Extramedullary Haematopoiesis in Axillary Lymph Nodes of Breast Carcinoma Patients Receiving Neoadjuvant Chemotherapy: A Potential Diagnostic Pitfall

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Established Facts

- Extramedullary haematopoiesis (EMH) is mainly hosted by the lymphoid tissue and organs. However, it could be seen elsewhere.
- The process may be tri-lineage or involve one haematological lineage.
- EMH is considered a compensatory process that may indicate a haematological disorder. Other factors such as drugs may induce EMH. The precise mechanisms underlying EMH, however, remain unclear.

Novel Insights

- EMH could be seen in axillary lymph nodes of breast carcinoma patients following neoadjuvant chemotherapy especially with trastuzumab treatment.
- EMH in axillary lymph nodes should be differentiated from metastatic carcinoma and from multinucleate histiocytes. Awareness of the entity and careful attention to the morphological features including the presence/absence of atypia is crucial.
- A suggested panel of immunohistochemistry includes pan epithelial and histiocytic markers as well as markers of different blood derivatives such as CD42b, myeloperoxidase, and factor VIII.

Keywords

Extramedullary haematopoiesis · Megakaryocytes · Breast carcinoma · Axillary lymph node · Neoadjuvant chemotherapy · Herceptin

Abstract

Extramedullary haematopoiesis (EMH) in the axillary lymph node of breast cancer patients is extremely rare but when encountered can represent a diagnostic challenge. We aim to highlight this incidental finding as a diagnostic pitfall which can be mistaken for metastatic carcinoma (particularly of the metaplastic type). We report the case of a 68-year-

old Caucasian female with a family history of cancer. Core biopsy revealed that she had grade II oestrogen receptor-negative, Her2-positive invasive ductal carcinoma. She was offered neoadjuvant chemotherapy with Herceptin and subsequently underwent breast-conserving surgery. Microscopic examination of the post-treatment breast surgical specimen showed a partial pathological response with large areas of tumour regression. The sentinel lymph node showed frequent large single and multinucleate giant cells with hyperchromatic nuclei located predominantly within the subcapsular and medullary sinuses. The morphological differentials of metastatic carcinoma, sinus histiocytosis and extramedullary haematopoiesis were considered. A panel of immunohistochemistry showed these large cells to be negative for epithelial markers and CD68. They were strongly positive for CD42b (megakaryocyte marker). Smaller myeloperoxidase and factor VIII-positive cells were identified. The findings confirmed EMH. Sentinel nodes are often well scrutinised by pathologists for evidence of metastatic carcinoma as an important prognostic parameter both in the standard and neoadjuvant setting. Nodal megakaryocytes have been described in response to neoadjuvant chemotherapy particularly in association with Herceptin treatment. Pathologists' awareness of this finding in the neoadjuvant setting is crucial to avoid a mistaken diagnosis of malignancy. A relevant immunohistochemical panel together with careful attention to morphology should help establish the correct diagnosis.

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Introduction

Extramedullary haematopoiesis (EMH) is defined as the presence of one or more haematopoietic cell tri-lineages outside of the bone marrow [1]. It is regarded as a compensatory process associated with variable haematologic disorders that may be due to either primary or secondary aetiologies [2, 3]. EMH may be induced by drugs, such as after the administration of granulocyte colony-stimulating factor (G-CSF) [4]. Recently, it has been reported that this phenomenon may be associated with chemotherapy [5]. The main host of EMH is the reticuloendothelial system that includes liver, spleen, and lymph nodes [6]. EMH was also reported in other organs, including kidney, adrenals, dura mater, gastrointestinal tract, lung, pleura, and skin [3, 7, 8]. The breast was reported to host EMH as an incidental finding [7, 9, 10] with a rare occurrence in axillary lymph node [1]. We report a case with EMH in a sentinel lymph node of a wide local excision specimen after treatment with neoadjuvant

chemotherapy and trastuzumab. Our aim is to highlight this incidental finding as a diagnostic pitfall which can be mistaken for metastatic carcinoma.

Case Presentation

A 68-year-old, white British woman presented with a radiologically malignant mass lesion in her left breast on screening mammogram. She is a heavy smoker. She had a family history of cancer (1 of her sisters had cervical cancer at the age of 34 years and breast cancer aged 40 years; the other sister suffered from a malignant bone tumour at the age of 31 years). The breast lesion was clinically palpable. Ultrasound identified the lesion and ultrasound-guided core biopsies revealed grade II ER-negative Her2-positive invasive ductal breast cancer of no special type. Axillary nodes were normal on both clinical examination and imaging. Following multidisciplinary team discussion, the decision was made to proceed with neoadjuvant chemotherapy and trastuzumab after clip insertion to downstage the lesion and subsequently offer breast-conserving surgery. She was treated with docetaxel-FEC (2 cycles of docetaxel, 3 cycles of FEC) with concurrent trastuzumab. Imaging showed an excellent tumour response and the patient underwent ultrasound wire-guided left wide local excision with sentinel lymph node.

Pathological Features Breast

The specimen weighed 48 g and measured 80 × 65 × 20 mm. Microscopic examination showed a partial pathological response in the form of very few patches of grade II ductal invasive carcinoma with marked pleomorphic nuclei. There were multiple areas of tumour regression. The residual tumour was ER negative and HER2 positive.

The Sentinel Lymph Node

Throughout the node there were scattered large single and multinucleate giant cells located within the sinusoids of the cortical area as well as the medullary sinuses of the lymph nodes. The cells in places showed large nuclei with hyperchromasia and a high nucleocytoplasmic ratio, and were histologically suspicious of metastatic carcinoma (Fig. 1a, b). The presence of multiple nuclei also raised the possibility of a histiocytic proliferation. An immunohistochemistry panel for epithelial markers and macrophages, as well as haematopoietic markers, was created. These large cells were positive for CD42b (Fig. 1c), and negative for broad spectrum cytokeratin AE1/3 (Fig. 1d) and CD68 (Fig. 1e). Smaller myeloperoxidase (Fig. 1f) and factor VIII- (Fig. 1g) positive cells were identified in the background. However, both markers were negative in megakaryocytes. Careful attention to the morphological features and immunohistochemical profile confirmed these cells to be megakaryocytes. The lymph node showed no evidence of metastatic carcinoma. The diagnosis was reviewed and agreed by a haematopathologist.

Follow-Up and Outcome

Histology revealed that the medial margin of the wide local excision was positive. Therefore, a medial cavity shave was taken and showed no residual malignancy. The patient underwent adjuvant radiotherapy with adjuvant trastuzumab. She remains well after 31 months of follow-up.

Fig. 1. Histological and immunohistochemical characteristics of nodal megakaryocytes. Low- (**a**) and high-power (**b**) magnification showing scattered large multinucleate cells from the sentinel lymph node biopsy. **c** CD42b-positive megakaryocytes. **d** Cytokeratin AE1/3-negative megakaryocytes. **e** CD68-negative megakaryocytes. **f** Myeloperoxidase positive for myeloid precursors and negative for megakaryocytes. **g** Background small cells positive for factor VIII and negative for megakaryocytes. **c, d** Original magnification. $\times 40$.

Discussion

The presence of megakaryocytes in the axillary lymph node of breast carcinoma patients is extremely rare, but when encountered can represent a diagnostic pitfall. The foci of nodal EMH could potentially be mistaken for metastatic invasive carcinoma, metaplastic carcinoma [5], or

multinucleated histiocytes [11]. Histiocytes are comparatively larger and have more abundant cytoplasm with multiple vesicular nuclei, in contrast to megakaryocytes that contain multilobulated nuclei [12]. In our case, some of these cells were cytologically atypical. The presence of multinucleation, hyperchromasia, and a high nucleocytoplasmic ratio may lead to an erroneous diagnosis of me-

Table 1. Summary of previous studies reporting EMH associated with breast cancer

Article	Year	Patients, <i>n</i>	Age, years	Screen vs. symptomatic	Neoadjuvant chemotherapy	Herceptin	Node procedure
Hoda et al. [12]	2002	1	36	Symptomatic	No	No	Sentinel lymphadenectomy
Wang and Darvishian [17]	2006	2	43, 38	1 patient symptomatic, and 1 routine screening	Yes (doxorubicin and cyclophosphamide followed by paclitaxel)	No	EMH was seen in mastectomy specimens, not dissected lymph nodes
Millar et al. [13]	2009	1	36	Symptomatic	Yes (docetaxel, doxorubicin, cyclophosphamide followed by docetaxel with G-CSF)	Yes, post-operatively	Axillary node dissection
Prieto-Granada et al. [5]	2013	3	41–47	All symptomatic	Yes	No	1 patient, sentinel lymphadenectomy 1 patient, axillary lymph node dissection 1 patient, core biopsy of an enlarged node
Takhar et al. [1]	2013	1	53	Symptomatic	Yes (epirubicin and cyclophosphamide followed by docetaxel with G-CSF)	No	Axillary node dissection
Wilsher and Bonar [14]	2014	1	46	Symptomatic	Yes (5-fluorouracil, epirubicin and cyclophosphamide followed by docetaxel with G-CSF)	Yes	Sentinel lymphadenectomy

tastasis in the context of chemotherapy. These cells should also be distinguished from multinucleated giant cells. CD68 immunohistochemistry should also be performed to exclude this possibility. Nodal megakaryocytes have been reported only for 9 individuals in 6 previous articles (summarised in Table 1). In 2002, it was first described by Hoda et al. [12] who reported the presence of megakaryocytes in axillary lymph nodes following mastectomy and axillary node sampling without prior neoadjuvant chemotherapy. All subsequent cases were reported after neoadjuvant chemotherapy with co-administration of G-CSF in 3 of those cases [1, 13, 14]. Similar to our case, 2 previously reported cases had Her2 amplification [13, 14], with 1 of them receiving Herceptin on an adjuvant basis [13]. The underlying mechanism of EMH in the breast is still unknown. In 1994, Redmond et al. [4] suggested that stem cells can differentiate into haematopoietic cells in response to changes in the microenvironment. Growth factors or cytokines produced by inflammatory cells stimulated by local injury, such as biopsy or surgery, may also play a role in the pathogenesis of EMH [3, 15, 16]. Functional disruption of bone marrow causes circulating stem cells to home and proliferate in a favourable microenvironment outside the bone marrow [17]. Lymph nodes may harbour EMH as they normally sup-

port the proliferation of haematopoietic progenitors, which are derived haematogenously from the bone marrow [12]. EMH was reported in other solid tumours, such as renal, lung, colonic, hepatic, endometrial, adrenal, skin, ovarian carcinomas, and melanoma [18–43]. However, the aetiology of EMH in these patients remains unclear. One hypothesis is that EMH may be induced by G-CSFs that are administered along with chemotherapy [4] or radiotherapy. As a growth factor, G-CSF can stimulate the bone marrow to produce granulocytes by promoting stem cell production, thereby increasing the release of granulocytes into the blood. In addition, doxorubicin, which is widely used as a neoadjuvant chemotherapy for breast cancer, was linked to the pathogenesis of EMH in animal models [44]. An anaemic status results in the production of cytokines and growth factors that promote haematopoiesis. These chemical mediators may enter the blood circulation and assist in generating favourable microenvironments [17] that can elicit a natural homeostatic response to increase the production of red blood cells through a compensatory mechanism. EMH was also reported in patients receiving concurrent trastuzumab with neoadjuvant chemotherapy [13]. Poon et al. [45] suggested that trastuzumab emtansine has a cytotoxic effect that causes transient cyclic anaemia in non-

clinical species (especially monkeys). Platelet formation experiments using human haematopoietic stem cells showed that T-DM1 can impair megakaryocyte maturation as well. Furthermore, some abnormal cytokine or paracrine growth factors may be secreted by tumour cells, which may stimulate stem cell differentiation into haematopoietic cells and evoke regional hyperplasia of circulating haematopoietic progenitors [46].

Conclusion

Axillary node sampling (either by sentinel node biopsy or axillary clearance) is considered as a standard for all breast carcinomas. Those nodes are often well scrutinised by pathologists for evidence of metastatic carcinoma, which is an important prognostic parameter both in the

standard and neoadjuvant setting. It is important for pathologists reporting such cases to be aware of the phenomenon of EMH, particularly in the neoadjuvant setting, to avoid mistaking the large haematopoietically derived cells for metastatic carcinoma. We recommend that megakaryocytes should be included on the list of differential diagnoses of multinucleated giant cells in axillary lymph nodes of breast carcinoma. Epithelial immunohistochemistry as well as markers of blood derivatives (such as CD42b, myeloperoxidase, factor VIII) together with careful attention to morphology should help establish the correct diagnosis.

Disclosure Statement

The authors declare that no conflicts of interest exist.

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