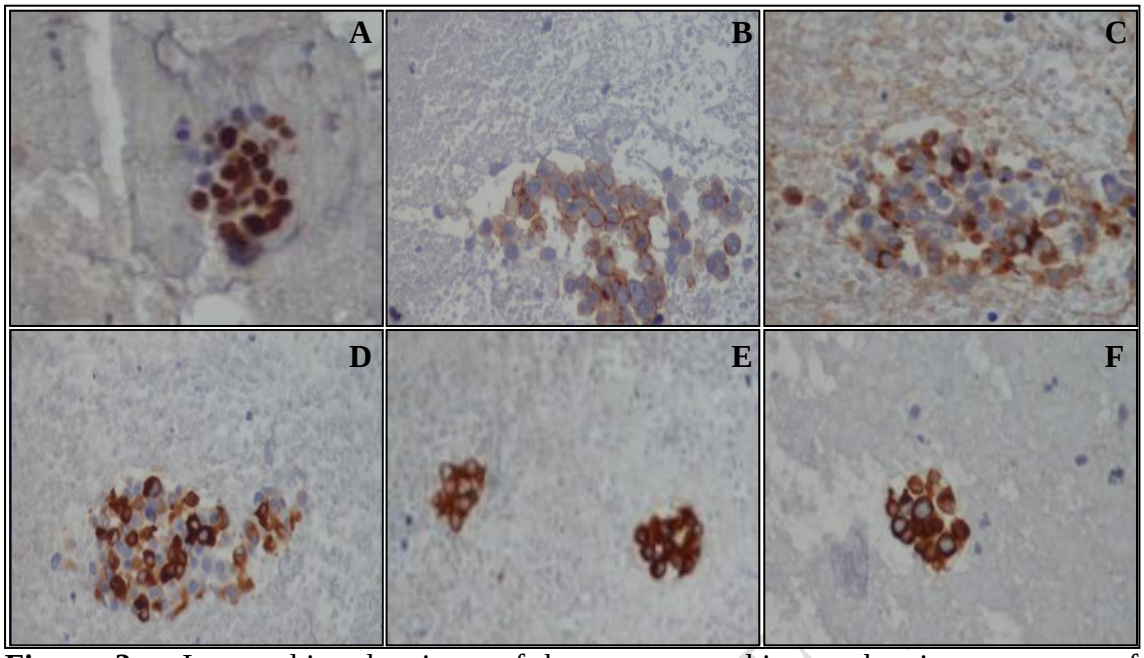

Figure 3 - Immunohistochemistry of bone marrow biopsy showing presence of epithelial cells with positive reaction to anti-estrogen receptor (A), anti-HER2 (B), anti-GCDFP15 (C), anti-mammaglobin (D), anti-CK pool (E), and anti-CK7 (F) at 100× magnification.

Subsequent physical examination revealed a nodule in the right breast, which was biopsied and analyzed. H&E-stained microscopic images showed invasive mammary carcinoma. Immunohistochemistry was carried out using a prognostic panel for breast cancer (ER, PR, HER2, and Ki67) as well as CK7 and GATA3 antibodies, and positivity for CK7 and GATA3 was observed (**Error! Reference source not found.**4A to 4F).

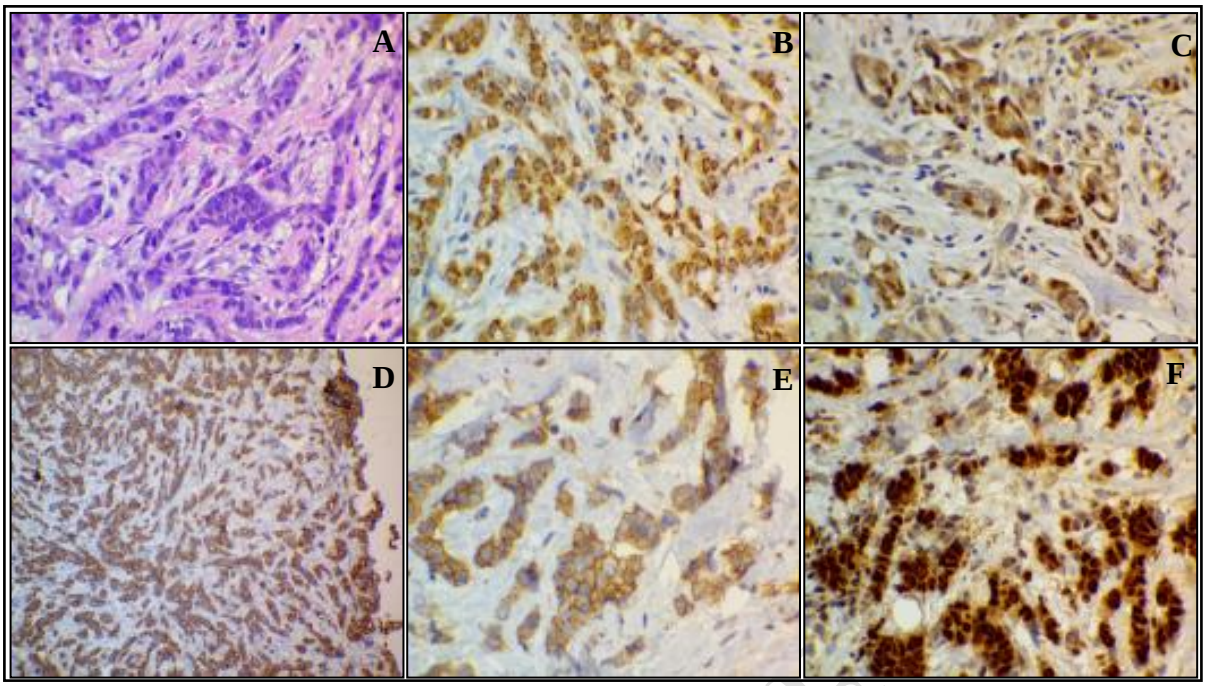

Figure 4 - (A) Breast biopsy specimen stained with hematoxylin and eosin showing features characteristic of invasive mammary carcinoma (×100). (B–F) Immunohistochemistry of breast biopsy specimens at 100× magnification. (B) Breast tissue with positive expression of progesterone receptor (+/Allred 6). (C) Breast tissue with positive expression of estrogen receptor (+/Allred 8). (D) Mammary gland tissue with positive expression of CK7 (+/diffuse). (E) Breast tissue with positive expression of HER2 (++/indeterminate). (F) Breast tissue with positive expression of GATA3 (+/diffuse).

The results confirmed the presence of primary breast tumor. Late diagnosis contributed to disease progression and a poor outcome. The patient died six months after diagnosis.

3 DISCUSSION

Here we report the case of a young patient diagnosed with metastatic breast carcinoma in bone marrow who died six months after diagnosis. Aggressive phenotypes of neoplasms commonly occur in young women, aged less than 45 years, resulting in poor prognosis and high risk of death. The Brazilian government, aiming to reduce breast cancer-related mortality rates, recommends screening from 40 years of age onward for women in the low-risk group and 30 years onward for women in the high-risk group. The level of risk is determined by family history, environmental factors, parity, and lifestyle habits.[4,5]

In the case of the patient presented here, her young age, unknown family history, and the overall clinical picture made the diagnosis difficult. The initial suspicion of lymphoma misled

the multidisciplinary team. Flow cytometry helped the team achieve quick results, despite the advanced stage of the disease. This scenario emphasizes the importance of laboratory techniques that assist in rapidly establishing the correct diagnosis. We highlight that the use of anti-HER2 antibody for the differential diagnosis of lymphoma by flow cytometry was an important step in reaching a final diagnosis. Detection of $HER2^{+}$ expression in non-hematopoietic cells in bone marrow alerted to the presence of metastasis, suggesting malignant breast neoplasm, the most common neoplasm in women.

Flow cytometric immunophenotyping is a relevant method that has made great contributions to the diagnosis of hematological malignancies.[6] The technique is able to phenotypically characterize and differentiate abnormal cell populations from normal populations, even at low concentrations, by means of antigen–antibody reactions. Reliable results are obtained in less than 4 h of processing.[7] Although this method is widely used as a routine diagnostic tool for hematological disorders, its application in solid tumor diagnosis remains limited, to a large extent, to research purposes.[8]

4 CONCLUSION

Flow cytometry showed to be an important tool in cancer diagnosis. Its application as a routine laboratory test for the diagnosis of solid tumors, such as breast cancer, can help provide fast results while increasing diagnostic coverage.

5 COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this case report.

6 CONSENT

All authors declare that ‘written informed consent was obtained from the patient for publication of this paper and accompanying images.

7 ETHICS

The patient agreed to participate in this study and signed an informed consent form approved by the Human Research Ethics Committee of the Federal University of Santa Catarina, Brazil - CEPSH/USFC no. 1.691.983/2016 - (Supplementary File).

REFERENCES

1 Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2018; 68(6):394-424.

2 Winters S, Martin C, Murphy D, Shokar NK. Breast Cancer Epidemiology, Prevention, and Screening. Prog Mol Biol Transl Sci 2017; 151:1-32.

3 Jafari SH, Saadatpour Z, Salmaninejad A, Momeni F, Mokhtari M, Nahand JS, Rahmati M, Mirzaei H, Kianmehr M. Breast cancer diagnosis: Imaging techniques and biochemical markers. J Cell Physiol 2018;233(7):5200-5213.

4 Klarenbach S, Sims-Jones N, Lewin G, Singh H, Thériault G , Tonelli M, Doull M, Courage S, Garcia AJ, Thombs BD. Recommendations on screening for breast cancer in women aged 40–74 years who are not at increased risk for breast cancer. CMAJ 2018; 10; 190(49).

5 Rojas K, Stuckey A. Breast Cancer Epidemiology and Risk Factors. Clin Obstet Gynecol 2016;59(4):651-672.

6 Betters DM. Use of Flow Cytometry in Clinical Practice. J Adv Pract Oncol 2015;6:435–440.

7 Adan A, Alizada G, Kiraz Y, Baran Y, Nalbant A. Flow cytometry: basic principles and applications. Crit Rev Biotechnol 2017; 37(2):163-176.

8 Bhagwat N, Dulmage K, Pletcher CHJ, Wang L, Demuth W, Sen M, Balli1 D, Yee SS, Silin Sa, Tong F, Yu L, Moore JS, Stanger BZ, Dixon EP, Carpenter EL. An integrated fow

198 cytometry based platform for isolation and molecular characterization of circulating tumor
199 single cells and clusters. Sci Rep. 2018;8(1):5035.
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