Bcl-2 oncogene expression in estrogen receptor-positive and negative breast carcinoma

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Summary

Purpose: The aim of this study was to evaluate Bcl-2 oncogene expression in estrogen receptor (ER)-positive and negative breast carcinomas. *Methods:* A study involving 72 cases of infiltrating ductal carcinoma of the breast in postmenopausal women divided into two groups: Group A (ER positive, n = 37) and Group B (ER negative, n = 35). Immunohistochemical analysis of bcl-2 expression was carried out semiquantitatively based on the percentage of stained tumoral cells and the intensity of staining. The chi-square test was used in the statistical analysis of the data and significance was established at $p < 0.05$. *Results*: Bcl-2 oncogene expression was statistically greater in tumors of Group A (59.5%) compared to those of Group B (8.6%), ($p < 0.001$). *Conclusion*: Bcl-2 had a significantly greater expression in the ER-positive breast tumors compared to ER-negative tumors.

Key words: Breast; Cancer; Apoptosis; Estrogen receptor; Bcl-2.

Introduction

Breast carcinoma is a heterogeneous disease with a wide variety of histopathological characteristics, presenting unpredictable clinical behavior and prognosis [1]. A considerable number of patients with early breast cancer treated with local therapy alone suffer recurrences; however, there are, unfortunately, still no predictive and/or prognostic factors that are able to unequivocally identify which patients will respond to adjuvant therapy and which will have an unfavorable prognosis [2].

Estrogen receptors are expressed in around 60-65% of breast cancer cases, and in these cases, a relatively better prognosis can be expected compared with tumors that do not express them [3, 4]. This association of estrogen (ER)-positive tumors with a better prognosis may involve other molecular markers related to the proliferation and apoptosis of tumor cells [5-7]. A significant association has been shown between Ki-67 expression and negativity for estrogen receptors [8]. On the other hand, a positive expression of the bcl-2 protein has been associated with ER-positive breast cancer cells and a more favorable prognosis [9].

The bcl-2 oncogene, although suppressing apoptosis, paradoxically appears to be associated with a better prognosis, and its expression may be a marker of the response to endocrine therapy [10]. This association between bcl-2 expression and breast cancer prognosis has not yet been clarified. A study in women with ER-positive breast cancer treated with tamoxifen reported an increase in bcl-2 expression and a reduction in cell proliferation [9]. However, there is a scarcity of studies correlating bcl-2 with hormone receptor-positive and negative breast cancer tumors, which led to the conception of the present study.

Material and Methods

This study includes tumor samples from 72 patients who had been postmenopausal for at least two years and who were receiving care at the Mastology Department of the Federal University of Piauí. The patients were submitted to surgical treatment between 1999 and 2004 for ER-positive or ER-negative infiltrating ductal carcinoma. None of the patients had undergone any prior treatment. The study was approved by the Institutional Review Board of the Federal University of Piauí. Following hematoxylin-eosin staining and confirmation of the diagnosis of invasive ductal carcinoma, paraffin blocks containing the samples underwent histochemical analysis to evaluate estrogen and progesterone receptor status. Tumors with nuclear staining measured semiquantitatively as high (> 10% immunoreactive cells) were considered positive [8]. The cases were then divided into two groups: Group A: ER-positive, n = 37; and Group B: ER-negative, n = 35. Patients ranged in age from 52 to 82 years (mean 69.73) in Group A and from 51 to 86 years (mean 63.49) in Group B. The size of the tumors in the two groups ranged from 2 to 5 cm, Stages I and II. Immunohistochemistry (Dako ABC) was carried out to identify bcl-2 oncogene expression (mouse anti-human bcl-2 oncoprotein clone 124, Dako, code number M0887, 1:50). Bcl-2 expression was evaluated under light microscopy by two independent observers, who were blinded with respect to group identification. These observers performed semiquantitative counts of the cells with positively stained cytoplasm (magnification 400x) using a Nikon optical microscope, model Eclipse E200. Bcl-2 immunoreaction was evaluated according to the criteria established by Van Slooten *et al.* [11], taking the following parameters into consideration: intensity of cell coloration (I) and fraction of stained neoplastic cells (F). Intensity of cell staining was classified as: 0 (negative), 1 (weakly stained), 2 (moderately

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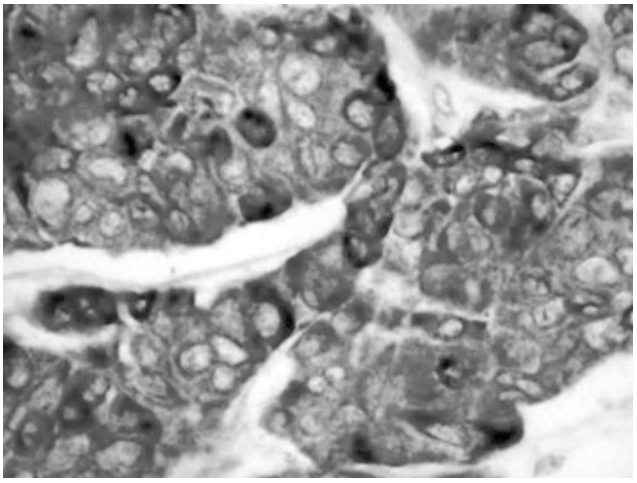
Fig. 1



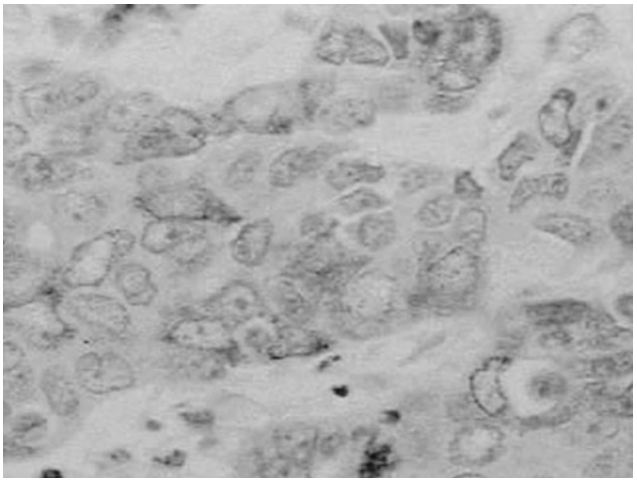


Fig. 2

Figure 1. — Photomicrograph of a histological section of invasive ductal carcinoma, case #34, ER-positive (Group A), showing a positive immunohistochemical reaction for the bcl-2 oncogene, as expressed by the numerous cells with cytoplasm intensely stained in brown.

Figure 2. — Photomicrograph of a histological section of an invasive ductal carcinoma, case #7, ER-negative (Group B), showing a negative immunohistochemical reaction for the bcl-2, as expressed by sparse cells with cytoplasm weakly stained in brown.

stained) and 3 (strongly stained). The fraction of stained cells was classified as: I (0-25%), II (25-75%) or III (75-100%). The final score was the result of the combination of the two parameters (I and F) and ranged from 0 to 6. Cases with a final score ≥ 3 were classified as positive for bcl-2 (11). In all cases, brownish staining in the cytoplasm was adopted as the standard for positivity. Evaluation began at the location that had the greatest quantity of stained cells, after which the other microscopic fields were selected at random, and staining intensity and the percentage of stained tumor cells were assessed, resulting in a final score [11]. In all cases, lymphoid cells accompanying the tumors served as internal positive controls of the immunohistochemical reaction. Statistical analysis was carried out on the bcl-2 data using the chi-square test. Significance was established at $p < 0.05$.

Results

Under light microscopy, the ER-positive tumors had an immunohistochemical reaction for the bcl-2 oncogene, as expressed by numerous cells containing cytoplasm with intense brown staining. In comparison, the hormone receptor-negative tumors showed no such reaction (Figures 1 and 2). Analysis of bcl-2 oncogene expression was positive in 22 (59.5%) ER-positive tumors (Group A) compared with only three (8.6%) tumors in Group B (Table 1). This difference was statistically significant ($p < 0.001$).

Table 1. — *Percentage of cases with bcl-2 expression in Groups A and B.*

Group	Negative n (%)	Positive n (%)	Total n (%)
A	15 (40.5)	22 (59.5)	37 (100.0)
B	32 (91.4)	3 (8.6)	35 (100.0)
Total	47 (65.3)	25 (34.7)	72 (100.0)

The percentage of cells with positive bcl-2 expression in Group A was significantly greater when compared to those of Group B ($p < 0.001$).

Discussion

The classic morphological prognostic factors of breast cancer, although subject to diverse influences, still play a relevant role in predicting the biological behavior of some of these cancers [5]. Nevertheless, there is a continual search for molecular markers capable of predicting therapeutic response and prognosis in breast cancer [2].

ER and PR, expressed in around 60-70% of cases of breast cancer, have been used with the principal objective of predicting response to adjuvant therapy [12, 13]; however, it is known that ER-positive tumors are more likely to be well-differentiated and to present a better prognosis than ER-negative tumors [4]. This difference between ER-positive and ER-negative tumors may be associated with the expression of molecular markers such as those related to apoptosis of breast cancer cells [9].

In the present study, there was a highly significant expression of the bcl-2 oncogene in ER-positive breast tumors compared with hormone receptor-negative tumors. Likewise, some studies have shown that there is a strong positive correlation between ER and bcl-2 immunoreactivity [5, 10, 12], raising the hypothesis that bcl-2 is an ER-regulated gene [6]. On the other hand, an inverse correlation has been reported between bcl-2 protein expression and cell proliferative activity [9].

Some authors have failed to confirm any relationship between bcl-2 oncogene and cell proliferation and prognosis in breast cancer [14]. A tentative explanation for this lack of correlation may be in the difficulty to quantify bcl-2 expression, which would result in low accuracy and reproducibility of this marker [2]. The hypothesis that loss of antigenicity may result from material having been kept in storage [15] was not observed in the blocks used in this study. Thus, the present study showed that ER-positive breast cancer cells significantly express bcl-2 compared to negative tumors.

Bozzetti *et al.* [16] reported a correlation in bcl-2 expression, evaluated by immunocytochemistry on fine-needle aspirates from primary breast carcinoma, with favorable prognostic features such as ER and PR expression, p53 negativity, a low Ki-67 index, and high tumor differentiation [16]. Bcl-2 oncogene expression occurs in 40-80% of breast carcinomas [17] and its strong correlation with estrogen receptor status suggests a potential role for bcl-2 expression as a modulator of the response to adjuvant therapy in breast cancer [18, 19]. ER-positive tumors treated with tamoxifen have shown an increase in bcl-2 expression and a reduction in cell proliferation, which in this case may explain the association between bcl-2 and a favorable prognosis in breast cancer [9].

Studies involving the effects of selective estrogen receptor modulators (SERMs), both in normal breast tissue and in neoplastic tissue, may help clarify the correlation between cell proliferation, apoptosis and prognosis in breast cancer [20]. Bcl-2, despite being an anti-apoptotic protein, is associated with a favorable prognosis; moreover, in the present study, bcl-2 expression in ER-positive tumors was significantly greater compared to negative tumors. Further studies are, therefore, required to better clarify the association between bcl-2 and other markers and prognosis in breast cancer.

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