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.

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34 ## 2 PRESENTATION OF CASE

35 The patient is a 28-year-old woman, adopted, mother of three children, with a history of 36 postpartum depression, undergoing treatment for lactation mastitis in the right breast. The 37 patient presented with five months of worsening bilateral lumbar pain radiating to the thorax 38 and lower limbs. Two months later, she returned to the hospital with epistaxis, alopecia, 39 lymphadenopathy, exertional dyspnea, petechiae in the lower limbs, and weight loss of 20 kg. 40 Laboratory examination revealed bicytopenia. The patient was admitted to the hospital with 41 fever and night sweats. Clinical findings and patient history favored initial hypothesis of 42 lymphoproliferative neoplasm. Bone marrow aspirate and biopsy were collected for 43 immunophenotypic, histological, and immunohistochemical examination.

44 A standardized panel of monoclonal antibodies for samples suspected of 45 hematological neoplasms (anti-CD45-V500, anti-CD34-PerCP-Cy5.5, anti-CD3-APC, anti-46 CD19-PE-Cy7, anti-CD56-PE, anti-CD38-APC, anti-CD20-PB, and anti-CD8-FITC) was 47 used for flow cytometry. Samples were also labeled with an anti-HER2-PE antibody using a 48 protocol standardized for samples from female patients with suspected non-hematologic 49 malignancies of unknown primary. Sample acquisition was performed on a FACSCanto II 50 (BD Biosciences, San Jose, CA, USA), and data were analyzed using Infinicyt® version 1.7 51 (Cytognos, Spain).

52 Cytology of MGG-stained bone marrow aspirate from patient of this case report 53 revealed non-hematological cells with atypical, enlarged nuclei (**Error! Reference source 54 not found.**A to C).

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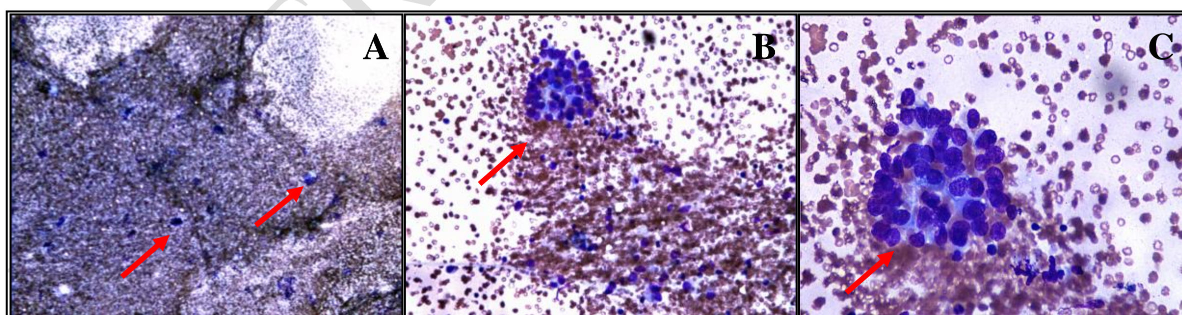

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62 **Figure 1** - Myelogram of bone marrow aspirate stained with May–Grünwald–Giemsa at 4× 63 (A), 10× (B), and 40× magnification (C).

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65 Morphological and flow cytometric analysis of bone marrow aspirate negated the initial 66 hypothesis of lymphoproliferative neoplasm. In the following step, diagnosis of malignant

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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.

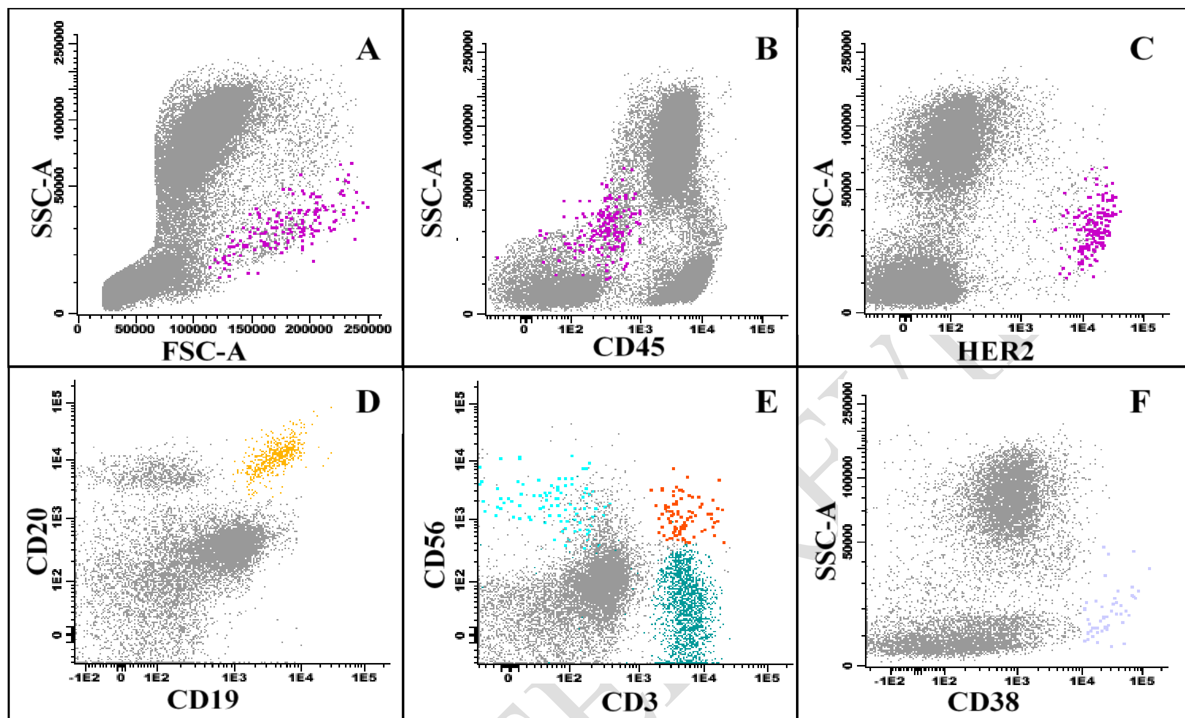

Figure 1 - Immunophenotypic profile of bone marrow aspirate cells by flow cytometry. (A–C) Size (FSC) and granularity (SSC) of non-hematopoietic cells ($CD45^{-}/HER2^{+}$), shown in pink. (D) B lymphocytes ($CD19^{+}/CD20^{+}$) highlighted in yellow. (E) T lymphocytes ($CD3^{+}$) highlighted in green,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

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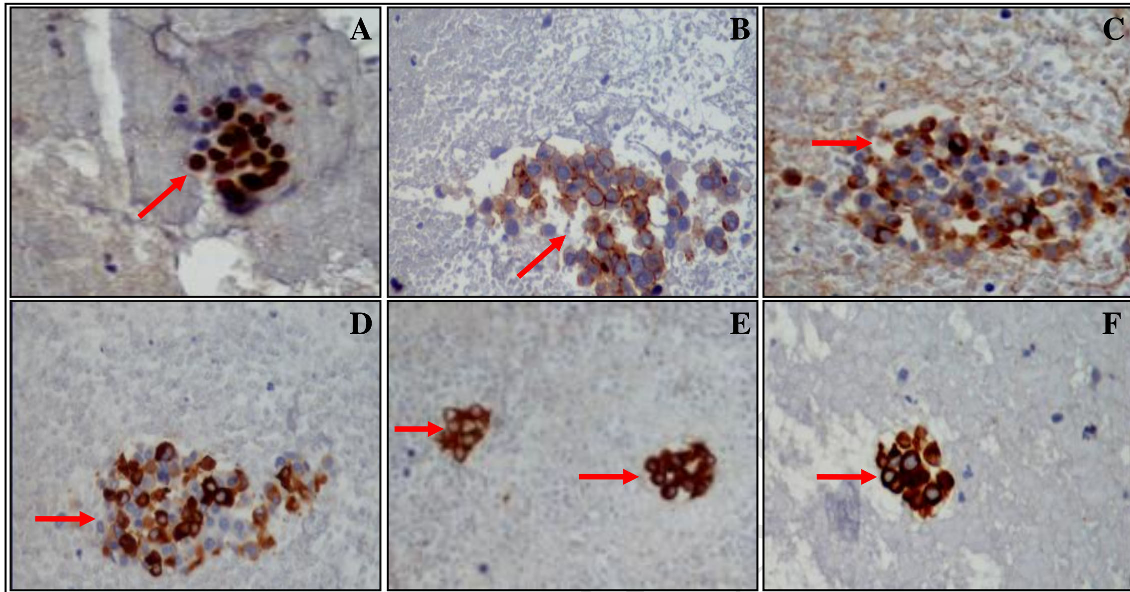

100 **Figure 3 -** Immunohistochemistry of bone marrow biopsy of the reported case showing
101 presence of epithelial cells with positive reaction to anti-estrogen receptor (A), anti-
102 HER2 (B), anti-GCDFP15 (C), anti-mammaglobin (D), anti-CK pool (E), and anti-CK7
103 (F) at 100× magnification.
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105 Subsequent physical examination revealed a nodule in the right breast, which was
106 biopsied and analyzed. H&E-stained microscopic images showed invasive mammary
107 carcinoma. Immunohistochemistry was carried out using a prognostic panel for breast cancer
108 (ER, PR, HER2, and Ki67) as well as CK7 and GATA3 antibodies, and positivity for CK7
109 and GATA3 was observed (**Error! Reference source not found.**4A to 4F).

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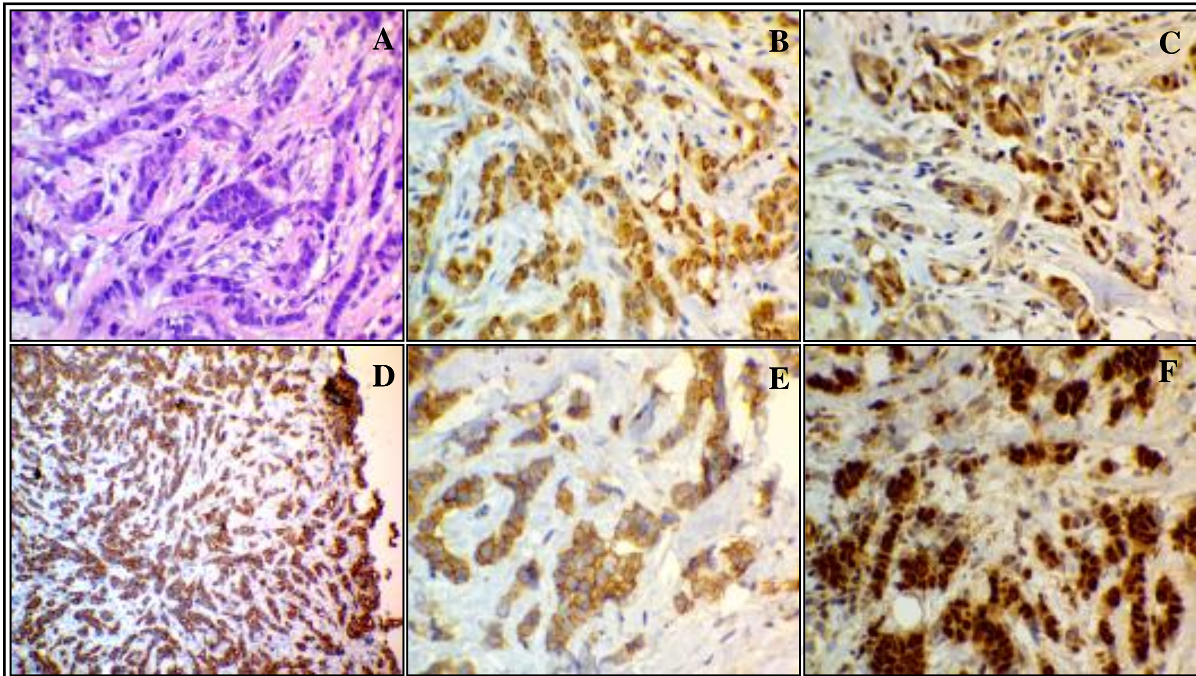

110 **Figure 4** – Morphological and immunohistochemical evaluation of breast biopsy specimen
111 of the reported case (A) Breast biopsy specimen stained with hematoxylin and eosin
112 showing features characteristic of invasive mammary carcinoma (×100). (B–F)
113 Immunohistochemistry of breast biopsy specimens at 100× magnification. (B) Breast tissue
114 with positive expression of progesterone receptor (+/Allred 6). (C) Breast tissue with
115 positive expression of estrogen receptor (+/Allred 8). (D) Mammary gland tissue with
116 positive expression of CK7 (+/diffuse). (E) Breast tissue with positive expression of HER2
117 (++/indeterminate). (F) Breast tissue with positive expression of GATA3 (+/diffuse).

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

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123 ## 3 DISCUSSION

124 Here we report the case of a young patient diagnosed with metastatic breast carcinoma in
125 bone marrow who died six months after diagnosis. Aggressive phenotypes of neoplasms
126 commonly occur in young women, aged less than 45 years, resulting in poor prognosis and
127 high risk of death. The Brazilian government, aiming to reduce breast cancer-related
128 mortality rates, recommends screening from 40 years of age onward for women in the low-
129 risk group and 30 years onward for women in the high-risk group.[4] The level of risk is
130 determined by family history, environmental factors, parity, and lifestyle habits.[5,6]

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

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133 the multidisciplinary team. Flow cytometry helped the team achieve quick results, despite the
134 advanced stage of the disease. This scenario emphasizes the importance of laboratory
135 techniques that assist in rapidly establishing the correct diagnosis. We highlight that the use
136 of anti-HER2 antibody for the differential diagnosis of lymphoma by flow cytometry was an
137 important step in reaching a final diagnosis. Detection of $HER2^{+}$ expression in non-
138 hematopoietic cells in bone marrow alerted to the presence of metastasis, suggesting
139 malignant breast neoplasm, the most common neoplasm in women. [7,8]
140 Flow cytometric immunophenotyping is a relevant method that has made great
141 contributions to the diagnosis of hematological malignancies.[9,10] The technique is able to
142 phenotypically characterize and differentiate abnormal cell populations from normal
143 populations, even at low concentrations, by means of antigen–antibody reactions. Reliable
144 results are obtained in less than 4 h of processing.[11] Although this method is widely used as a
145 routine diagnostic tool for hematological disorders, its application in solid tumor diagnosis
146 remains limited, to a large extent, to research purposes.[12, 13, 14] Thus cases reports should be
147 describe showing the importance of the flow cytometry to diagnosis of solid tumors.
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149 ## 4 CONCLUSION
150 The results presented in this case report show the importance of flow cytometry in the
151 laboratory routine. In this case, the initial diagnostic hypothesizes lymphoproliferative
152 neoplasia, however the inclusion of the HER2 immunostaining on the screening panel by
153 flow cytometric was essential for directing the diagnosis of the of metastatic breast carcinoma
154 in the bone marrow. Thus, the flow cytometry was an essential tool for the quick and rapid
155 conclusion of this case. The results, obtained by flow cytometry, guided the markers of breast
156 carcinoma investigation by immunohistochemistry in early bone marrow biopsy and allowed
157 the correct clinical management of the case. In this sense, the flow cytometry showed to be an
158 important tool in cancer diagnosis. Its application, as a routine laboratory test for the
159 diagnosis of solid tumors, can help provide fast results while increasing diagnostic coverage.
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162 ## 5 COMPETING INTERESTS
163 The authors declare that there is no conflict of interest regarding the publication of this
164 case report.
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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).

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