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Expression of *c-erbB-2*, *p53* and *c-myc* proteins in male breast carcinoma. Comparison with traditional prognostic factors and survival

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Abstract

There are few data evaluating biological markers for men with breast cancer. The purpose of the present study was to analyze the expression of the oncogenes *c-erbB-2* and *c-myc* and of the suppressor gene *p53* by immunohistochemical techniques in archival paraffin-embedded tissue blocks of 48 male breast cancer patients, treated at the A.C. Camargo Cancer Hospital, São Paulo, SP, Brazil. The results were compared with clinicopathological prognostic features. Immunopositivity of *c-erbB-2*, *p53* and *c-myc* was detected in 62.5, 16.7 and 20.8% of the cases analyzed, respectively. Estrogen and progesterone receptors were positive in 75 and 69% of the cases, respectively. Increasing staging was statistically associated with *c-erbB-2* (P = 0.04) and weakly related to *p53* positivity (P = 0.06). No significant correlation between specific survival rate (determined by the log rank test) and the molecular markers analyzed was found, whereas the number of compromised lymph nodes and advanced TNM (tumor, node, metastasis) staging were associated with diminished survival.

Key words
- *p53*
- *c-myc*
- *c-erbB-2*
- Male breast cancer

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Introduction

Male breast carcinoma (MBC) is a rare condition representing less than 1% of breast malignancies. Despite differences in the hormonal milieu, it follows a course of invasion and metastasis similar to that of female breast cancer (1). Several clinicopathological characteristics of male breast carcinoma are considered to be prognostic factors (2-10). Evaluation of the expression of proto-oncogenes, suppressor genes and cell regulatory proteins in female breast cancer has led to remarkable progress in our understanding of key issues in tumor development and progression. For men with breast cancer there are few data on the prognostic value of molecular marker expression.

The aims of the present study were threefold. First, to evaluate clinical and pathological features in a group of Brazilian male patients with breast cancer. Second, to determine by immunohistochemistry the expression of genes that are frequently involved in

female breast cancer, i.e., *c-erbB-2*, *c-myc* and *p53.* Third, to analyze associations between these features and specific patient survival.

Material and Methods

Patients

According to the tumor registry, 102 patients with male breast cancer were diagnosed at the A.C. Camargo Hospital, USP, São Paulo, SP, Brazil, between 1957 and 1998. The clinical files were reviewed and all cases were submitted to a histopathological review of tumor slides in order to confirm diagnosis. Conventional clinicopathological features of each case including tumor size, histologic grade and lymph node status were reviewed. All cases were classified according to the TNM (tumor, node, metastasis) classification system (UICC). Histological grade was established according to Elston and Ellis (11). Morphological analysis of the surgical specimen slides and paraffin-embedded blocks showed that only 48 cases had adequate tumor samples and the most representative sample of each tumor was submitted to immunohistochemical analysis.

Immunohistochemical methods

Antigens c-myc, p53, c-erbB-2, estrogen (ER) and progesterone receptors (PR) were assessed with the following antibodies: mouse *c-myc* antibody (9E10; Oncogene Science Inc., Manhasset, NY, USA), *p53* antibody (DO-7), anti-human *c-erbB-2* oncoprotein and ER (ID_5; Dako A/S, Glostrup, Denmark), and PR (1A6; Novocastra, Newcastle upon Tyne, UK). Positive and negative control slides were used in all reactions. We used previously known female breast cancer cases as positive controls. Negative controls were performed by incubating slides with PBS instead of primary antibody.

Briefly, after deparaffinization in xylol and alcohol, slides (3 µm thick) were immersed in PBS, pH 7.4, for 5 min and submitted to microwave treatment for epitope retrieval with citric acid solution, pH 6.0, with two 9-min cycles. The material was submitted to endogenous peroxidase blocking treatment (10 vol. H_2O_2) followed by another PBS immersion, and by overnight incubation in a humid chamber at 4°C with the primary antibody at 1/50 dilution for *c-myc*, 1/150 for *p53*, 1/100 for *c-erbB-2* and ER and at 1/20 dilution for PR. Slides were then rinsed in PBS solution and incubated with the biotinylated secondary antibody (1:200 dilution; Dako) for 30 min and again rinsed and incubated with the ancillary antibody (StrepAB complex/HRP Duet, mouse/rabbit) for another 30 min. The material was developed by immersion in 2,3 diaminobenzidine tetrahydrochloride (DAB, Sigma Chemical Co., St. Louis, MO, USA), 60 mg% + 1% dimethyl sulfoxide at 37°C for 5 min and by hematoxylin counterstaining. Positivity was characterized by dark brown staining of nucleus (p53, c-myc, ER and PR) or cell membrane and cytoplasm (c-erbB-2). A case was considered positive if more than 20% of tumor cells were stained. Sections of female breast cancer with known results for the molecular markers analyzed were included as positive and negative controls.

Statistical analysis

Fisher's exact test at the 95% significance level was used to assess the univariate association of clinicopathological characteristics with *p53*, *c-erbB-2* and *c-myc*. The time from the date of surgery to the last contact available (for live patients) or to the date of death was used to calculate the survival rate and the survival curves. Patients who died of causes other than breast cancer were treated as censored observations. The distribution of specific survival was estimated using the Kaplan-Meier method. The

log rank test was used to determine whether any tumor feature, patient characteristic or molecular marker was significantly associated with specific survival.

Results

Patient and tumor characteristics for the entire cohort and for patients with adequate tissue are shown in Table 1. No significant differences were observed between groups.

Treatment protocols were slightly heterogeneous due to the large time span. In the group of 48 patients for whom tissue was available, modified radical mastectomy was performed in 22 (46%), radical mastectomy in 16 (33%) and simple mastectomy in 4 (8%). According to the staging during diagnosis, the majority of patients in clinical stage IV (6/8) underwent another type of surgery or received only systemic treatment. The remaining two patients (4%) with metastasis in the homolateral supraclavicular lymph nodes were submitted to radical mastectomy (Halsted operation). Adjuvant therapy was performed as chemotherapy with cyclophosphamide, methotrexate and 5-fluorouracil (CMF) in 4 patients with stage I or II disease. For 2 other patients (stage I and stage II, respectively) 5-fluorouracil, adriblastine and cyclophosphamide (FAC) were prescribed. Five patients with advanced disease were treated with CMF or FAC. Four patients (stages I and II) received hormonal therapy (tamoxifen). Postoperative adjuvant radiation therapy was administered to 23 patients (clinical stages I, II and III). Orchidectomy was performed in 4 patients to treat metastatic disease.

Regional and axillary lymph nodes were evaluated in 38 patients when lymphadenectomy was performed during surgery. The average number of axillary lymph nodes dissected per patient was 22.9, with a median number of 22.5. In 16 (42.10%) patients, no neoplastic involvement of lymph nodes was identified. Tumor metastasis in lymph nodes was detected in 22 cases: 10 (26.3%) showed 1-3 involved nodes, 5 (13.2%) showed 4 to 10 and 7 (18.42%) showed more than 10.

Expression of *c-erbB-2*, *p53* and *c-myc* was positive (i.e., ≥20% of cells displaying

Table 1. Patient and tumor characteristics.

	All patients (N = 102)	Patients with available tissue (N = 48)
Median age (range) (years)	60 (35-84)	62 (35-84)
Family history		
Positive	18 (17.6%)	10 (20.8%)
Negative	52 (51.0%)	31 (64.6%)
Unknown	32 (31.4%)	7 (14.6%)
Surgery		
Biopsy	5 (5.0%)	2 (4.2%)
Lumpectomy	8 (7.8%)	4 (8.3%)
Simple mastectomy	5 (5.7%)	4 (8.3%)
Modified radical mastectomy	36 (35.3%)	22 (45.8%)
Radical mastectomy	48 (47.0%)	16 (33.3%)
Histological features		
Ductal carcinoma *in situ* (DCIS)		
Papillary	2 (4.0%)	1 (2.1%)
Paget's disease	1 (1.0%)	1 (2.1%)
Invasive ductal carcinoma		
Papillary	6 (6.0%)	4 (8.3%)
Colloid	2 (2.0%)	1 (2.1%)
Microinvasive	1 (1.0%)	1 (2.1%)
NOS*	88 (86.0%)	40 (83.3%)
Stage		
DCIS	3 (3.0%)	2 (4.2%)
I	6 (6.0%)	3 (6.0%)
IIA	10 (9.8%)	6 (13.0%)
IIB	12 (11.8%)	8 (17.0%)
IIIA	14 (13.7%)	3 (6.0%)
IIIB	37 (36.3%)	18 (37.0%)
IV	20 (19.6%)	8 (17.0%)
Chemotherapy		
Yes	25 (24.5%)	11 (23.0%)
No	77 (75.5%)	37 (77.0%)
Adjuvant hormonal therapy		
Yes	10 (9.8%)	4 (8.3%)
No	92 (90.2%)	44 (91.7%)
Adjuvant radiotherapy		
Yes	51 (50.0%)	23 (48.0%)
No	51 (50.0%)	25 (52.0%)
Histologic grading (HG)		
HG I	18 (17.6%)	10 (20.8%)
HG II	62 (60.8%)	30 (62.5%)
HG III	22 (21.6%)	8 (16.7%)

*NOS = not otherwise specified.

staining positivity) in 30 (62.5%), 8 (16.7%) and 10 (20.8%) of the cases analyzed, respectively. Representative illustrations of the immunostainings performed are presented in Figure 1. A total of 36 (75%) and 33 (68.8%) of the 48 cases were positive for ER and PR, respectively.

The expression of *c-erbB-2*, *c-myc* and *p53* was analyzed in relation to clinical, pathological and biochemical parameters such as age, stage of disease, lymph node involvement, ER and PR (Table 2). A significant association was found between positive staining for *c-erbB-2* and increased staging (P = 0.04). There was also a trend to a higher proportion of *p53*-positive tumors in advanced stages of the disease (P = 0.06). The positive factors ER and *p53* showed a trend to interdependence (P = 0.07). The *c-myc*-positive tumors were generally *c-erbB-2* negative (P = 0.09), whereas no correlation was found between *p53* and *c-myc* or *p53* and *c-erbB-2*.

At the last follow-up (August 1999) 18 patients were alive and only 2 had evidence of disease, 14 had died of cancer, 11 had died of other causes, and 5 had no follow-up information. The mean length of follow-up was 67.5 ± 58.3 months. For the 14 patients who died of cancer the specific survival rate was estimated to be 75.8% at 5 years and 60.8% at 10 years. The specific survival rate was analyzed according to clinical, pathological, biological and treatment parameters

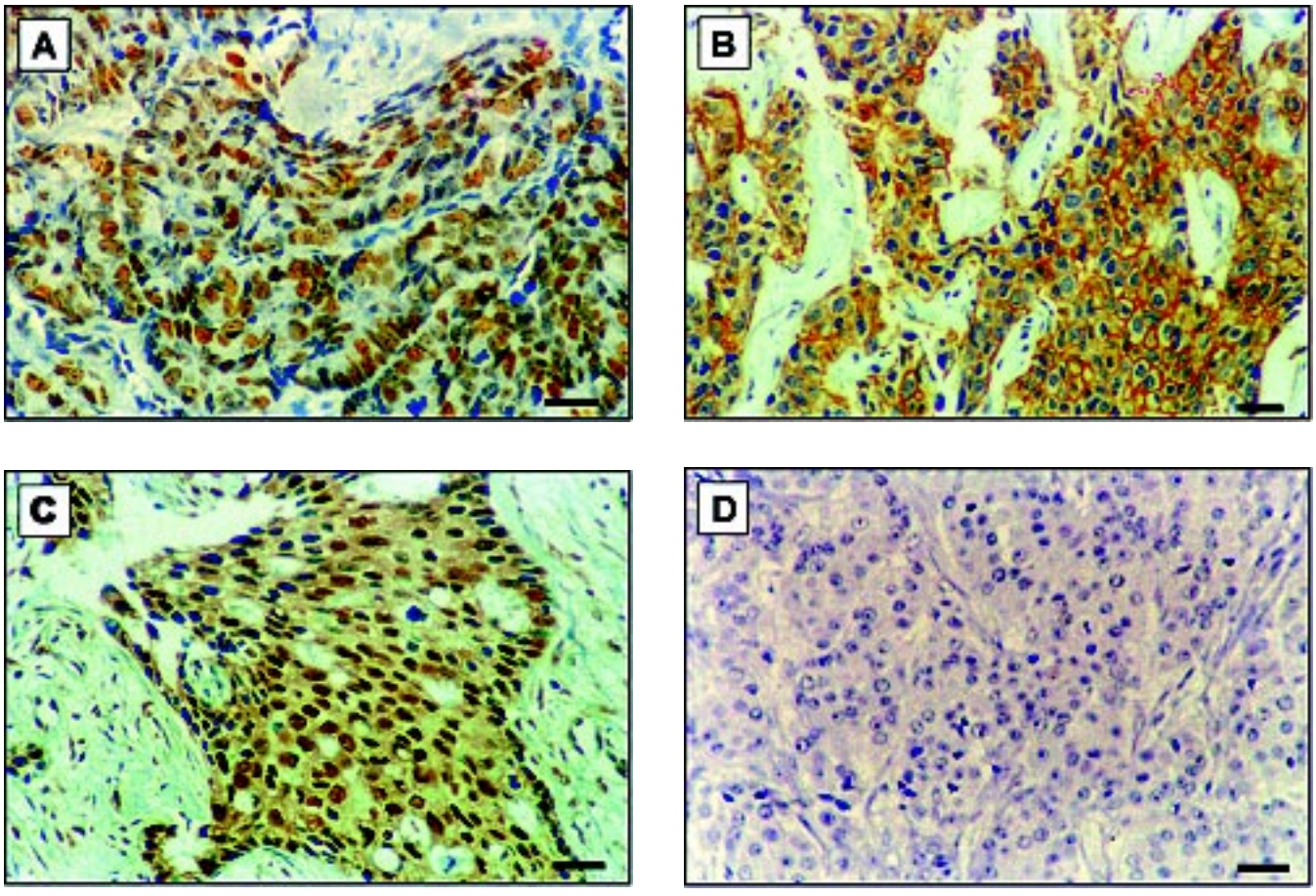

Figure 1. Immunohistochemical detection of *p53* (A), *c-erbB-2* (B), and *c-myc* (C) in male breast carcinoma (original magnification 240X). *D*, Negative control. Bar = 50 µm.

(Table 3). Specific survival rates were found to be significantly decreased for patients in more advanced stages of disease and presenting increasing numbers of positive lymph nodes. No significant associations between *c-erbB-2*, *p53* or *c-myc* immunohistochemical staining and specific survival probability were observed (Figure 2). Though the analysis did not achieve statistical significance, a worse outcome was found to be correlated with *c-erbB-2* positivity.

Discussion

The present report confirms the high median age of MBC patients at the onset of disease, the preponderance of advanced stage tumors and the high incidence of lymph node positivity reported by others. The overall survival rate was also within the range reported in the literature for males (reviewed in 1-3,9,10). Familial breast cancer in males is a noteworthy occurrence. It was recorded

Table 2. Relationship between immunohistochemical features and clinicopathological variables.

Variable	*c-myc*		P	*c-erbB-2*		P	*p53*		P
	+	-		+	-		+	-	
Age (years)									
35-60	2	20	0.06	15	7	0.65	3	19	0.60
61-70	4	11		8	7		2	13	
>70	4	7		7	4		3	8	
Stage									
DCIS	-	2	0.89*	1	1	0.04	-	2	0.06*
I	-	3		1	2		-	3	
II	4	10		7	7		1	13	
III	4	17		14	7		3	18	
IV	2	6		7	1		4	4	
ER status									
Positive	9	27	0.21	23	13	0.73	8	28	0.07
Negative	1	11		7	5		0	12	
PR status									
Positive	8	25	0.39	21	12	0.81	7	26	0.21
Negative	2	13		9	6		1	14	
Number of lymph nodes									
Negative	4	12	0.57	10	6	0.69	3	13	0.72
1-3	1	9		5	5		1	9	
4-10	2	3		2	3		0	5	
>10	1	6		5	2		0	7	

*Considering clinical stages II, III and IV. Fisher exact test (95% significance).
DCIS, ductal carcinoma *in situ*; ER, estrogen receptor; PR, progesterone receptor.

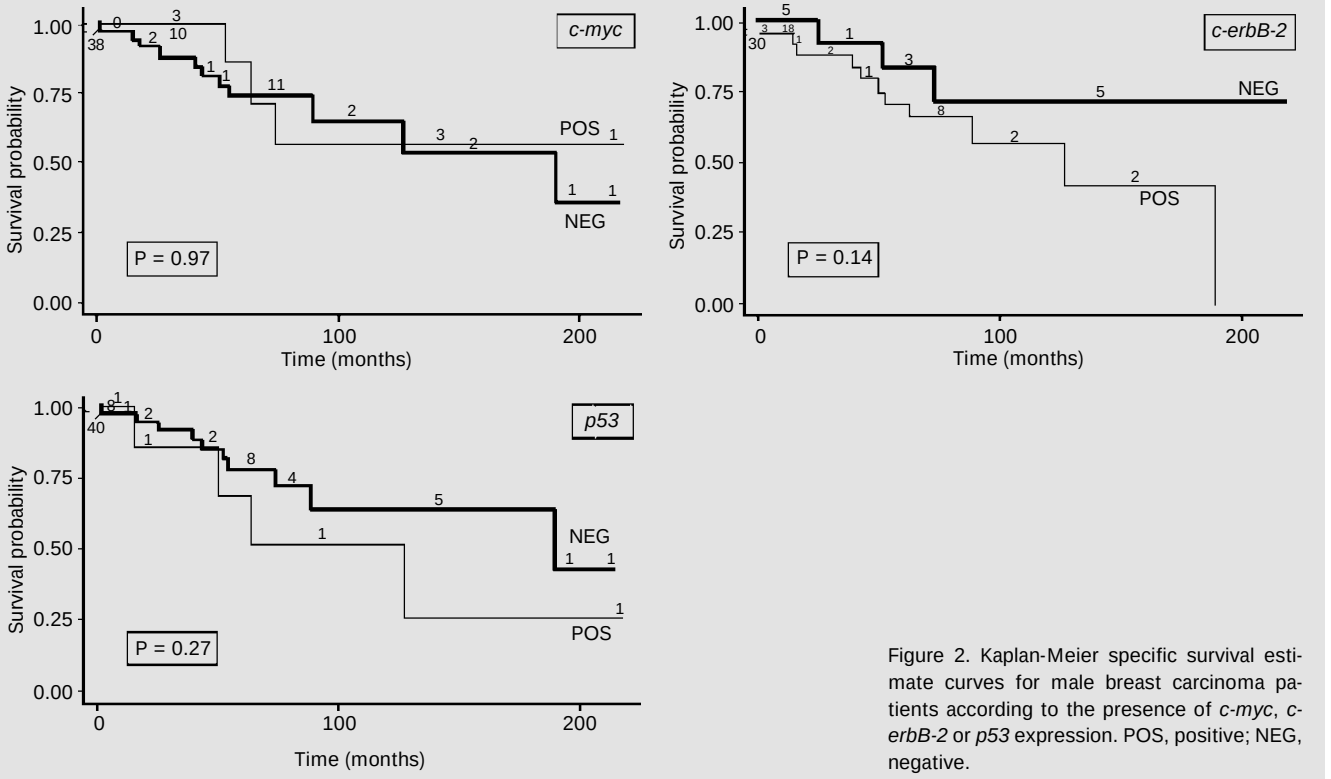

Figure 2. Kaplan-Meier specific survival estimate curves for male breast carcinoma patients according to the presence of *c-myc*, *c-erbB-2* or *p53* expression. POS, positive; NEG, negative.

in 18 patients of our series (17.6%), a value similar to that reported by Salvadori et al. (2). We observed that ER and PR positivity was conspicuously high, in agreement with several previous studies (2-5,12-20).

In female breast cancer, numerous studies have reported *c-erbB-2* amplification and overexpression and some of them found a positive correlation with earlier relapse and poorer overall patient survival (21). In addition, experimental approaches have provided evidence that the *c-erbB-2* oncogene plays an important role in cancer metastasis (22). Data available for MBC indicate a wide range of positivity of 0-95% (8,12,16,19,20, 23-28). We found a high proportion of *c-erbB-2* positivity (62.5%) which probably reflects the high number of patients with advanced stage at presentation, since we have verified in our series an association between *c-erbB-2* and more advanced stage of the disease (P = 0.04). The results of studies that have evaluated the prognostic importance of *c-erbB-2* in MBC have been contradictory. Some reports indicated a correlation between *c-erbB-2* and a poorer outcome (10), which could not be established in other studies (14,19,20,26,27). We found only a slight positive correlation between *c-erbB-2* positivity and specific survival probability (P = 0.14); however, this may have resulted from a small sample size.

Immunohistochemical detection of *p53* has been obtained in a low percentage of male breast tumors (8,12,17,20,28-30). Our result (16.7%) is within the range previously reported. Although some studies indicated a correlation between *p53* and a poorer prognosis (27,28), no such statistical correlation could be established in the current study, in agreement with other reports (4,12,16,19,20). However, we determined a trend to a higher proportion of *p53*-positive tumors in advanced stages (P = 0.09).

c-myc is a key cellular proliferative signal in breast tumorigenesis (31). *c-myc* amplification or overexpression has been reported in female breast tumors but the prognostic significance remains controversial, partly because of discrepancies among different methodologies used for the detection of oncogene amplification or overexpression. The reported frequency of *c-myc* overexpression is as high as 100% in some studies and as low as 12% in others (reviewed in 32). We found that only 20% of MBC tumor specimens analyzed expressed *c-myc* and

Table 3. Association of clinical, pathological and molecular markers and treatment features with specific survival.

Prognostic variable	N	5-year survival rate (%)	10-year survival rate (%)	P*
Number of lymph nodes				
Negative	16	88.2	88.2	<0.01
1-3	10	100.0	100.0	
4-10	5	80.0	80.0	
>10	7	53.6	53.6	
Stage				
DCIS	2	100.0	100.0	<0.01
I	3	100.0	100.0	
II	14	100.0	100.0	
III	21	71.8	48.4	
IV	8	35.7	17.9	
c-myc				
Positive	10	85.7	53.6	0.97
Negative	38	73.5	54.0	
c-erbB-2				
Positive	30	71.5	42.0	0.14
Negative	18	83.5	69.6	
p53				
Positive	8	67.7	25.4	0.27
Negative	40	77.5	62.7	
ER				
Positive	36	72.9	59.6	0.29
Negative	12	82.5	66.0	
PR				
Positive	33	78.6	61.3	0.90
Negative	15	66.0	66.0	
Radiotherapy				
Yes	23	84.0	67.0	0.14
No	25	67.0	54.0	
Chemotherapy				
Yes	11	78.0	63.0	0.88
No	37	75.0	56.0	

*Log rank test with significance of 95%.
DCIS, ductal carcinoma *in situ*; ER, estrogen receptor; PR, progesterone receptor.

the *c-myc*-positive tumors generally were *c-erbB-2* negative. Similarly, the simultaneous overexpression of *c-myc* and *c-erbB-2* seems to occur at a very small percentage in female breast cancer (33). Deregulated expression of *c-myc* and loss of wild-type *p53* function may cooperate in tumor development (34). In our series the co-expression of *c-myc* and *p53* occurred in 7% of MBC specimens, but the small number of patients in this category precluded the analysis of association with outcome. Recently, patients with mutations of the *p53* gene showed a predisposition for a shorter survival in MBC (29). Many of the sections in the present study were far from optimum because they were prepared from old archival tissue with variations in tissue fixation, hampering this sort of analysis.

The current study evaluated the prognostic significance of *c-erbB-2*, *p53* and *c-myc* in 48 cases of MBC and verified that such biological markers have no statistically significant correlation with specific overall survival. The present study confirms the importance of staging and axillary lymph node status in the prognosis of male breast cancer patients (2,3,5-7). Our series covers a period of 41 years during which the therapies applied to a small portions of patients have changed, and no firm conclusions can be drawn about the impact of adjuvant treatments on survival. The small number of patients studied precluded multivariate analysis of the data. However, due to the rarity of MBC, reports from single institution series, although including small numbers of patients, will contribute to the advancement of our understanding of this disease.

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