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High Ki67 predicts unfavourable outcomes in early breast cancer patients with a clinically clear axilla who do not receive axillary dissection or axillary radiotherapy

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Abstract ***Aim:*** Axillary dissection is increasingly forgone in early breast cancer patients with a clinically negative axilla. The GRISO 053 randomised trial recruited 435 patients of age over 45 years, tumour ⩽1.4 cm and clinically negative axilla, to assess the importance of axillary radiotherapy versus no axillary radiotherapy in patients not given axillary dissection. In the present study on a subgroup GRISO cases our aim was to assess the prognostic importance of tumour biological factors after more than 10 years of follow-up.

Methods: We retrospectively assessed biological factors in a subgroup of 285 GRISO cases (145 given axillary radiotherapy; 140 not given axillary radiotherapy) with complete biologic, therapeutic and follow-up information, using multivariable Cox proportional hazards regression modelling.

Results: Only 10-year cumulative incidence of distant metastasis was lower in the axillary radiotherapy (1%) than no axillary radiotherapy arm (7%) ($p = 0.037$). Irrespective of study arm, hormone receptor positivity had significantly favourable effects on 10-year disease-free

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survival (DFS) and overall survival. human epidermal growth factor receptor 2 (HER2)-positive and triple-negative subtypes were associated with lower 10-year DFS (60% and 76%, respectively) than luminal A (96%) and B (91%) ($p = 0.001$). Ten-year DFS for high ($\geqslant 14\%$) Ki67 cancers was lower than for low Ki67 cancers ($p = 0.027$); however, this effect was mainly confined to the no axillary radiotherapy arm.

Concluding statement: For patients with clinically node-negative small breast cancer not given axillary dissection, 10-year DFS is worsened by HER2 positivity, triple-negative phenotype and high Ki67. Axillary radiotherapy counteracts the negative prognostic effect of high Ki67 in patients not receiving axillary dissection.

1. Introduction

There is a growing trend, in patients with early breast cancer and a clinically clear axilla, not to clear the axillary nodes if the sentinel node is minimally involved.[1] The findings of a recent trial support this practice, although the authors recommend systemic therapy for patients not undergoing axillary dissection.[2]

We carried out the GRISO 053 multicentric randomised trial[3] in 1995–1998 to address a closely similar issue: We recruited women over 45 years with small cancers (up to 1.4 cm) and no palpable axillary nodes. Patients were not given axillary dissection (AD), but randomised to axillary radiotherapy (RT) versus no axillary treatment. After a median follow-up of 63 months, there were no significant differences between the arms, and rates of distant metastasis and local failure (including axillary failure) were low.

In the present study we investigated a subset of GRISO patients with the aim of assessing the influence of oestrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2), tumour proliferation index (Ki67) and molecular subtype on outcomes after over 10 years of follow-up. Our hypothesis was that these factors might predict long-term outcomes in early breast cancer patients with a clinically negative axilla who did not receive AD, and hence identify subgroups who may benefit from axillary RT or other adjuvant treatment.

2. Patients and methods

2.1. Study population

Four hundred thirty-five patients over 45 years with axilla negative on palpation and pathological tumour size up to 1.4 cm were recruited to GRISO;[3] 214 patients were randomised to breast-conserving surgery and no axillary treatment; 221 were randomised to breast-conserving surgery plus axillary RT. No patient received AD. All patients received breast radiotherapy with X-rays from a 6 MeV linear accelerator producing two opposed non-parallel tangential fields to avoid posterior beam divergence and minimise lung and heart (for left breast) irradiation. The fields were also designed to minimise irradiation to the axilla. The 100% tumour dose (50 Gy in 25 equal fractions) was specified at the isocenter (ICRU point). A 10 Gy boost with 6–15 MeV electrons was given in five equal fractions to all patients, with irradiation field confined to the tumour bed.

In the axillary RT group, the axilla was irradiated by two parallel opposed (antero-posterior and postero-anterior) X-rays from a 6 MeV linear accelerator. The dose was 50 Gy in 25 equal fractions. The isocenter was at the mid-plane of the axilla or slightly anterior. The shoulder joint was shielded.

In the present study a subset of 285 cases (66% of GRISO patients) was evaluated (153 from the European Institute of Oncology, Milan; 88 from the Pascale Cancer Institute of Naples, and 44 from Careggi University Hospital, Florence). These were the only cases for which complete biologic, therapeutic and follow-up (to December 2009) information was available. Two pathologists (MGM and GV) reviewed the specimens.

2.2. Pathological investigations

ER and PgR expression was recorded as the percentage of immunostained cells[4,5] and categorised as highly positive (ER and PgR $\geqslant 50\%$ of cells positive), moderately positive (ER or PgR 1–49% of cells positive) or negative (ER and PgR $<1\%$ of cells positive).

HER2 immunoreactivity was determined using Herceptest kit following the manufacturer's instructions (Dako, Glostrup, Denmark). HER2 status was assigned on a 0 to 3+ scale. Tumours with 3+ (intense circumferential staining in $>10\%$ of cells) were considered HER2-positive; all other results were considered HER2-negative.

Ki67 was assessed immunohistochemically using the Mib-1 monoclonal antibody (1:200 dilution; Dako) and automated immunostainer (Autostainer, Dako).[6] The percentage of positive cells (nuclear immunoreactivity) among 2000 randomly selected tumour cells viewed at 400× at the periphery of the tumour, was calculated; Ki67 was categorised as low ($<14\%$) or high ($\geqslant 14\%$).[7]

2.3. Adjuvant treatments

Patients with positive ER tumour were assigned tamoxifen for 5 years; patients with negative ER, high grade (G3) and high proliferation rate (Ki67 $>20\%$)