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- 1 <u>Title:</u>
- 2 Higher rate of progesterone receptor positivity in skeletal metastases of breast cancer
- 3 with a pathological fracture versus those without fracture
- 4 <u>Short title:</u>
- 5 Pathological fractures linked to increased progesterone receptor positivity in breast cancer metastases
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- 23 <u>Preprint:</u>
- 24 Influence of receptor status and proliferation index in skeletal metastases of breast carcinoma on pathological
- 25 fracture occurrence
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- 28
- 29 <u>Abbreviation list:</u>
- 30 ASCO/CAP American Society of Clinical Oncology/College of American Pathologists
- 31 BC Breast cancer
- 32 CT Computed tomography
- 33 ER Estrogen receptor
- 34 FISH Fluorescence In Situ Hybridization
- 35 HER2 Human epidermal growth factor receptor 2
- 36 Ki67 Ki67 proliferative index
- 37 MRI Magnetic resonance imaging
- 38 PR Progesterone receptor
- 39 PET Positron emission tomography
- 40 SM Skeletal metastases
- 41 SINS Spinal instability neoplastic score
- 42 SPSS Statistical Package for the Social Sciences
- 43
- 44 <u>Novelty and Impact:</u>
- 45 Few studies so far investigated the relationship between hormone and HER2 receptors in breast cancer metastatic
- tissue with the occurrence of a pathological fracture. Our results showed that skeletal metastases of breast cancer
- 47 with a pathological fracture have a significantly higher rate of progesterone receptor positivity compared to those
- 48 with no fracture. Determining the progesterone receptor status in skeletal metastases may identify high-risk
- 49 groups for fracture occurrence and guide surgical and hormonal therapy.
- 50

51 ABSTRACT

Identifying risk factors for fracture occurrence in breast cancer (BC) skeletal metastases (SM) may guide the 52 53 management of such bone deposits. There is sparse evidence regarding receptor status in SM and their 54 relationship to fracture occurrence. This study aimed to determine the relationship between estrogen (ER), 55 progesterone (PR), and HER2 receptor status and Ki-67 index and fracture occurrence in SM of BC. 152 samples 56 of SM of BC obtained from individual patients were evaluated. The status of the aforementioned receptors and 57 Ki67 index were determined in SMs samples. Their expression was compared between SM that did and did not 58 develop a fracture. Ninety-one cases had pathological fracture at the SM site, and 61 did not. Patients who 59 sustained a pathological fracture had a higher rate of PR positivity at their SMs as compared to those with no 60 fracture. There was no significant difference between the two groups concerning ER, HER2+, or Ki67 status. 61 SMs secondary to BC with a fracture are more likely to be PR positive than those with no fracture. Determining 62 the receptor status in SMs may identify high-risk groups for fracture occurrence, and determining the PR status 63 may also guide surgical and hormonal therapy.

64 Keywords:

65 pathological fracture; skeletal metastases; breast cancer; hormone receptor; progesterone

66

67 1. INTRODUCTION

Breast cancer (BC) is the leading cancer site in women with an incidence in Europe and North America of 85-94/100,000. [1] Although early diagnosis and contemporary treatment have improved the survival rates of BC patients remain second to lung cancer as a cause of cancer deaths. Such a high mortality rate is due to the early dissemination of cancer cells, especially in bone, with skeletal metastases (SM) reported in 65-75% of patients. In 5-6% of patients SMs are identified at the same time as the initial diagnosis of BC. [2]

A devastating complication of SM is the occurrence of a pathological fracture which impairs the patient's quality of life and can adversely affect the survival rate. [3] Hence, great effort is put into the early diagnosis and treatment of SM using radiological investigations such as whole-body bone scintigraphy, positron emission tomography (PET) along with a more focussed assessment of suspicious lesions using plain radiographs, computed tomography (CT) scan and magnetic resonance imaging (MRI).

78 Identifying SM at high risk for fracture allows early intervention to minimize the risk of fracture occurrence. 79 Fracture risk scores for skeletal metastases have been described and are used in clinical practice including the 80 Mirels' score for long bones and the spinal instability neoplastic score (SINS) for vertebrae. [4, 5] Mirels'score 81 is based on plain radiographs and scores the site of metastasis, its size, the radiological type of lesion (lytic, 82 mixed, blastic), and the pain it causes, each scored from 1 to 3 points. The SINS scores 6 components namely the 83 location, pain type (none, mechanical, non-mechanical), radiological type, spine alignment, vertebral body 84 collapse, and posterolateral involvement each scored from 0 to 3. Both scores are used for treatment guidance, 85 especially in decision-making for prophylactic surgery of SM.

86 In addition to clinical and radiological parameters, it may also be that histological analysis of metastatic 87 tissue may guide as to the risk of pathological fracture occurrence. It is recognized that the status of various 88 tissue receptors and other cellular markers in the breast cancer tissue is related to the aggressiveness of the 89 disease and the effectiveness of breast cancer treatment which raises the possibility that the status of these in 90 metastatic disease may influence the risk of pathological fracture occurrence. These include the progesterone 91 receptor (PR), estrogen receptor (ER), the human epidermal growth factor receptor 2 (HER2), and Ki67 92 proliferative index. [6, 7, 8, 9] However, although the status of these receptors is routinely reported in primary 93 breast cancer biopsy analysis and used to guide treatment, this is not a routine practice for SM. Studies reported 94 that the receptor status in SM may differ from that in their primary tumour, [10, 11, 12, 13] which emphasizes 95 the importance of pathohistological and immunohistochemical analysis of metastatic tissue.

96 There is sparse evidence regarding receptor status in SM and the relationship of such receptors to fracture 97 occurrence, hence this study was performed. The aim of this study was to determine the ER, PR, HER2 status, 98 and Ki67 index in SM of BC with fracture vs. those with no fracture to thus determine whether there is any role 99 in these factors in guiding as to the risk of pathological fracture occurrence.

100

101 2. MATERIAL AND METHODS

102 2.1 Clinical data

103 This was a retrospective, observational study that evaluated clinical, radiological, and pathohistological data 104 in patients presenting with SM secondary to breast cancer. Patients treated at a regional reference centre for bone 105 and soft tissue oncology over ten-year period (June 2011 to June 2021) were evaluated. Female patients with 106 biopsy-proven metastatic breast cancer were included. Male patients and those surgically treated outside the 107 reference center, as well as those with incomplete medical data, were excluded. Similarly, only lesions with 108 completely determined ER, PR, HER2, and Ki67 status in the skeletal metastasis were included.

Bone lesions that presented with a pathological fracture were routinely biopsied at the fracture site in most cases along with the operative procedure of fracture treatment. Furthermore, bone lesions not associated with a fracture were biopsied if their primary origin was unknown or uncertain. SM with no fracture was biopsied if the time interval from the primary breast cancer diagnosis was long and was thus considered that the lesion may not be related to that primary or if the patient had been diagnosed earlier with more than one primary tumour. In those with multiple SM, the most suitable bone with regards to accessibility was selected for biopsy.

115 Clinical data were collected from patients' records. Radiological evaluation of SM was performed using 116 plain radiographs, CT, and skeletal scintigraphy, and the results for these were assessed and reported. The 117 immunohistochemical profile (PR, ER, and HER2 status) and Ki67 proliferative index were analyzed as 118 described below.

2.2 Immunohistochemistry

120 Serial sections, 5 µm thick, were cut for immunohistochemical analysis of ER, PR, HER2, and Ki67 using 121 US Food and Drug Administration approved primary rabbit monoclonal antibodies (Ventana Medical Systems, 122 Oro Valley, Arizona, USA; PR [1E2 clone], ER [6F11 clone], HER2 [4B5 clone], and Ki-67 [M7240 Clone 123 MIB-1, dilution 1 : 100; Dako]). Positive and negative controls were included for each case. The slides were 124 evaluated according to the ASCO/CAP guidelines. [14, 15] For PR and ER the percentage and intensity of 125 positively stained tumour cells were measured and the score was calculated by adding these two values for a 126 total of 0 to 8. The percentage of positive tumour cells was divided into 6 categories, 0 - negative, 1 indicating 127 <1%, 2 - 1%-10%, 3 - 11%-33%, 4 - 34%-66%, and 5 - 67%-100% (Figure 1). The intensity of positive tumour 128 cells was averaged across the predominant area and scored 0 indicating no staining, 1 - weak staining, 2 -129 moderate staining, and 3 - strong staining. HER2 slides were analyzed for the intensity of staining and 130 percentage of stained cells and classified as negative (score 0 or 1+), equivocal (score 2+), or positive (score 3+). 131 For equivocal cases, immunohistochemical analysis was repeated, and if still unchanged, fluorescence in situ 132 hybridization (FISH) was performed. Tumour cells with nuclear staining were considered positive for Ki-67 and 133 were reported as a percentage of the overall cells. [16]

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135 2.3 Statistical analysis

Statistical analysis was performed using SPSS v.28.0 software (SPSS Inc., Chicago, IL, USA). Descriptive data are expressed as a percentage of a group for discrete measures. A normal distribution of continuous numerical data was analyzed using the non-parametric Kolmogorov-Smirnov test. For categorical data t-test was used and where it was not applicable, non-parametric statistical Pearson's chi-squared test and Mann-Whitney test were utilized. The odds ratio and 95% Confidence Intervals were also calculated. Statistical significance was established at the p<0.05 level.</p>

142

143 **3. RESULTS**

144 **3.1 Demographic and clinical characteristics**

145 152 patients were included for analysis, with a mean age of 61.4 years (range 33-83, SD=10.37, 95%CI 146 ± 1.649). Localized bone pain and limitation of the movement were the most common complaints at the SM site. 147 In this series 99/152 (65.1%) patients had a confirmed diagnosis of breast cancer prior to the SM biopsy, whilst 148 in 53/152 (34.9%) the diagnosis of BC was made for the first-time following bone biopsy. In those with 149 previously diagnosed breast cancer, the mean time from the primary breast cancer diagnosis to the diagnosis of 150 SM was 75.7 months (range 4-264, SD=61.47, CI95% ±15.99). The duration of symptoms related to the SM 151 until pathological fracture varied from 1 day (in patients with sudden pathological fractures and no previous 152 complaints) to 60 months (mean of 4.4 months, SD=8.15, CI95% ±1.297). A total of 91 (59.9%) cases had a 153 pathological fracture at their skeletal metastasis whereas 61 did not. The demographics between these groups 154 were similar (Table 1.).

At the time of the SM diagnosis, 81 patients (53.3%) had a SM in only one bone and 71 patients (47.7%) in two or more bones. The most frequently involved bone was the femur in 71 cases (46.7%), followed by the spine in 67 (44.1%) and the pelvis in 39 (25.6%). The femur was the most frequently fractured bone, in 55 cases (60.4%). Mixed SM (with osteoblastic and osteolytic components) were seen in 101 cases (66.4%) and lytic SM (isolated osteolysis), in 51 (33.6%) (Table 2.). No relationship was found between the lesion's radiological appearance and fracture occurrence (p=0.117).

In 67 (44.1%) cases an isolated SM biopsy was performed whereas in 85 (55.9%) cases other concomitant surgical procedures were performed as part of the fracture treatment. These additional surgical procedures included stabilization with or without corpectomy of the affected vertebra (21), stabilization of long bone with or without resection (18), resection of the long bone segment and implantation of a tumor mega-prosthesis (26), and hemiarthroplasty or total hip arthroplasty (20).

- 166 **3.2 Immunohistochemistry**
- 167 Overall, 71 (46.7%) biopsy samples were positive for PR (PR+), 117 (76.9%) for ER (ER+), and 57 (37.5%)
 168 for HER2 (HER2+).

In the group with pathological fracture PR+ metastases were seen 49 (53.9%) patients versus 22 (36.1%) in those without fracture (p<0.05). We further analyzed the PR score along with the intensity of expression and percentage of positive cells (Table 3.). The PR score was significantly higher in those with a pathological</p>

172 fracture (p<0.05), and this was mainly due to the percentage of positive cells (p<0.05) rather than staining 173 intensity (p=0.066).

In the group with pathological fracture 71 (78%) were ER+ and in the group without fracture 46 (75.4%)
were positive (OR=1.16, CI95%=0.54-2.49, p=0.708). HER2 was positive in 32 (35.2%) cases with fracture and
in 25 (41%) without (OR=0.78, CI95%=0.40-1.52, p=0.468). The mean Ki67 value was 23.4 for all SM (range
1-90, SD 17.94, CI95% ±2.852). The mean value was 22.9 in SM with pathological fracture and 24.2 in those

178 179

180 4. DISCUSSION

without fracture (p=0.542).

181 Our results showed that SMs of breast cancer with a pathological fracture had a significantly higher rate of 182 PR positivity and PR scores as compared to those with no fracture. Moreover, our findings show that the 183 association between the PR score and fracture occurrence depends on the percentage of stained cells and not on 184 the staining intensity. The latter is in line with previous reports that recommend that $\geq 1\%$ of hormone receptor-185 positive tumour cells presence should be considered as hormone receptor-positive tumor. [14]

Breast cancer is the most common type of cancer in women, with 2.3 million women diagnosed globally with the disease and 685 000 deaths in 2020. [17] The occurrence of distal metastatic disease is the main cause of breast cancer-related mortality. Breast cancer can metastasize to several organs in the body, with bone being the most common site, accounting for 60-80% of metastatic cases [18] and with a median survival of 3 – 5 years. [19]

Metastatic breast cancer can cause destruction of the normal skeletal structure and function, resulting in skeletal-related events, including severe bone pain, pathological bone fracture, spinal cord compression, and hypercalcemia, which may lead to reduced quality of life and survival. [20] Under normal circumstances, bone metabolism is a well-balanced process between osteoblasts and osteoclasts, regulated by various cytokines and steroid sex hormones, including estrogen and progesterone. [21] Disrupted levels of these hormones together with their downstream receptor signalling may lead to an abnormal bone composition and facilitate the development of SM.

198 Primary breast cancers and/or metastatic deposits may be biopsied before any treatment to confirm the 199 diagnosis of malignancy and also to establish the expression of three receptors namely PR, ER, and HER2. The 200 expression of these receptors may help clinicians formulate an individualized management plan for each breast 201 cancer patient, and determine the order of the various treatment modalities in this plan (such as surgery, 202 chemotherapy, endocrine suppression therapy, and biological anti-HER2 treatment). Discordance in the PR 203 and/or ER, and HER2 status between primary and metastatic breast cancer has been described more than 30 204 years ago, [22] but only recently this important feature of the disease is coming into focus. [23] With discordant 205 rates ranging from 18% to 56% for PR and ER status and 6% to 48% for HER2 status, obtaining a biopsy from 206 metastatic lesions rather than simply relying on the characteristics of the primary breast tumour seems of 207 substantial clinical importance. [24] When the status of primary and metastatic tissues are discordant, the 208 consensus of the American Society of Clinical Oncology (ASCO)/ College of American Pathologists (CAP) clinical practice guideline is to use the PR, ER and HER2 status of the metastasis to direct therapy if supportedby the clinical scenario of the individual patient. [25]

211 PR and ER are both critical steroid hormone receptors employed to predict response and hence the suitability 212 of endocrine suppression therapy (including tamoxifen, aromatase inhibitors such as Anastrazole, Letrozole, and 213 Exemestane), as well as provide prognostic information. About 70-80% of both primary breast cancers and 214 metastatic breast cancers to the bones are hormone receptor-positive. Hormone receptor-positive breast cancers 215 tend to have the highest probability of developing bone metastases. [26] HER2 is a receptor tyrosine kinase on 216 the plasma membrane of breast cells, it is overexpressed in 25-30% of breast cancers and it is usually associated 217 with the amplification of the Erb-B2 gene. HER2 overexpression plays an important pathogenic role in breast 218 cancer, as it provides the cancerous cells with potent proliferative and anti-apoptosis signals, being the main 219 driver of tumour development and progression in this subset of breast cancer. [27]

220 Breast cancer is a heterogeneous disease that encompasses multiple biologically distinct entities with specific 221 biological behaviours and pathological features. It was previously shown that the receptor status of primary BC 222 tissue may influence the development of SMs and their radiological features (sclerotic or lytic) [8]. The influence 223 of receptor status on patient survival after pathological fracture and the outcome of surgical treatment is also well 224 documented. Triple-negative tumours (PR/ER/HER2) are the most aggressive, whilst PR-positive primary 225 breast cancer shows more aggressive features in comparison to ER-positive lesions. [28, 29] Since PR and ER 226 regulate each other's expression, various combinations of these receptors and HER2 receptor differ in their 227 prognosis, with single PR+ (ER-) and HER2- patients having poorer prognosis than PR+ and ER+, also than 228 single ER+ (PR-) in same HER2- subtype. [30]

229 Although various national and international guidelines exist as to the management of early and metastatic 230 breast cancer, to the best of our knowledge, none of these guidelines provides any guidance as to how the 231 receptor status of the SMs of these patients may help in the selection process by the orthopaedic surgeon of the 232 patients who are at high risk of pathological fractures and therefore require bone fixation. Our results showed 233 that SMs of breast cancer with a pathological fracture had a significantly higher rate of PR positivity and PR 234 scores as compared to those with no fracture. Moreover, our findings show that the association between the PR 235 score and fracture occurrence depends not on the staining intensity but on the percentage of stained cells. These 236 findings raise the possibility that the progesterone status of metastatic bone lesions may help identify SM at high 237 risk for fracturing. Such an approach may supplement existing predictive clinical and radiological scores such as 238 the Mirels' and SINS scores. [4, 5]

Limitations of this study include its retrospective design and the lack of a complete cohort of breast cancer cases. However, despite its limitations, this study assessed a large number of patients to allow meaningful conclusions and guide clinical decision-making. Since tumour tissue in SM can differ from the primary tumour, the significance of this study is that it shows the potential value of routine biopsy of SMs and establishing their receptor status. Long bone SMs found to be PR+, especially SMs in the femur, may benefit from prophylactic stabilization to minimize the risk of fracture. PR positivity may also guide hormone therapy for such bone lesions either as the principal or post-surgery treatment. In SMs of BC, PR positivity may confer an increased risk for fracture, especially in the femur. This may be
taken into consideration with regard to the prophylactic surgical treatment of SM or guide hormone therapy of
such lesion.

249

250 5. ETHICS STATEMENT

The study was approved by the Ethical Committee of the Medical Faculty, University of Belgrade, number 1322V-3, and the research was carried out in compliance with the 1964. Declaration of Helsinki. It was conducted retrospectively as data analysis of an existing data bank without any additional experiment on human or animal tissue. Informed Consent to Participate and Consent to Publish were obtained from all participants or if participants are under 18, from a parent and/or legal guardian.

256

257 6. REFERENCES

- Sancho-Garnier H, Colonna M. Breast cancer epidemiology. Presse Med. 2019 Oct;48(10):1076-1084. doi:
 10.1016/j.lpm.2019.09.022.
- Zhang H, Zhu W, Biskup E, Yang W, Yang Z, Wang H, et al. Incidence, risk factors and prognostic
 characteristics of bone metastases and skeletal-related events (SREs) in breast cancer patients: A systematic
 review of the real-world data. Journal of bone oncology. 2018; 11: 38–50; doi: 10.1016/j.jbo.2018.01.004
- 3. Yang M, Liu C, Yu X. Skeletal-related adverse events during bone metastasis of breast cancer: current
 status. Discov Med. 2019 May; 27(149): 211-220
- 4. Mirels H. Metastatic disease in long bones. A proposed scoring system for diagnosing impending
 pathologic fractures. Clin Orthop Relat Res. 1989;(249):256-264.
- Fisher CG, DiPaola CP, Ryken, TC, Bilsky M, Shaffrey CI, Berven SH, et al. A Novel Classification
 System for Spinal Instability in Neoplastic Disease: An Evidence-Based Approach and Expert Consensus
 From the Spine Oncology Study Group. Spine 2010, 35, E1221–E1229; doi:
- 270 10.1097/BRS.0b013e3181e16ae2.
- 6. Ferguson NL, Bell J, Heidel R, Lee S, Vanmeter S, Duncan L, et al. Prognostic value of breast cancer
 subtypes, Ki-67 proliferation index, age, and pathologic tumor characteristics on breast cancer survival in
 Caucasian women. The breast journal. 2013; 19(1): 22–30; doi: 10.1111/tbj.12059
- Yang H, Wang R, Zeng F, Zhao J, Peng S, Ma Y, et al. Impact of molecular subtypes on metastatic
 behavior and overall survival in patients with metastatic breast cancer: A single-center study combined with
 a large cohort study based on the Surveillance, Epidemiology and End Results database. Oncology letters.
- **277** 2020; 20(4): 87; doi: 10.3892/ol.2020.11948
- Wu Q, Li J, Zhu S, Wu J, Chen C, Liu Q, et al. Breast cancer subtypes predict the preferential site of distant
 metastases: a SEER based study. Oncotarget. 2017; 8(17): 27990–27996; doi: 10.18632/oncotarget.15856
- 280 9. Johnson RW, Suva LJ. Hallmarks of Bone Metastasis. Calcified tissue international. 2018; 102(2): 141–
- 281 151; doi: 10.1007/s00223-017-0362-4

- 282 10. Vecchi M, Confalonieri S, Nuciforo P, Viganò MA, Capra M, Bianchi M, et al. Breast cancer metastases
 283 are molecularly distinct from their primary tumors. Oncogene. 2008; 27(15): 2148–2158; doi:
 284 10.1038/sj.onc.1210858
- 11. Broom RJ, Tang PA, Simmons C, Bordeleau L, Mulligan AM, O'Malley FP, et al. Changes in estrogen
 receptor, progesterone receptor, and Her-2/neu status with time: discordance rates between primary and
 metastatic breast cancer. Anticancer research. 2009; 29(5): 1557–1562.
- 288 12. Grinda T, Joyon N, Lusque A, Lefèvre S, Arnould L, Penault-Llorca F, et al. Phenotypic discordance
 289 between primary and metastatic breast cancer in the large-scale real-life multicenter French ESME cohort.
 290 NPJ breast cancer. 2021; 7(1): 41; doi: 10.1038/s41523-021-00252-6
- 13. Kao JY, Tsai JH, Wu TY, Wang CK, Kuo YL. Receptor discordance and phenotype change in metastatic
 breast cancer Asian J Surg. 2021 Jan;44(1):192-198. doi: 10.1016/j.asjsur.2020.05.032.
- 14. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of
 Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical
 testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol. 2010; 28: 2784- 2795.
- Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH et al. American Society of
 Clinical Oncology; College of American Pathologists. Recommendations for human epidermal growth
 factor receptor two testing in breast cancer: American Society of Clinical Oncology/College of American
 Pathologists clinical practice guideline update. J Clin Oncol. 2013; 31: 3997- 4013.
- Hashmi AA, Hashmi KA, Irfan M, Khan SM, Edhi MM, Ali JP, et al. Ki67 index in intrinsic breast cancer
 subtypes and its association with prognostic parameters. BMC research notes. 2019; 12(1): 605; doi:
 10.1186/s13104-019-4653-x
- 303 17. Fact Sheet: Breast Cancer [Internet]. World Health Organisation; 2021 March 26. Available from:
 304 https://www.who.int/news-room/fact-sheets/detail/breast-cancer
- 305 18. Coleman RE, Croucher PI, Padhani AR, Clezardin P, Chow E, Fallon M, et al. Bone metastases. Nat Rev
 306 Dis Primers. 2020; 6(1):83. doi: 10.1038/s41572-020-00216-3
- 307 19. D'Oronzo S, Coleman R, Brown J, Silvestris F. Metastatic bone disease: Pathogenesis and therapeutic
 308 options: Up-date on bone metastasis management. J Bone Oncol. 2019; 15:004–4. doi:
 309 10.1016/j.jbo.2018.10.004
- 310 20. Jiang X, Chen G, Sun L, Liu C, Zhang Y, Liu M, et al. Characteristic and survival in bone metastatic breast
 311 cancer patients with different hormone receptor status: A population-based cohort study. Front Oncol.
 312 2022; 12:977226.
- 21. Parkes A, Clifton K, Al-Awadhi A, Oke O, Warneke CL, Litton JK, et al. Characterization of bone only
 metastasis patients with respect to tumor subtypes. NPJ Breast Cancer. 2018; 4:2. doi: 10.1038/s41523018-0054-x
- 316 22. Holdaway IM, Bowditch JV. Variation in receptor status between primary and metastatic breast cancer.
 317 Cancer. 1983; 52:479-485.
- 23. Lindström LS, Karlsson E, Wilking UM, Johansson U, Hartman J, Lidbrink EK, et al. Clinically used
 breast cancer markers such as estrogen receptor, progesterone receptor, and human epidermal growth factor

- 320 receptor 2 are unstable throughout tumor progression. J Clin Oncol. 2012; 30:2601-2608. doi:
- **321** 10.1200/JCO.2011.37.2482
- 22. Lindstrom L, Howell S, Astrom G. Controversies in the management of metastatic breast cancer: biologic
 23. evaluation of breast cancer-should metastases be biopsied? In: American Society of Clinical Oncology
- **324** 2010 Educational Book. American Society of Clinical Oncology; 2010:e7-e12.
- 325 25. Van Poznak C, Somerfield MR, Bast RC, Cristofanilli M, Goetz MP, Gonzalez-Angulo AM, et al. Use of
- biomarkers to guide decisions on systemic therapy for women with metastatic breast cancer: American
 Society of Clinical Oncology Clinical Practice Guideline. J Clin Oncol. 2015; 33:2695-2704. doi:
 10.1200/JCO.2015.61.1459.
- 329 26. Hofbauer L, Rachner T, Coleman R, Jakob F. Endocrine aspects of bone metastases. Lancet Diabetes
 330 Endocrinol. 2014; 2(6):500–12. doi: 10.1016/s2213-8587(13)70203-1
- 331 27. Moasser M. The oncogene HER2: its signaling and transforming functions and its role in human cancer
 332 pathogenesis. Oncogene. 2007 Oct 4; 26(45):6469-87. doi: 10.1038/sj.onc.1210477.
- Rakha EA, El-Sayed ME, Green AR, Paish EC, Powe DG, Gee J, et al. Biologic and clinical characteristics
 of breast cancer with single hormone receptor-positive phenotype. Journal of clinical oncology: official
- journal of the American Society of Clinical Oncology. 2007; 25(30): 4772–4778. doi:
 10.1200/JCO.2007.12.2747
- 29. Zhao H, Gong Y. The Prognosis of Single Hormone Receptor-Positive Breast Cancer Stratified by HER2
 Status. Frontiers in oncology. 2021; 11:643956. doi: 10.3389/fonc.2021.643956
- 30. Li Z, Wei H, Li S, Wu P, Mao X. The Role of Progesterone Receptors in Breast Cancer. Drug Des Devel
 Ther. 2022; 16: 305–314. doi: 10.2147/DDDT.S336643.s.

341

342 7. ADDITIONAL INFORMATION

343 7.1 Authors' contributions

344 SR was responsible for designing and writing the protocol, conducting the search, collecting surgical data, 345 reviewing other studies in this field, extracting and analyzing data, interpreting results, and writing the report. 346 MC contributed to analyzing and interpreting the results. CC analyzed and interpreted the results and helped 347 write the report. LS was responsible for pathologic diagnostics, collecting pathological data, analyzing and 348 interpreting results, and writing report. GD took part in collecting and analyzing radiological data and analyzing 349 and interpreting results. DD contributed to collecting pathological data and took part in analyzing and 350 interpreting results. LM helped collect surgical data and review studies at writing the report. BM conducted the 351 statistical analysis and helped analyze and interpret the results. JS took part in designing and writing the 352 protocol, supervising pathological data, reviewing previous studies, and analyzing and interpreting results. The 353 work reported in the paper has been performed by the authors, unless clearly specified in the text.

7.2 Data availability

355 The data that support the findings of this study are available from the corresponding author upon reasonable

356 request.

357 7.3 Competing interests

358 The authors declare that they have no conflict and competing interests.

359

360 8. FIGURE LEGEND

Figure 1. (A) Typical morphology of metastatic breast cancer into the bone (H&E, 4x), (B) Bone trabeculae
surrounded by tumor tissue (H&E, 20x) (C-H) Percentage of PR positive tumor cells were divided into 6
categories: (C) 5/ indicating 67%-100%; (D) 4/ 34%-66%; (E) 3/ 11%-33%; (F) 2/ 1%-10%, (G) 1/ <1%, (H) 0/
negative) (PR, 20x).