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Prognosis, stage and oestrogen receptor status of contralateral breast cancer in relation to characteristics of the first tumour, prior endocrine treatment and radiotherapy

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Abstract ***Aim:*** A contralateral breast cancer (CBC) is today treated as an independent primary tumour, although recent data suggest risk and prognosis of CBC to be influenced by characteristics of and treatment given for the first tumour (BC1). We hereby investigate phenotypical and prognostic features of the second tumour (BC2) in relation to prior endocrine treatment and radiotherapy.

Methods: From a well-defined population-based cohort of CBC-patients, we have constructed a unique tissue-microarray including 600 pairs of primary tumours and CBCs. Breast cancer mortality was primary end-point for prognosis.

Results: Both oestrogen receptor (ER) status and stage was strongly correlated between BC1 and BC2 within CBC-pairs. Although BC2 had the highest prognostic impact, BC1 continued to influence prognosis after diagnosis of CBC. Patients diagnosed with two high stage tumours within a short time-interval had a particularly bad prognosis. Prior endocrine therapy and radiotherapy both correlated to ER-negativity of BC2. An ER-negative BC2 was associated with an inferior prognosis compared to an ER-positive BC2 regardless of ER-status of BC1 or prior endocrine therapy.

Conclusions: Our results suggest that both the residual prognostic impact of BC1, the possibility of contralateral metastasis, as well as prior treatment given, need to be considered when determining appropriate diagnostic work-up and treatment of CBC. In addition, radiation to

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the contralateral breast and risk of inducing CBC with an aggressive ER-negative phenotype should be considered when establishing new radiation treatment techniques. This study indicates loss of ER-expression as an important 'endocrine treatment escape mechanism', although further studies are warranted.

1. Introduction

Prior breast cancer patients have a life-time risk of 2–20% of developing a contralateral breast cancer (CBC) [1–3]. A CBC is today treated as a new independent primary tumour, although recent data suggest that the second tumour (BC2) may in some cases be a metastasis of the first (BC1) [4,5]. In addition, CBC diagnosed in close connection to prior adjuvant treatment is presumably resistant to the treatment given. Indeed, prior endocrine therapy, chemotherapy and radiotherapy have all been associated with a worse prognosis once diagnosed with CBC [4,6,7]. CBC may hence be used as an *in vivo* model for studies of adjuvant treatment resistance. In addition, with new radiotherapy techniques becoming clinically available, importance of scattered dose to the contralateral breast and risk of radiation induced CBC need to be further evaluated.

We have hereby studied TNM-stage, oestrogen (ER) and progesterone receptor (PR) status of BC2 in relation to characteristics of BC1 and prior treatment, using a unique tissue-microarray (TMA) including >700 CBC-patients. This is to our knowledge the largest cohort of CBC-patients with access to detailed patient, tumour and treatment information as well as tumour tissue ever studied. We hereby wish to clarify the biological relationship between CBC-pairs, and find indications as to when contralateral metastasis should be suspected and clonal relationship further investigated. We also want to investigate phenotypical and prognostic features of BC2 in relation to prior treatment. This could not only give us important information on how to optimise treatment for patients with CBC, but also increase our knowledge on treatment escape mechanisms *in vivo*.

2. Patients and methods

2.1. Tissue microarray and immunohistochemistry

Inclusion criteria and data abstraction have been described before [4]. Briefly all patients within the Southern Swedish Healthcare Region with two breast cancers reported in the Swedish Cancer Registry, and BC2 diagnosed between 1977 and 2007 were included. Clinical data were abstracted from individual charts and paraffin-embedded tissue collected. We focused on patients with metachronous CBC ($\geqslant$3 months between tumours), excluding patients with synchronous CBC, patients with distant metastasis or another malignancy diagnosed before BC2, and patients with BC2 found only in the axilla. For the remaining 764 patients, paraffin blocks were available for 643 BC1 and 685 BC2, giving a total of 728 patients included in the TMA (Fig. 1). After exclusion according to predefined criteria 688 patients were considered in the main statistical analysis. From representative areas of the invasive breast cancers, tissue-core-biopsies (diameter 1.0 mm) were punched out and mounted into the recipient block using a tissue-array-machine (Beecher Instruments, USA).

ER and PR were reevaluated by a pathologist (AE), using immunohistochemistry (Ventana Benchmark system, 790-4324 clone SP1 and 790-2223 clone 1E2) [8]. In line with Swedish clinical standard during this period, tumours with $\geqslant$10% stained nuclei were considered positive. The project was approved by the Regional Ethical Review Board of Lund University (LU240-01) and carried out in accordance with the code of ethics of the World Medical Association. All data were handled confidentially according to Sweden's Personal-Data-Act.

2.2. Statistical analysis

Survival-data and cause of death were retrieved from the Swedish National Board of Health and Welfare (March 2014), and breast cancer mortality (BCM) chosen as primary end-point. BCM includes breast cancer death or death after metastasis as a primary event. Event-free survival was measured from diagnosis of CBC.

For statistical calculations, the software package Stata 11.2 (StataCorp. 2009. TX, USA) was used. General comparisons between groups of BC1 and BC2 were done with McNemar's test, Wilcoxon matched-pairs signed-ranks test or McNemar-Bowker's test of symmetry (Table 1). Associations between tumour-pairs or treatment groups were evaluated with χ^2-test or χ^2-test for trend (Table 2). Prognosis after BC2 was summarised graphically as cumulative BCM and cause-specific Cox-regression, treating competing events (death not related to breast cancer) as censoring, was used to estimate hazard ratios (HR). Plots were curtailed when $\leqslant$5 individuals remained at risk. When relating stage of BC1 and BC2 to prognosis after BC2 (Table 3), a full factorial Cox-model with 8 parameters was fitted.

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