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CASE REPORT

# Diffuse osteosclerosis as a presentation of recurrent breast cancer: role of endothelin 1

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## Abstract

**Summary** We report a 46-yr-old woman with a history of breast cancer who presented with diffuse myalgias, bone pain, and osteosclerosis. She was found to have recurrent breast cancer producing endothelin-1.

**Introduction** Acquired osteosclerosis can be caused by various disorders. Endothelin -1 is believed to contribute to osteosclerosis caused by breast cancer.

**Methods** Although the bone marrow biopsy did not reveal breast cancer, she developed skin lesions consistent with metastatic breast cancer. She ultimately died from progressive disease. At autopsy immunohistochemistry for endothelin-1 was performed on a section from the L5 vertebral body.

**Results** The section from the L5 vertebral body showed small foci of cells consistent with metastatic carcinoma and a prominent sclerotic response. Immunohistochemistry for endothelin-1 was strongly positive.

**Conclusions** Recurrent breast cancer may present with diffuse osteosclerosis. Endothelin-1 may be a paracrine factor responsible for increased bone formation and osteosclerosis.

**Keywords** Bone metastases · Breast cancer · Endothelin 1 · Osteosclerosis

## Introduction

Bone is a common site of cancer metastasis. Metastatic bone lesions can be osteoblastic, osteolytic, or mixed. Osteoblastic metastases are lesions that have both increased bone formation and resorption [1]. These metastatic lesions become more radiodense since bone formation exceeds resorption [2] and are described as osteosclerosis radiographically. Osteoblastic bone metastases occur most commonly in prostate cancer [3]. Breast cancer metastases are typically osteolytic, but 15–20% of cases are osteoblastic [1].

Tumor-related factors that may induce bone formation at osteoblastic metastases include insulin-like growth factors 1 and 2, transforming growth factor (TGF)-β, prostate-specific antigen, urokinase-type plasminogen activator, fibroblast growth factors (FGF)-1 and -2, bone morphogenic proteins (BMPs), and endothelin-1 (ET1) [4–9].

Endothelins, their convertases, and their receptors, referred to as the endothelin axis, have developmental and physiological functions in normal tissue, acting as modulators of vasomotor tone, tissue differentiation, development, cell proliferation, and hormone production [10]. The endothelin axis also functions in the growth and progression of tumors by modulation of proliferation, apoptosis, angiogenesis, and invasion

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[11]. Guise et al. identified three breast cancer cell lines in a mouse model that cause osteoblastic metastases and secrete ET1. The tumor-produced ET1-stimulated bone formation in vitro and osteoblastic metastases in vivo via endothelin A receptor. Treatment with an endothelin A receptor antagonist decreased bone metastases and tumor burden [12].

Recently, it was shown that patients with ET1 enriched phenotype continue experiencing relapses years after diagnosis and that silencing of the endothelin A receptor has induced apoptosis in both hormone receptor-negative and hormone receptor-positive breast cancer cells [13].

We report a patient that presented with diffuse osteosclerosis and was found to have breast cancer recurrence. Her disease progressed quickly and autopsy stains of the metastatic disease in bone revealed positivity for ET1.

## Methods

A post-mortem specimen from L5 was obtained after IRB approval. Histological evaluation was done using 5 μm sections of paraffin embedded tissue and stained with ET1 antibody using standard procedure. Rabbit polyclonal IgG against ET1 (SC-21625) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). We used dilution of 1:200 for primary antibody. We used biotinylated goat anti rabbit IgG (BA-1000) from Vector Laboratories (Burlingame, CA) at a concentration of 7.5 μg/ml as a secondary antibody. Vectastain ABC and DAB peroxidase substrate kit, 3, 3′-diaminobenzidine (PK6100 and SK-4100, Vector Laboratories, Burlingame, CA) were used as recommended by the manufacturer to visualize the antibody binding.

## Case report

A 46 year-old female was evaluated for diffuse osteosclerosis. Five months earlier, the patient completed a 26-mile walk. Subsequently, she developed fatigue, myalgias, fevers, and bilateral hip and upper extremity pain. Imaging done at an outside institution before our evaluation revealed diffuse sclerosis throughout the skeleton. Additional imaging before our evaluation included a total body bone scan which revealed increased uptake in proximal long bones, spine, sternum, and pelvis and a positron emission tomography–computed tomography (PET/CT) which showed heterogeneous diffuse sclerosis throughout the skeleton. A bone marrow biopsy, also done at an outside institution before our evaluation, revealed osteosclerosis with absent hematopoietic bone marrow. No cancer cells were seen and pancytokeratin stain was negative. Her past medical history was significant for invasive lobular breast carcinoma, T1cN0M0, 1.8 cm, and estrogen receptor (ER) positive/progesterone receptor (PR)-positive diagnosed 4 years earlier treated with bilateral mastectomy and tamoxifen.

Her weight was 52.6 kg and height 1.63 m. There were multiple matted, firm, supraclavicular, and cervical nodes. There was no bone tenderness to palpation.

Baseline laboratory evaluation (Supplemental Table 1) revealed an initially mildly elevated serum parathyroid hormone (PTH) and a subsequent normal measurement with normal serum calcium, phosphorus, and creatinine. The serum total alkaline phosphatase was about 3.5 times the upper limit of normal (ULN) and bone-specific alkaline phosphatase 7.4 times ULN. The c-telopeptide was 1.6 times ULN. Serum and urine protein electrophoresis, hepatitis C serology, plasma fluoride, biochemical tests for mastocytosis, serum vitamin A, serum 25(OH) vitamin D, and serum parathyroid hormone-related protein were normal.

A skeletal survey revealed diffusely sclerotic osseous structures with sparing of the distal extremities, rounded sclerotic areas of the proximal tibiae and femora, and a sclerotic focus within the right proximal radius. These findings were new compared to radiographs obtained 3 years earlier. Representative images are seen in Fig. 1.

A bone density study done by DXA confirmed high bone density (spine Z-score 7.4, mean total hip Z-score 7.6) and is shown in supplemental Table 2.

During the course of the evaluation, new, multiple, firm pink-colored dermal nodules ranging in size from 3 mm to 1 cm and located on the back, neck, left cheek, left eyebrow, angle of jaw, abdomen, and arms were noted (Supplemental Figure). Biopsy of one of these lesions revealed metastatic poorly differentiated adenocarcinoma consistent with lobular breast cancer. A brain MRI revealed dural enhancement with a subsequent CSF analysis revealing leptomeningeal carcinoma. A left deep axillary lymph node biopsy confirmed metastatic carcinoma with extranodal extension ER negative/PR negative and human epidermal growth factor receptor 2 (HER-2 Neu) negative. The patient received chemotherapy with paclitaxel. Her condition progressed and she expired 5 months after presentation.

Autopsy showed extensive metastatic carcinoma with involvement of skin of the anterior chest wall and neck, visceral/parietal pleura, pericardium, diaphragm, lungs, liver, gallbladder, gastrointestinal tract, and genitourinary tract. A section from L5 vertebral body showed small foci of crushed tissue consistent with metastatic carcinoma and prominent sclerotic response. Immunohistochemistry stains of post-mortem tissue of the L5 vertebra (obtained after IRB approval) were positive for ET1 (Fig. 2). ET1 staining was not done on other tissues or the initial tumor.

## Discussion

The patient presented with painful diffuse osteosclerosis 4 years after the diagnosis of breast cancer. Although she

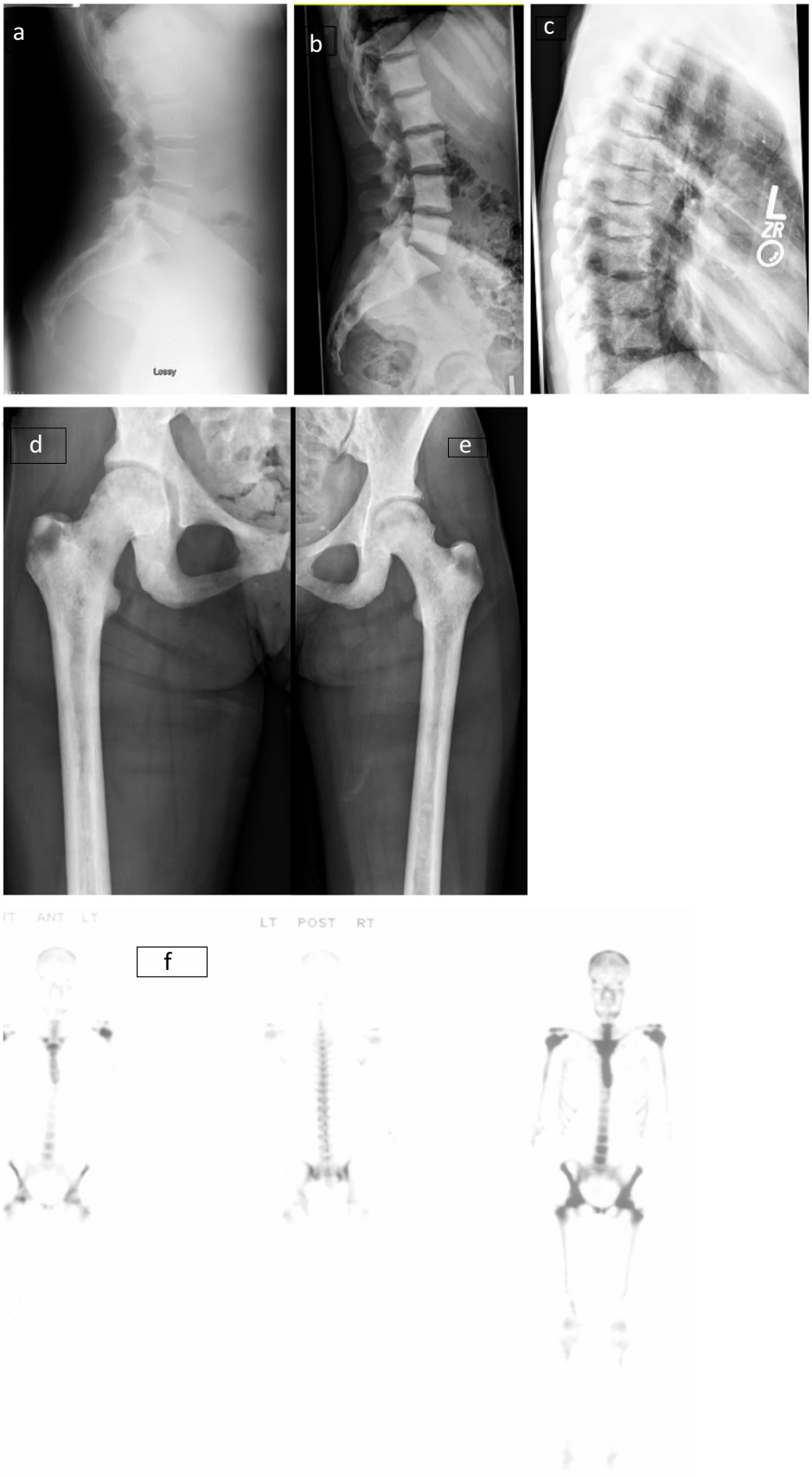


**Fig. 1** Imaging **a** 3-years before presentation. **b**–**e** At presentation. **f** Bone scan at presentation

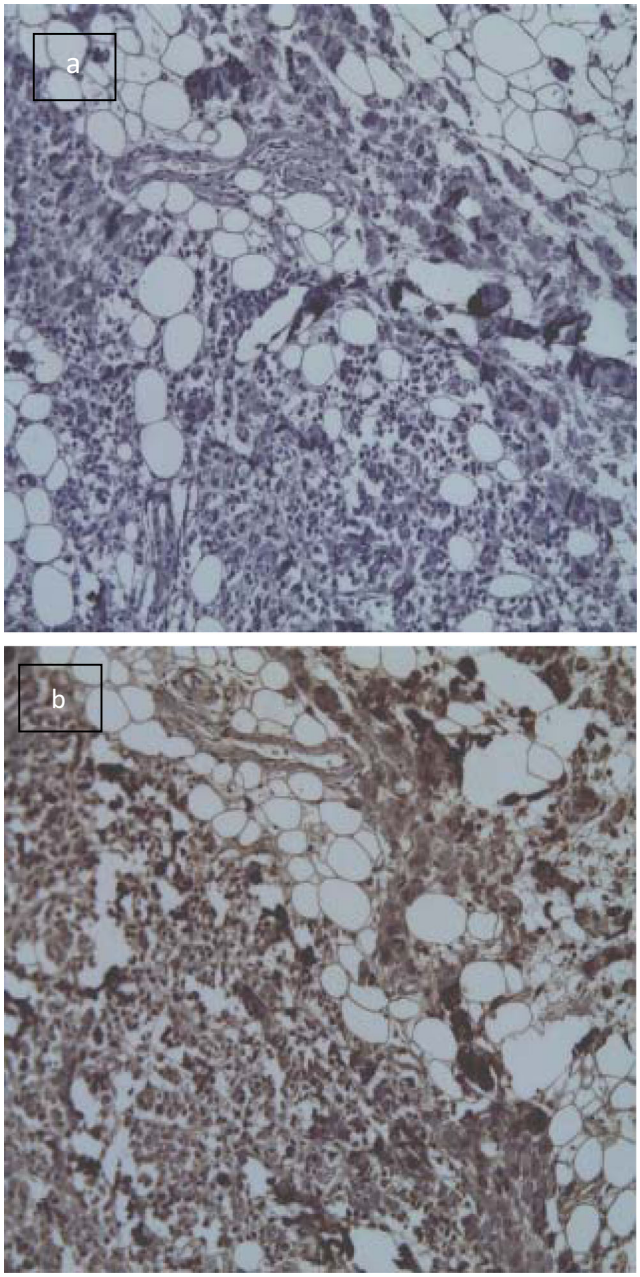


**Fig. 2** Immunocytochemistry for ET1. **a**. Control. **b**. ET1

developed diffuse bone metastases, the initial bone marrow was negative for cancer cells.

Osteosclerosis may be associated with hepatitis C, fluoride toxicity, hypervitaminosis A or D, plasma-cell dyscrasias, hyperparathyroidism, Paget's disease, osteopetrosis, skeletal dysplasias, mastocytosis, and sclerotic metastases [2]. These conditions were excluded during the evaluation. Bone marrow biopsy was done and was negative for cancer cells and pancytokeratin staining. The diagnosis of recurrent breast cancer was made with the appearance of metastatic skin nodules. This suggested that the diffuse osteosclerosis was caused by an endocrine or paracrine factor produced by cancer cells.

Tamkus et al. [13] showed that ET1-enriched tumor phenotype predicts breast cancer recurrence with higher nodal involvement and advanced stage. ET1 is a potent vasoactive peptide that signals through a pair of G protein-coupled receptors, endothelin A (EDNRA) and B (EDNRB)-type receptors [14]. ET1 is primarily synthesized in endothelium [14] and is secreted in response to shear stress [15]. ET1 is initially secreted as an inactive intermediate, (big ET1), which is cleaved by endothelin converting enzymes 1 and 2 to yield active ET1 [16]. ET1 is also a known mitogenic factor for both smooth muscle cells [14] and cancer cells [11]. In addition to its essential function in the cardiovascular system, ET1 is secreted by glandular tissue with increased expression measured during pregnancy [17] and lactation [18].

Based on prior work by Guise et al. [12], it was speculated that a possible mediator of osteosclerosis was ET1. In our case, post-mortem analysis of the vertebra stained positive on immunohistochemistry for ET1.

A proposed mechanism of endothelin action suggests that metastatic tumor cells secrete ET1. ET1 binds to the endothelin A receptor in osteoblasts. This stimulates osteoblast proliferation and new bone formation most likely by derepressing the Wnt signaling pathway through suppression of the Wnt pathway inhibitors *Dickkopf homolog 1 (DKK1) and sclerostin (SOST)* [19, 20].

The stimulation of osteoblast activity enriches the local microenvironment with growth factors, which in turn could increase tumor burden and ET1. The net effect is a vicious cycle that increases osteoblastic bone metastases [12].

Our patient's presentation was unusual because she initially presented with ER-positive/PR-positive disease while her recurrent disease was ER negative /PR negative /HER-2 Neu negative. It is also possible that cancer cells were present but missed in the at the time of bone marrow examination. That said, the cause of the sclerotic bone is likely ET1.

Further studies are needed to examine whether ET1 expression should serve as a prognostic biomarker for stratification of patients with hormone-positive breast cancer for adjuvant therapy and to determine if ET1 receptor blockade has a role in treatment of osteoblastic metastatic disease. Clinical trials of endothelin antagonism in metastatic prostate cancer have been largely unsuccessful [21]. We do not know of clinical trials of endothelin antagonism in breast cancer.

We conclude that recurrent breast cancer may present with diffuse osteosclerosis that is partly caused by secretion of ET1 by cancer cells with resultant stimulation of osteoblastic bone formation.

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## Compliance with ethical standards

**Conflict of interest** None directly related to this work, Jasmin Kristianto Employee: Unity Biotechnology John Charlson Advisory Board:Immune Design Clinical trial funding: Lilly Oncology Robert D. Blank Consultant: Amgen, Novo-Nordisk, Radius Health, Ultragenyx Authorship Royalties: UpToDate, McGraw-Hill Ownership Interest: JangoBio, Abbott Laboratories, Abbvie Joseph L. Shaker Consultant Shire, Alexion, Ultragenyx Authorship Royalties: McGraw-Hill.

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