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Original Research

Breast cancer in young women and prognosis: How important are proliferation markers?

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Abstract ***Aim:*** Compared to middle-aged women, young women with breast cancer have a higher risk of systemic disease. We studied expression of proliferation markers in relation to age and subtype and their association with long-term prognosis.
Methods: Distant disease-free survival (DDFS) was studied in 504 women aged <40 years and 383 women aged ≥40 years from a population-based cohort. Information on patient characteristics, treatment and follow-up was collected from medical records. Tissue microarrays were produced for analysis of oestrogen receptor, progesterone receptor (PR), Her2, Ki-67 and cyclins.
Results: Young women with luminal tumours had significantly higher expression of Ki-67 and cyclins. Proliferation markers were prognostic only within this subtype. Ki-67 was a prognostic indicator only in young women with luminal PR+ tumours. The optimal cut-off for Ki-67 varied by age. High expression of cyclin E1 conferred a better DDFS in women aged <40 years with luminal PR− tumours (hazard ratio [HR] 0.47 [0.24–0.92]). Age <40 years

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was an independent risk factor of DDFS exclusively in women with luminal B PR+ tumours (HR 2.35 [1.22–4.50]). Young women with luminal B PR− tumours expressing low cyclin E1 had a six-fold risk of distant disease compared with luminal A (HR 6.21 [2.17–17.6]).
Conclusions: The higher expression of proliferation markers in young women does not have a strong impact on prognosis. Ki-67 is only prognostic in the subgroup of young women with luminal PR+ tumours. The only cyclin adding prognostic value beyond subtype is cyclin E1. Age is an independent prognostic factor only in women with luminal B PR+ tumours.

1. Introduction

Although early breast cancer generally has an excellent prognosis, breast cancer in young women is associated with a high risk of systemic disease at long-term follow-up [1–6]. Young women tend to be diagnosed at a later stage with highly proliferative, high-grade tumours with the presence of lymphovascular invasion (LVI) [2–4,7–11]. The breast cancer subtypes associated with a worse prognosis; luminal B, human epidermal growth factor receptor 2 (Her2)–positive and triple-negative (TN), are more common in young relative to middle-aged women [3,10]. The prognostic importance of age seems to differ between subtypes, being independently significant in oestrogen receptor positive (ER+) breast cancer [12,13] and specifically in the luminal B subtype [1,3,13,14].

Proliferation markers are highly expressed in young women with breast cancer [1,3,5,10,11,13,15] and have proven to be particularly prognostic in luminal tumours [16,17]. In the original description of molecular subtypes by Sorlie and Perou, proliferation genes are highly important [18] and have also been shown to play a central role in the prognostic capacity of commercially available gene-assays such as MammaPrint and Oncotype DX [19]. In clinical treatment decision-making, proliferation markers such as Ki-67 are heavily relied upon, particularly when gene expression analyses are not available or cost prohibitive. Furthermore, the level of Ki-67 is used to separate the luminal A and B subtypes by immunohistochemistry (IHC) [20,21].

For tumour cells to sustain proliferative signalling, a hallmark of cancer, they must first circumvent the tightly regulated cell division cycle. Central to the cell cycle are the cyclin family of proteins that regulate cellular growth and division in both normal and malignant cells. Cyclins display subtype-specific expression in breast cancer [22,23] and are generally expressed in higher levels in young women [24–26], potentially contributing to age-related differences in disease-specific survival. Despite their central role in breast cancer oncogenesis, it is currently unclear whether cyclins retain their prognostic value in young women when taking other strong prognostic indicators such as age and subtype into account.

To get further insight into the biology behind the age-related differences in prognosis in breast cancer, we performed IHC analysis of Ki-67, cyclin A2, B1, D1 and E1 in a population-based cohort of women with stage I–III breast cancer and related the expression to prognosis in different age groups and subtypes. As a secondary aim, we determined whether any of the cyclins add prognostic information to standard biomarkers in young women with breast cancer.

2. Patients and methods

2.1. Study design

From a Swedish population-based registry cohort of 22,017 women diagnosed with primary invasive breast cancer from 1992 to 2005 at age 69 or younger [2], a smaller cohort including all women aged <35 years (n = 471) and random sampled groups of women aged 35–39 years (n = 200), 40–49 years (n = 200) and 50–69 years (n = 300) was constructed. The sample size was decided after power calculations based on effect sizes in the full cohort [2], with the aim to over-sample young women but still with a reasonable possibility to collect detailed clinical data from the medical records as well as tumour tissue for both cases and comparison group. This smaller cohort consisted of 1120 women with full information on patient and tumour characteristics, including treatments given and follow-up until the end of 2012 or until death. Tumour tissues were retrieved from 88% of the women (983/1120) and protein expression centrally evaluated on tissue microarrays (TMAs) [3]. Women with full information on ER, progesterone receptor (PR), Ki-67 and Her2 at central re-analyses were selected for the present study, which thereby consisted of 887 women stage I–III in ages <35 (n = 352), 35–39 (n = 152), 40–49 (n = 155) and 50–69 years (n = 228) (Supplementary Fig. 1). Analyses were performed stratified by age <40 and ≥40. The study conforms to the STROBE and REMARK guidelines [27,28].

2.2. Tumour material

Archival haematoxylin and eosin–stained sections and corresponding formalin-fixed and paraffin-embedded