European Journal of Cancer (2014) **50**, 2916–2924

Available at www.sciencedirect.com

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journal homepage: www.ejcancer.com

Original Research

Breast cancer during follow-up and progression – A population based cohort on new cancers and changed biology

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Received 31 March 2014; received in revised form 3 August 2014; accepted 18 August 2014
Available online 17 September 2014

KEYWORDS
Breast cancer
Oestrogen receptor
Progesterone receptor
Human epidermal growth factor receptor 2
Biomarker

Abstract ***Background:*** Emerging data indicate an important role for biopsies of clinically/radiologically defined breast cancer 'recurrences'. The present study investigates tumour related events (relapses, other malignancies, benign conditions) after a primary breast cancer diagnosis.
Patients and methods: The cohort includes 2102 women, representing all patients, with primary invasive breast cancer during 2000–2011 in the county of Värmland, Sweden. A comparative analysis of oestrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and proliferation (Ki67) between the primary tumour and the relapse was performed and related to outcome.
Results: With a mean follow-up time of 4.8 years, 1060 out of 2102 patients have had a biopsy taken after the initial breast cancer diagnosis demonstrating 177 recurrences, 93 other malignancies (colorectal, lung, skin), 40 cancer *in situ* (skin, breast) and 857 benign lesions. Approximately 70% (177 out of 245) of all cases of relapsed breast cancer underwent a biopsy during this time period. For patients with recurrences, ER ($n = 127$), PR ($n = 101$), HER2 ($n = 73$) and Ki67 ($n = 55$) status in both primary tumour and the corresponding relapse were determined. The discordance of receptor status was 14.2%, 39.6%, 9.6% and 36.3%, respectively. Loss of ER or PR in the relapse resulted in a significant increased risk of death (hazard ratio (HR) 3.62; 95% confidence interval (CI), 1.65–7.94) and (HR 2.34; 95% CI, 1.01–5.47) compared with patients with stable ER or PR positive tumours. The proportion of patients losing ER was bigger in the group treated with endocrine therapy alone or in combination with

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http://dx.doi.org/10.1016/j.ejca.2014.08.014

chemotherapy, 16.7% and 13.3%, respectively, compared with the group treated with chemotherapy alone or that which received no treatment 4.3% and 7.7%, respectively.
Conclusion: Discordance of biomarkers between the primary tumour and the corresponding relapse was seen in 10–40% of the patients, adjuvant therapies seem to drive clonal selections. Patients with tumours losing ER or PR during progression have worse survival compared with patients with retained receptor expression.

1. Introduction

The standard prognostic and therapy predictive factors oestrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and proliferation (Ki67) status guide clinicians to optimise and individualise the management of breast cancer patients. In many institutions, the treatment following on from the diagnosis of the primary cancer and possible metastasis and/or local relapses is based on this primary tumour marker assessment. ER and HER2 are of particular interest since they are both important predictors of the likeliness of response to endocrine therapy and efficacy of HER2 directed therapies, respectively. However emerging data indicate discordances in biomarkers between the primary tumour and the relapse [1–7], and indeed, a few studies (including data from our group) have reported a prognostic value of such a change in receptor status [1,3,8,9]. Most studies in this field have been retrospective however three prospective studies have reported considerable discordances of ER (10–16%), PR (24–40%) and HER2 (3–10%) between the primary tumour and corresponding relapse [2,4,10]. Today, in spite of these findings, morphological evaluations of suspicious relapses are not always carried out as part of clinical routine. In addition, such biopsies could harbour important clinical information such as new malignancies which could change the management of the patient/treatment offered to the patient [5,6].

In this study, we wanted to investigate the characteristics of tumour related events (e.g. relapses, other malignancies, benign conditions) after primary breast cancer in a population based primary breast cancer cohort. Furthermore, for patients with a confirmed breast cancer relapse we aimed to perform a comparative analysis of ER, PR, HER2 and Ki67 status between the primary tumour and corresponding relapse. Any discordance in hormonal receptor status was to be correlated to survival and the role of adjuvant therapy on such discordances was to be evaluated.

2. Patients and methods

2.1. Study population

The study was approved by the Central ethics committee at the Swedish Research Council in Stockholm (Dnr. Ö 15-2012). The cohort includes 2102 consecutive women diagnosed with primary invasive breast cancer between 1st January 2000, and 31st December 2011 in the county of Värmland, Sweden. These patients were retrospectively identified from the population-based Breast Cancer Registry for Uppsala-Örebro region, established in 1992, (by using the unique 12-digit id-number given to all individuals living in Sweden). Of these 2102 patients, 1060 had been subjected to an unspecified biopsy after the initial breast cancer diagnosis. It is worth clarifying that these biopsies were not necessarily related to their previous cancer. In addition, data from the cervical cancer screening tests are excluded in this study.

2.2. Tissue sample and biomarker measurement

ER and PR status were assessed by immunohistochemistry (IHC) or immunocytochemistry (ICC) (Autostainer Link 48, DAKO) with commercially available antibodies, 6F11 (Novocastra) and SP1 (DAKO) for ER and polyclonal PGR (Novocastra) and PGR636 (DAKO) used for PR analysis respectively. ER and PR, tumours with a threshold $\geqslant 10\%$ were classified as receptor positive Proliferative activity was assessed by same IHC/ICC-methods with Ki67 (clone MIB1, DAKO). The cut-off level for high Ki67 was set at >10%. Tumours with a threshold >10% were classified as Ki67 positive. Positive controls were included in all staining runs. Fixation and staining procedure were performed according to manufacturer's instructions. Fine needle aspirates were air-dried and fixed in buffered formalin before immunocytochemistry. Autostainer Link 48 was used for IHC/ICC.

HER2 status was assessed by using IHC/ICC analysis with antibody c-erb2 (polyclonal, DAKO) initially in a validated protocol according to the manufacturer's instructions and later with Hercept-kit Sk001 containing the same antibody. Internal controls of three standardised cell lines; MDA-231, MDA-175 and SK-BR-3 from the Hercept-kit were run with each staining. Staining of the membrane was set at four levels, according to the manufacturer's instructions, 0, 1+, 2+ and 3+ (in accordance with both international and national standards). IHC/ICC was classified as positive at the 3+ protein level. Confirmatory molecular genetic analysis/*in situ* hybridisation (FISH) was carried out for all probes