

Very young women (<35 years) with operable breast cancer: features of disease at presentation

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Background: Breast cancer rarely occurs in young women. Our knowledge about disease presentation, prognosis and treatment effects are largely dependent upon older series.

Materials and methods: We evaluated biological features and stage at presentation for 1427 consecutive premenopausal patients aged ≤ 50 years with first diagnosis of invasive breast cancer referred to surgery at the European Institute of Oncology from April 1997 to August 2000. A total of 185 patients (13%) were aged <35 years ('very young') and 1242 (87%) were aged 35–50 years ('less young'). The expression of estrogen receptors (ER), progesterone receptors (PgR), presence of vascular invasion (VI), grading (G), expression of Ki-67, HER2/neu overexpression, pathological stage according to TNM staging system (pTNM), pathological tumor size and number of axillary lymph node involvement were evaluated.

Results: Compared with less young patients, the very young patient group had a higher percentage of tumors classified as ER negative ($P < 0.001$), PgR negative ($P = 0.001$), higher expression of Ki-67 $\geq 20\%$ of cells stained; 62.2% versus 53%, ($P < 0.001$), vascular or lymphatic invasion (48.6% versus 37.3%, $P = 0.006$), and pathological grade 3 ($P < 0.0001$). There was no difference between the two groups for pT, pathological tumor size (pN) and number of positive lymph nodes.

Conclusions: We conclude that compared with less young premenopausal patients, very young women have a greater chance of having an endocrine-unresponsive tumor, and are more likely to present with a higher grade, more extensively proliferating and vessel invading disease. Pathological tumor size, nodal status and number of positive axillary lymph-nodes have a similar distribution among the younger and the older cohorts, thus not supporting previous data indicating more advanced disease in younger patients at diagnosis of operable disease.

Key words: breast cancer, prognostic features, very young women

Introduction

About 2% of the patients with breast cancer are <35 years old at diagnosis [1]. Breast cancer at a young age has been reported to have a more aggressive biological behavior and to be associated with a more unfavorable prognosis compared with the disease in older patients. Specifically, in previously published reports, tumors in younger women were less well differentiated (higher grade), had a higher proliferating fraction and had more vascular invasion than those occurring in older patients [2–5]. Two population-based studies yielded a

relationship between age at diagnosis and risk of death, with the youngest and the oldest having a higher risk than patients of intermediate age [3, 4]. Moreover, a review of the National Cancer DataBase revealed that patients younger than 35 years had more advanced disease at diagnosis and a poorer 5-year survival than older premenopausal patients [5]. Similar findings have been reported in the past from the US National Cancer Institute SEER database [1], the Finnish Cancer Registry [6] and other sources [7–9]. More positive axillary lymph nodes and higher incidence of local recurrences were detected in younger compared with older patients [10, 11]. However, data on treatment effects are largely dependent upon older series collected over several years, and extrapolation of data from older age cohorts. Staging procedures, attention given to small metastases in axillary lymph nodes, assessment of over-

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expression of HER2/neu, immunohistochemical determination of estrogen and progesterone receptors (and not ligand-binding assay), are features that underwent a more or less substantial change in recent years. Thus, the aim of this study was to investigate the most recently available details of biological characteristics and stage at disease presentation in a large group of very young patients (<35 years of age) with operable breast cancer.

Patients and methods

We collected information on all consecutive breast cancer patients operated at the European Institute of Oncology between April 1997 and August 2000. Data on the patient's medical history, concurrent diseases, surgery, pathological evaluation and results of staging procedures (blood chemistry, hematological values, bone scan, chest film and upper abdominal ultrasound examination) were required. Pathological assessment included evaluation of the primary tumor size, histological type and of lymph nodes status, including a sentinel node biopsy [12], when applicable. Tumor grade was evaluated according to Elston et al. [13] and peritumoral vascular invasion (PVI) was assessed according to Rosen et al. [14]. Estrogen (ER) and progesterone receptor (PgR) status, Ki-67 labeling index, determined with the MIB1 monoclonal antibody, and HER2/neu overexpression were evaluated immunocytochemically as reported previously [15]. In particular, HER2/neu overexpression was evaluated using a 1/100 dilution of a polyclonal antiserum (Dako, Glostrup, Denmark) and considering only complete membrane staining of at least 10% neoplastic cells as evidence of overexpression. For evaluation of ER and PgR status and Ki-67 labeling index, the percentage of cells exhibiting definite nuclear staining over at least 2000 neoplastic cells examined at 400× magnification was recorded. The stained slides were evaluated independently by two of the authors. Only nuclear immunoreactivity was evaluated for ER, PgR and MIB1. The threshold for HER/neu overexpression, and ER and PgR positivity was 10%, and 20% for MIB1 positivity, as previously published [15]. Data were entered by surgeons into a 'user-friendly' database designed with Microsoft Access® once a week on a mean of 25 patients per week, and checked by a data manager. The database was then used for an interdisciplinary discussion (among surgeons, medical and radiation oncologists, and pathologists) resulting in a proposal of an adjuvant treatment program. Typically, a medical oncologist (and a radiation oncologist, if applicable) discussed the proposed treatment with the patient and verified the accuracy of the items entered into the database (internal quality control).

Statistical analysis

The aim of this analysis was to compare tumor characteristics and biological markers in two groups of patients: the 'very young', aged <35 years, and the 'less young', aged between 35 and 50 years. Several items among the main prognostic features were incomplete in some of the patients. Some of these were subsequently retrieved. Grading was not provided by pathologists in the group of patients submitted to preoperative chemotherapy due to the interference of treatment with the architectural and cytological features of the tumors not allowing a reproducible classification. Moreover, staining for HER2/neu overexpression was carried out on a routine basis only starting from September 1999.

ER, PgR and Ki-67 were categorized into their clinically accepted groups. There are no clinically relevant categories for HER2/neu, which was split into two groups at the 10% level. The associations between the

categorical variables and age group were measured in contingency tables using two sided χ^2 tests of association. Furthermore, we used the Mann-Whitney *U*-test to investigate whether the median values of the quantitative biological variables were associated with age. A first analysis included all the eligible patients. A second analysis excluded patients treated with preoperative chemotherapy (211 patients).

An analysis was also conducted by dividing the population in to four age groups (<35, 35–40, 41–45 and 46–50 years). In this case, the linear by linear interaction test was used to assess the association between the four age groups and then they were ordered in categories based on tumor size, and the presence or absence of lymph node metastases and their number. This test was also used to evaluate the association among the tumor biological characteristics. The Kruskal-Wallis test was used for the quantitative biological markers. We performed a binary logistic and multinomial regression analysis to estimate the interdependence between the tumor characteristics, biological features (both as categorical and continuous variables) and age.

Results

A total of 3890 patients with breast cancer were referred to the interdisciplinary evaluation and their data were included in the database. We selected 1837 premenopausal patients aged ≤50 years (47.2%). We subsequently excluded patients that presented with recurrent tumors (133), non-invasive breast cancers (93), bilateral tumors (12) and males (6). A total of 1427 patients were thus included in the analysis. One hundred and eighty-five were 'very young' and 1242 were classified 'less young'. The number of patients evaluable for each biological feature is given in Table 1.

In the 'very young' group, when compared with the 'less young' patients, there were higher percentages of tumors classified as ER negative (38.8% versus 21.6%, $P < 0.001$), PgR negative (49.1% versus 35.3%, $P = 0.001$), with a Ki-67 labeling index $\geq 20\%$ of the cells (71.4% versus 56%, $P < 0.001$), with PVI (48.6% versus 37.3%, $P = 0.006$), and classified as being of grade 3 (61.9% versus 37.4%, $P < 0.001$) (Table 1). Results by grade might be less reliable owing to a high percentage of missing data (27.6% versus 20.6%). No difference in the overexpression of HER2/neu was observed between the two age groups.

Similar results were observed when analyzing the groups without the inclusion of patients who had pre-operative chemotherapy (1211 patients: 'very young' 145, 'less young' 1066). Compared with the 'less young' group there was a higher percentage of patients with tumor characteristics thought to be associated with dire prognosis in the 'very young' group: ER negative (35.9% versus 20.3%, $P < 0.001$), PgR negative (45% versus 32.4%, $P = 0.004$), Ki-67 ≥ 20 (73.8% versus 57.2%, $P < 0.001$), VI present (44.8% versus 35.4%, $P = 0.045$), grade 3 (61.5% versus 37.4%, $P < 0.001$). The overexpression of HER2/neu (43.2% versus 36.8%, $P = 0.412$) was not different in the two groups.

According to the predefined age groups (<35, 35–40, 41–45 and 46–50 years) there was strong evidence of age gradients with regard to what we considered features associated with

Table 1. Biological factors distribution in evaluable patients

Factors	'Very young'	'Less young'	Two-sided <i>P</i> value
No. of patients evaluable for ER	170	1195	
Percentage ER <10%	38.8	21.6	<0.001
ER median	50	70	<0.001
No. of patients evaluable for PgR	169	1195	
Percentage PgR <10%	49.1	35.3	0.001
PgR median	10	30	<0.001
No. of patients evaluable for Ki-67	161	1176	
Percentage Ki-67 ≥20%	71.4	56	<0.001
Ki-67 median	28	22	<0.001
No. of patients evaluable for VI	173	1176	
Percentage VI present	48.6	37.3	0.006
No. of patients evaluable for grade (G)	134	986	
G1	8.2	16.9	<0.001
G2	29.9	45.6	
G3	61.9	37.4	
No. of patients evaluable for HER2/neu	58	425	
HER2/neu ≥10% ^a	39.7	37.4	0.741
HER2/neu median	0	0	0.442

^aPerformed in more recent cases only; refers to complete membrane staining.

dire prognosis: ER negative, PgR negative, Ki-67 ≥20%, presence of VI, high grade (grade 3) and overexpression of HER2/neu (Figure 1). Most strikingly the percentage of patients with ER-positive disease increased with increasing age.

All of the biological features (Ki-67, ER, PgR, HER2/neu, VI and grade) were correlated with each other with the absolute value of Spearman's correlations in the range 0.1–0.3, except for the correlation between grade and Ki-67 (0.67) and

that between ER and PgR (0.65). There was no evidence of any correlation between VI and ER or PgR. There were no strong correlations between the stage and nodal status and the biological features apart from a correlation of 0.37 between nodal involvement and VI.

In the logistic and multinomial regression analysis we investigated the independent association of age with the biological features using the quantitative values. Ki-67, VI, PgR

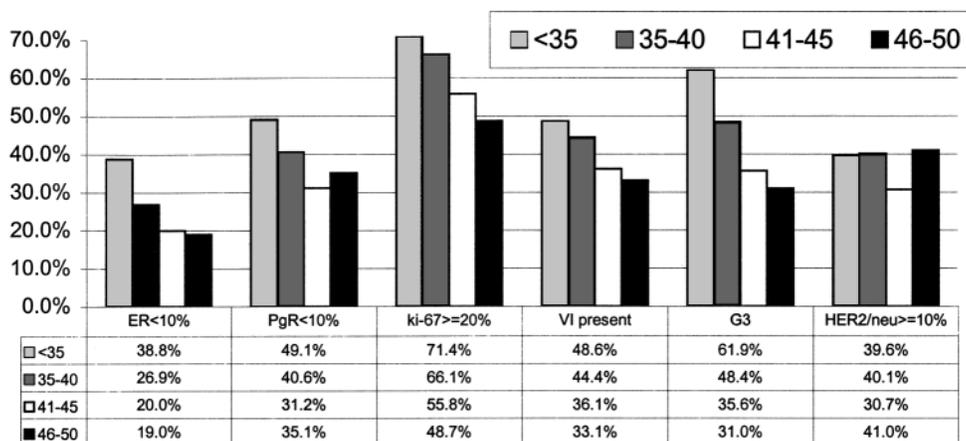


Figure 1. Expression of ERs, PgRs, presence of peritumoral VI, grading (G), expression of Ki-67, overexpression of HER2/neu, as percentage of the respective age cohorts.

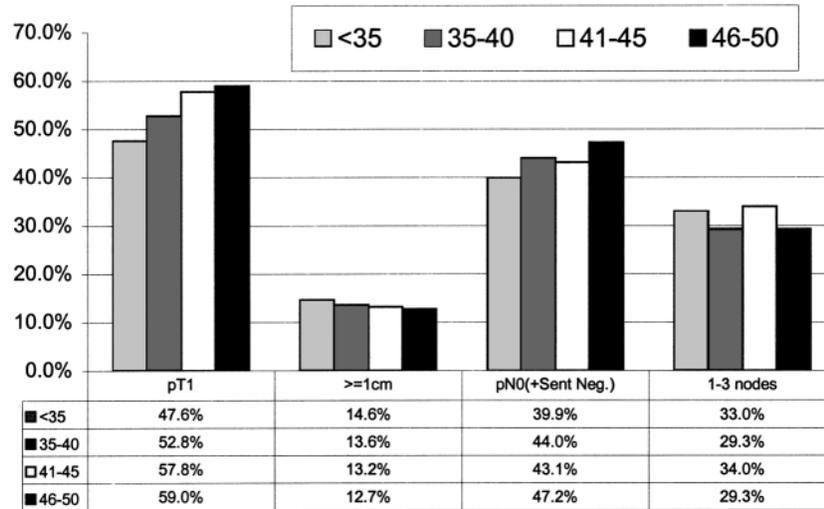


Figure 2. Pathological stage according to TNM (pTNM), tumor size and degree of axillary node involvement as a percentage of the respective age cohorts.

and ER were all associated with age, but in view of the high correlation between ER and PgR, the level of ER did not have an independent effect after considering the association between age and PgR. An ER-positive tumor is associated with patients aged 35–50, but the percentage of ER-positive cells is not. In contrast, a PgR-positive tumor is not associated with age, adjusting for ER positivity, but if over 75% of the tumor cells are PgR positive then this is associated with patients aged 35–50. If we further include the effect of grade, Ki-67 is not independently associated with age in addition to the association between grade and age. Grade, ER positivity, PgR and VI all had independent associations with age. We conclude that the association between Ki-67 and age is not independent of the association between grade and age. In fact, Ki-67 is known to be a surrogate indicator to grading [16].

Table 2 shows the characteristics of stage at presentation for ‘very young’ patients as opposed to the ‘less young’ group. No statistically significant difference was observed for stage of disease at diagnosis [according to the TNM (tumour–node–metastasis)] [17], for pathological tumor size and for the number of nodes involved. In fact, T1 was registered in 48% and 57% of the patients in the two groups, respectively. Tumors <1 cm in diameter were found in 15% and 13%. Similarly, node negative disease was recorded in 30% of the cases in both groups, 32% and 30% had one to three nodes involved and 10% had ≥ 10 nodes in both groups. No trends with age were observed for pathological stage according to TNM (pTNM), tumor size and degree of axillary node involvement when analyzing the data according to the predefined age groups (<35, 35–40, 41–45 and 46–50) (Figure 2).

Discussion

Breast cancer that develops at a young age is commonly considered to be different from that arising in older premeno-

pausal patients. Tumors occurring in very young patients are reported to have a particularly aggressive biological behavior leading to a somewhat unfavorable prognosis, which was described extensively in the pre-adjuvant systemic therapy era [3, 10]. Several reports about age and prognosis led investigators to conclude that features like higher grade [11] represent a relevant aspect for discriminating operable breast cancer between ‘very young’ and ‘less young’ premenopausal patients. Besides the feature of a more aggressive disease presentation, the age of 35 years, which was chosen as threshold between the two age groups, led to the identification of two groups with a different response of the ovaries to the ablative endocrine effect of chemotherapy. Patients below the age of 35 only rarely have amenorrhea after six courses of adjuvant cytotoxics [18].

Data from past series include information on several aspects of the disease collected in an earlier period, when neither systemic treatments nor various prognostic and predictive factors were available in the way they are today [19]. Adjuvant systemic therapies, increased attention to axillary lymph node involvement and determination of the degree of expression of steroid hormone receptors are probably the most relevant variations of features between current and past assessments.

Two important findings are related to responsiveness to endocrine therapy in very young patients with breast cancer. The first relates to the observation in the current series that very young patients had tumors with less immunoreactivity for ER and PgR than older premenopausal patients. Reliable information on large cohorts of patients with data on ER and PgR are rare, and usually receptor determination based on ligand-binding assay (LBA) is reported. This method of determination, directed towards the steroid hormone binding domain, is influenced by endogenous estrogens and progesterone much more so than immunohistochemical staining [20]. The relevance of immunohistochemical evaluation for predic-

Table 2. Staging distribution in evaluable patients

Factors	'Very young'	'Less young'	<i>P</i> value
No. of patients evaluable for pT stage	182	1224	0.071
pT1	53.9%	59.9%	
pT2	41.7%	33.2%	
pT3/4	4.3%	6.8%	
No. of patients evaluable for tumor dimension	157	1114	0.267
≤1 cm	14.6%	13.1%	
1.1–2 cm	32.4%	40.3%	
>2 cm	37.8%	36.2%	
No. of patients evaluable for nodal status	181	1215	0.288
pN0/sent neg.	39.8%	44.9%	
pN1	55.6%	52.2%	
pN2/3	4.5%	2.8%	
No. of patients evaluable for no. of positive nodes	179	1200	0.350
0	39.3%	43.4%	
1–3	31.9%	29.9%	
4–9	16.8%	13.4%	
≥10	9.7%	10%	

sent neg., sentinel node negative.

tion of response to endocrine treatment was recently reported, with the conclusion that immunohistochemical evaluation is superior to the LBA for predicting prognosis of patients who were treated with adjuvant endocrine therapy [21]. Response to endocrine therapy was postulated even when tumors expressed as few as 1% of immunohistochemically stained cells.

The second finding relates to the reduced efficacy of adjuvant chemotherapy for 'very young' patients with endocrine responsive tumors compared with 'less young' premenopausal women. It is well known that chemotherapy exerts some of its effect via an endocrine mechanism in premenopausal women with ER-positive tumors, as recently published [18]. The International Breast Cancer Study Group (IBCSG) assessed the course of the disease in 3700 pre- and perimenopausal patients treated in various trials of timing and duration adjuvant systemic therapy containing cyclophosphamide, methotrexate and fluorouracil (classical CMF) [22]. Three hundred and fourteen of these women were <35 years old at trial entry. Younger patients with ER-positive tumors had a significantly worse prognosis than younger patients with ER-negative tumors [10 year disease-free survival (DFS) 25% versus 47%, hazard ratio (HR) 1.49, *P* = 0.014]. The largest difference in 10-year DFS between younger and older women was observed for those with ER-positive tumors who did not achieve amenorrhea compared with those who had some cessation of menses [23% ± 6 versus 38% ± 3; HR 1.67; 95% con-

fidence interval (CI) 1.19–2.34; *P* = 0.003]. This retrospective analysis on treatment outcome leads to the hypothesis that the endocrine effects of chemotherapy alone were insufficient for patients in the younger age group with endocrine-responsive tumors, for whom suppression of estradiol production might be essential.

The results of our study, based upon an analysis of patients referred to a single center, clearly indicate that 'very young' patients presented more frequently tumors with high grade, PVI and high proliferating fraction than 'less young' premenopausal patients. Despite the fact that high grade is a controversial prognostic marker for invasive breast carcinoma [23–25], it is frequently used in the decision making process for offering adjuvant treatments, and its role within this context was recently specified [26].

A component for integration of histological grade is related to mitosis. Mitotic index was in fact shown to carry autonomously prognostic relevance [27]. Ki-67 staining might provide a more accurate figure than mitotic counts [16, 28, 29]. Also, VI was demonstrated to correlate with prognosis [30–32]. These features are all known to be related to baseline prognosis, but no data are available for very young patients.

The distributions of percentages of tumors with overexpression of HER2/neu in the two cohorts of 'very young' and 'less young' premenopausal women were similar. Others published results, based on small groups of patients, dismiss the association between age and overexpression of c-erbB-2 [33]. The

observation of this feature in invasive breast cancer is considered a relevant factor for dire prognosis [34–37]. However, although preliminary owing to the limited number of evaluable patients, our observation does not support the role of this specific feature as a marker for biological aggressiveness of the disease in very young patients with operable breast cancer.

Women diagnosed with breast cancer at the age of <35 years are likely to have germ-line BRCA1 or BRCA2 mutations in up to 15–30% of cases [38–40]. These mutations are more frequently associated with higher histological grade, lack of ERs and high proliferation rate. It might be hypothesized that germ-line mutations could partially explain the more aggressive breast cancer in young patients. Information is scarce on the efficacy of endocrine therapies (tamoxifen, ovarian suppression or a combination of both) or of the cytotoxics with respect to the presence of BRCA1 and BRCA2 mutations. Focused investigations might allow improvement of treatment indications, which cannot be otherwise extrapolated from trials on an older population.

The Experts' Consensus of the St Gallen Conference 1998 indicated age <35 to be a dire prognostic variable [41], while there has been a clear advancement throughout focusing on tailored treatments according to endocrine responsiveness (St Gallen 2001). The results of the present study indicate that 'very young' patients presented more frequently tumors with poor prognostic features such as high grade, PVI and high proliferating fraction than 'less young' premenopausal patients. Although data on prognosis and treatment outcomes are not available for the current series mainly due to the short follow-up, the observed results indicate that a large group of very young patients presents with endocrine responsive disease and therefore might enjoy the effect of tailored endocrine systemic therapies. On the other hand, endocrine therapies are not easy to offer to very young patients [42–46], and further investigations in this specific field are urgently needed [47].

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