Pergamon

European Journal of Cancer Vol. 30A, No. 1, pp. 16–22, 1994

0959–8049/94 $6.00 + 0.00

c-*erb*B-3 and c-*erb*B-2 Protein Expression in Node-negative Breast Carcinoma — an Immunocytochemical Study

Giampietro Gasparini, William J. Gullick, Sergio Maluta, Paolo Dalla Palma, Orazio Caffo, Elena Leonardi, Patrizia Boracchi, Franco Pozza, Nicholas R. Lemoine and Pierantonio Bevilacqua

The type I growth factor receptor family has been found to play an important role in the control of normal growth and differentiation. Moreover, the epidermal growth factor receptor and the c-*erb*B-2 oncogene seem to be implicated in the pathogenesis and behaviour of several cancers, including breast cancer. c-*erb*B-3 is a new member of the type I receptor family for which there is currently little information available on its expression in neoplastic tissues, and on its possible prognostic significance. This study was undertaken to define the prognostic value of c-*erb*B-3 expression in a series of node-negative breast cancer (NNBC) patients when compared, by multivariate analysis, with expression of the c-*erb*B-2 protein and conventional clinicopathological features. c*erb*B-3 was recognised by the novel monoclonal antibody RTJ1, whereas c-*erb*B-2 was detected by the polyclonal antibody 21N, using immunocytochemical methods. We found that overexpression of c-*erb*B-3 occurs frequently in NNBC. Overall, 138 of 212 carcinomas (65%) had some degree of membrane RTJ1 staining, and 28 (13%) showed strong and generalised positivity (+++). Twenty-four per cent of carcinomas had membrane 21N staining, and 12% presented strong and generalised positivity (+++). c-*erb*B-3 protein expression was significantly associated only with that of c-*erb*B-2 ($P = 0.05$), whereas 21N positivity was significantly associated with small tumour size ($P = 0.02$) and ductal histotype ($P = 0.04$). No significant correlation between expression of either receptor proteins or relapse-free survival was observed after a median follow-up of 63 months. Applying multivariate analysis, only tumour size approached significance. Our results indicate that analysis of expression of c-*erb*B-3 and c-*erb*B-2 alone do not seem to be useful in identifying patients with NNBC at different risk of relapse or death.
Eur J Cancer, Vol. 30A, No. 1, pp. 16–22, 1994

INTRODUCTION

THE FAMILY of the type 1 growth factor receptors currently includes three related members: the epidermal growth factor receptor (EGFR), c-*erb*B-2 and, more recently, the c-*erb*B-3 gene [1]. Members of this family are frequently implicated in human cancer, and they play an important role in the control of normal cell proliferation, differentiation [2] and in the pathogenesis of human cancer, particularly in breast carcinoma [3].

Slamon *et al.* [4] first reported an association between amplification of the c-*erb*B-2 oncogene (also called HER-2/*neu*) and poor prognosis in human breast cancer. These results have generated a great deal of interest and many publications because this study was one of the first in which molecular biology was applied in a clinical setting [5]. However, presently there is an unresolved controversy on the prognostic value of c-*erb*B-2 gene amplification or overexpression, particularly in node-negative breast cancer (NNBC; see [6] for a review), with less than half of the studies finding an association with poor prognosis.

More homogeneous are data on node-positive patients, with the majority of studies demonstrating a strong relationship between c-*erb*B-2 positivity and worse outcome [7].

At the present time, the prognostic role of the EGFR expression in patients with early stage breast carcinoma also remains contradictory [8], with little data available for node-negative patients [9].

The c-*erb*B-3 protein is a new member of the type I receptor family, recently cloned by two groups [10,11]. The gene for the c-*erb*B-3 receptor is located in the long arm of chromosome 12 (12q13), and is transcribed as a 6.2-Kb mRNA, which specifies a glycoprotein of about 160 000 daltons [10,11], and which is

Correspondence to G. Gasparini at the Department of Radiotherapy & Oncology, St Bortolo Regional Medical Center, 36100 Vicenza, Italy.
G. Gasparini, F. Pozza and P. Bevilacqua are at the St Bortolo Regional Medical Center, Vicenza, Italy; W.J. Gullick and N.R. Lemoine are at the Imperial Cancer Research Fund, Hammersmith Hospital, London, U.K.; S. Maluta, P.D. Palma, O. Caffo and E. Leonardi are at the St. Chiara Regional Medical Center, Trento, Italy; and P. Boracchi is at the Institute of Medical Statistic and Biometry, University of Milan, Istituto Nazionale Tumori, Milano, Italy.
Revised 27 July 1993; accepted 27 Aug. 1993.

still not fully characterised [1]. Kraus *et al.* [10] first reported that the mRNA for c-*erb*B-3 was expressed at high levels in some breast cancer-derived cell lines.

Lemoine *et al.* [12] recently evaluated the expression of the c-*erb*B-3 gene product in a series of primary human breast cancers. Using two different polyclonal antibodies (49.3 and 61.3), they found a strong immunoreactivity in 22% of the carcinomas, and a significant association of overexpression only with the presence of lymph node metastases. c-*erb*B-3 expression did not predict patient outcome.

Subsequently, Rajkumar *et al.* [13] have developed a sensitive and specific monoclonal antibody (RTJ1) which gives superior staining to the previously described polyclonal antibodies employed by Lemoine *et al.* [12].

We have, therefore, used the RTJ1 antibody for immunocytochemistry to analyse the relationship between c-*erb*B-3 protein overexpression, c-*erb*B-2 status and several conventional clinicopathological features, to investigate their prognostic value in a series of 212 NNBC patients.

PATIENTS AND METHODS

212 breast cancer patients with histologically confirmed invasive carcinoma and with negative axillary nodes, who underwent breast cancer surgery at the St. Bortolo Regional Medical Centre of Vicenza, Italy (112 cases) or at the St. Chiara Regional Medical Center of Trento, Italy (100 cases) from June 1986 to December 1988, were enrolled in the study.

All patients were staged according to the International Union Against Cancer tumour–node–metastasis (UICC-TNM) classification.

The eligibility criteria for the inclusion of patients were primary T1 to T3a, N0 (with at least I–II axillary levels cleared), M0, unilateral breast cancer, and no other primary cancer. Surgery had to be microscopically radical with a margin of normal tissue around the primary tumour.

No patient received systemic adjuvant therapy, and surgery was either radical modified mastectomy (RMM; according to Madden or Patey techniques) in 109 patients or quadrantectomy with complete axillary dissection according to Veronesi *et al.* [14] in 103 patients. Conservative surgery was followed within 4–6 weeks by radiation therapy with a dose of 50 Gy given by ^{60}Co over a period of 5–6 weeks in 25 daily fractions to all the residual breast by means of parallel opposed, tangential fields, and a boost was given to the primary bed site using a direct field to give a dose of 10 Gy in five daily fractions (QUART therapy). QUART was performed (as an alternative to RMM) only in patients with primaries with a diameter less than 3 cm.

The main clinicopathological characteristics of the patients are listed in Table 1.

By June 1992, the median follow-up of the patients was 63 months (range 1–100). The following clinical and pathobiological features were determined. Age and menopausal status were as defined previously [15].

Histology

Tumour size was recorded as the largest diameter of the primary at the time of trimming of the fresh specimens. Surgical specimens were fixed in buffered formalin for 24 h at 20°C, dehydrated through graded ethanol, and paraffin-embedded at 56°C for 30 min. Tumours were classified by histological type according to the criteria of the National Surgical Adjuvant Breast Project [16]. Nuclear and histological grading were performed according to the criteria described by Bloom and Richardson [17]. All identifiable lymph nodes in the I–II (minimum) axillary levels were examined by light microscopy (median number of cleared nodes, 14/specimen).

Table 1. Clinical and pathological characteristics of the patients

	No. of cases	%
Total asssessable	212	
Median age, years (range)	59 (28–84)	
Surgery		
Mastectomy	109	51
QUART	103	49
Menopausal status		
Premenopausal	54	26
Perimenopausal	20	9
Postmenopausal	138	65
Histotype		
Ductal	159	75
Lobular	36	17
Others	17	8
Tumour size		
1	133	63
2	77	36
3	2	1
Grading		
I	29	14
II	118	56
III	65	31
ER-ICA		
–	49	23
+	83	39
++	77	36
ND	3	1
PgR-ICA		
–	80	38
+	85	40
++	44	21
ND	3	1

ER-ICA, oestrogen receptor by immunocytochemical assay; PgR-ICA, progesterone receptor by immunocytochemical assay; ND, not done.

Steroid receptor assays

Immunocytochemical assays (ICA) on paraffin sections were performed. Oestrogen receptor (ER) ICA were analysed using the monoclonal anti-receptor antibody H 222 Sp2y with the kit purchased from Abbott Diagnostics (Abbott Lab, North Chicago, Illinois, U.S.A.) adopting a method similar to that described by Anderson *et al.* [18]. Sections were analysed for progesterone receptor (PgR) ICA using the rat monoclonal anti-receptor antibody IgG KD-68 using the kit assay of Abbott Diagnostics with a previously published method [19].

For both assays, a negative control was established by replacing the primary antibody with normal rat IgG at the same dilution. As positive controls, known ER-positive and PgR-positive specimens were incubated in parallel with the sections under evaluation. The staining features were evaluated by a semiquantitative score. At least 500 cells were counted from each tumour. Tumours were classified as ER−ICA− or PgR−ICA+ if any cell showed nuclear staining: + indicates heterogeneous and moderate staining with less than 50% of positive cells and ++ indicates strong staining and more than 50% positive cells.

Immunocytochemical assays to detect expression of c-erbB-3 and c-erbB-2 proteins

The expression of the c-*erb*B-3 protein was demonstrated in 5-μm sections of routine formalin-fixed, paraffin-embedded blocks using the IgM monoclonal antibody RTJ1, raised to the 49.3 peptide of the human c-*erb*B-3 protein. This peptide of 13 amino acids has the sequences ELEPELDLDLDLE representing residues 1004–1016 of the human c-*erb*B-3 protein [10]. It has been used previously to raise polyclonal antibodies in rabbits. The production and characterisation of the RTJ1 monoclonal antibody has been described in detail by Rajkumar *et al.* [13]. The sections were dewaxed and rehydrated using standard solutions. Endogenous peroxide activity was quenched with 0.3% hydrogen peroxide in phosphate buffered saline (PBS) for 30 min. The slides were washed with PBS and then the hybridoma supernatant was added at 1:100 dilution and incubated overnight at room temperature. Subsequent procedures were similar to those described by Rajkumar *et al.* [13].

c-*erb*B-2 protein was demonstrated in 5-μm sections of routine formalin-fixed, paraffin-embedded blocks using the polyclonal antibody isolated by Gullick *et al.* [20], and raised in rabbits against a synthetic polypeptide (21N) as reported previously [15].

For both the assays, tumours were scored by assessing the site of staining (membrane and/or cytoplasm) and the proportion of stained cells was scored by a semiquantitative score from 0 (negative) to +++ (strong and generalised positivity). For the purpose of this study, only tumours with membrane staining were considered c-*erb*B-3− and/or c-*erb*B-2+; all others, including those with generalised cytoplasmic staining, were considered negative. Positive controls include breast carcinomas known to exhibit high levels of membrane c-*erb*B-3 or c-*erb*B-2 proteins. Negative controls were obtained by omission of the primary antibody.

All pathobiological features were evaluated separately by two investigators, and all laboratory tests were performed without knowledge of clinical outcome of the patients, in a blind manner.

Follow-up

All patients were followed-up postoperatively. Physical examination was performed every 4 months in all women for the first 3 years and then twice per year. Radiographic studies, including chestroentgenogram, mammography and liver echotomography, were carried out every 12 months, or earlier whenever clinically indicated. Haematological tests, including 12-channel biochemical profiles and complete blood cell counts, were repeated every 6 months. The relapse-free survival (RFS) and the overall survival (OS) were calculated as the period from surgery until the date of the first recurrence or death, respectively.

Primary treatment failure was defined as the first documented evidence of new disease manifestation(s) in locoregional area(s), distant site(s), contralateral breast or a combination of the above. Any new disease involvement was accurately assessed by clinical, radiological and, whenever feasible, histological examination of the site(s) of first relapse.

Statistical methods

The association between oncogenes expression and other variables was investigated by a logistic regression model. Each of the regression coefficients (β) is the log of the odds ratio (OR). Odds is the probability of having c-*erb*B-3 (or c-*erb*B-2) positive over the c-*erb*B-3 (or c-*erb*B-2) negatives. For patients classified in two groups and under the null hypothesis of having the same probability of c-*erb*B-3 (or c-*erb*B-2) positivity, OR is expected to be 1.0.

The patterns of RFS were estimated by means of the product-limit method [21], on the basis of a 5-year follow-up period. The role of each of the prognostic variables (univariate analysis) and their joint effect (multivariate analysis) was evaluated using a log-logistic regression model by a relative measure of prognostic effects [22], as reported previously [15].

In this log-logistic regression model, each β is recognisable as the log in terms of OR and is constant with time [23]. The odds of relapse are the probability of the event divided by the probability of remaining free of an event, and the relative OR are the odds for a given prognostic category of patients divided by the odds for the reference group [22]. For patients classified into two prognostic categories and under the null hypothesis of having the same survival experience (or RFS experience), the OR is expected to be 1.0.

The hypothesis $\beta = 0$ was tested by the Wald statistic [24] both in logistic and log-logistic regression models. In the univariate analysis for each variable, unadjusted ORs [and their 95% confidence intervals (CI)] were estimated according to a regression model containing only that variable. In the multivariate analysis, the prognostic relevance of combined indicators was estimated, according to a multiple regression model containing, besides receptor gene expression, those variables that had unadjusted ORs significantly different from 1.0. Statistical analysis was performed using the Statistical Application System (SAS) package.

RESULTS

Immunocytochemical staining with the RTJ1 and 21N antibodies

The RTJ1 antibody gave a strong reaction in paraffin sections with variable intensity of staining of cell membranes (Fig. 1) in 138 carcinomas (65%), as reported in Table 2. Background was very low. One hundred and eighty-eight tumours (89%) showed cytoplasmic staining, and only 31 carcinomas had no evident staining.

We observed a highly significant association between membrane and cytoplasmic staining (OR = 7.07; $P = 0.0001$). Only membrane staining was considered positive for the purpose of this study.

The 21N antibody produced immunostaining of variable intensity of the membrane of tumour cells in 51 carcinomas

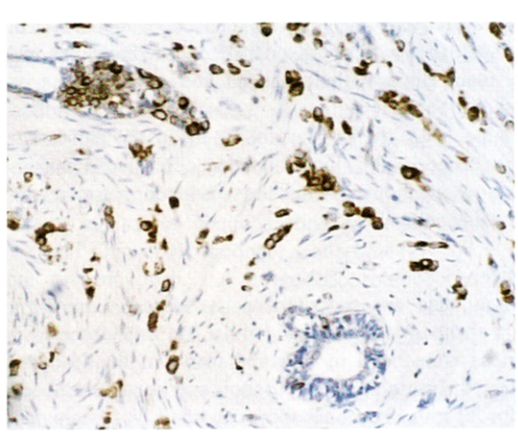

Fig. 1. Immunoreactivity for c-*erb*B-3 protein (RTJ1 antibody) in a human primary breast cancer. Picture shows generalised and high (+++) staining (× 250).

Table 2. Membrane c-erbB-3 and c-erbB-2 oncoprotein expression in breast carcinoma detected by immunocytochemical staining with RTJ1 and 21N antibodies, respectively

	c-*erb*B-3		c-*erb*B-2	
	No.	%	No.	%
Negative	74	35	161	76
Positive				
Overall	138	65	51	24
+	53		10	
++	57		16	
+++	28		25	

Score: +, less than 25% of stained cells; ++, 26–50% of stained cells; +++, > 51% of stained cells.

(24%; Table 2). One hundred and sixty-one tumours (76%) showed cytoplasmic staining of tumour cells, but we did not observe a significant association between membrane and cytoplasmic staining.

A significant association was observed between membrane staining with the two antibodies (OR = 2.035; $P = 0.05$). However, 12 cases were strongly positive for the 21N antibody and negative for RTJ1, whereas 20 cases were negative for 21N and strongly positive for RTJ1. In only 8 cases was there strong positivity for both the antibodies, but 62 cases were 21N− and RTJ1−. Thus, it seems unlikely that the association found between the two markers was due to antibody cross-reaction. Biochemical studies performed previously also support this behaviour [12,13].

Association of expression of the two receptor proteins with other features

c-*erb*B-3 protein positivity was not significantly associated with the other clinical (age and menopausal status) or pathological characteristics (histotype, tumour size, grading and steroid hormone receptors) analysed.

21N positivity was significantly associated with small tumour size (OR = 2.12; $P = 0.02$) and ductal histotype (OR = 3.12; $P = 0.04$). In fact, 28% of the ductal carcinomas (45/159) were 21N positive versus only 11% (4/36) of the lobular carcinomas and 12% (2/17) of the other rare histotypes.

Clinical results

After a 63-month median follow-up of the patients, the probabilities of OS and RFS were 89.1 and 82.2%, respectively. 38 patients had disease recurrence and 25 patients died, 8 from causes unrelated to their tumour.

The correlation between pathobiological features and outcome was investigated only with regard to RFS, in fact our follow-up period is too short to adequately evaluate OS.

Univariate analysis

We found that c-*erb*B-3 positivity was not associated with recurrence. These results were obtained both considering any degree of RTJ1 positivity (+++, ++ and + versus −; $\chi^2 = 0.72$; $P = 0.39$; OR = 0.73) and applying a more restrictive criteria considering c-*erb*B-3 staining as a dichotomous variable (+++ versus all other categories), in accordance with the criteria published previously by Lemoine *et al.* [12] (Fig. 2a and b; $\chi^2 = 0.38$; $P = 0.53$; OR = 1.36).

Moreover, we did not find that 21N positivity significantly correlated with RFS ($\chi^2 = 0.77$; $P = 0.37$; Fig. 3). Among all variables analysed, only tumour size and grade were significantly associated with clinical outcome (Table 3).

Multiple regression analysis

We considered a model including, besides c-*erb*B-3 and c-*erb*B-2 protein expression, those variables which were significant in the RFS univariate analysis.

As reported in Table 4, only tumour size retained a prognostic value near significance ($P = 0.07$), whereas grade and receptor gene expression give no additional information on prognosis. The results were similar when c-*erb*B-3 was entered as a dichotomous variable.

Overall, these suggest that the most important prognostic variable in this series is tumour size. The contribution of c-*erb*B-

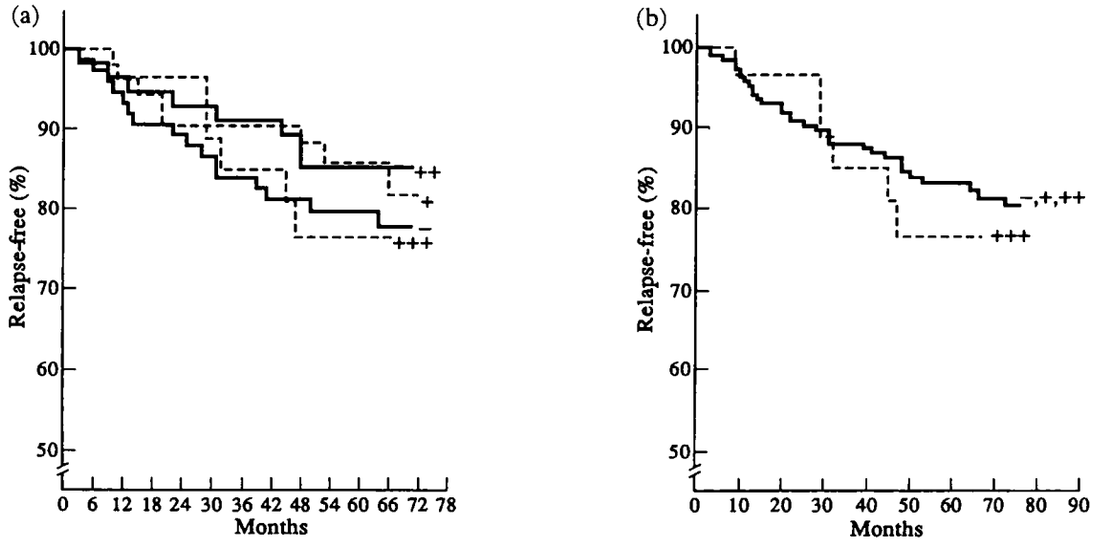

Fig. 2. (a) Relapse-free survival (RFS) in the three subsets of RTJ1 positive and RTJ1 negative patients: +, 53 patients; ++, 57 patients; +++, 28 patients; −, 74 patients: (b) RFS in RTJ1 +++ (28 patients) versus other categories (184 patients) of patients; $\chi^2 = 0.38$ and $P = 0.53$.

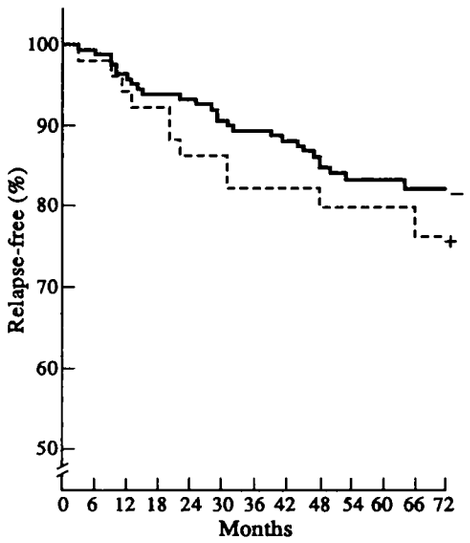

Fig. 3. Relapse-free survival in 21N positive (51 patients) versus 21N negative (161 patients) patients; $\chi^2 = 0.77$ and $P = 0.37$.

Table 3. Univariate analysis at 5 years on relapse-free survival (RFS)

Variable	RFS: Odds ratio (95% confidence intervals)	χ^2	P
c-*erb*B-3			
Negative* versus positive	0.73 (0.34–1.55)	0.72	0.39
c-*erb*B-2			
Negative* versus positive	1.42 (0.63–3.25)	0.77	0.37
Age			
<60* versus >60	0.88 (0.42–1.88)	0.10	0.74
Menopausal status			
Pre* versus peri	1.12 (0.46–2.70)	0.07	0.78
Pre versus post	0.39 (0.10–1.44)	0.07	0.78
Histotype			
Ductal* versus lobular	0.39 (0.10–1.44)	2.06	0.15
Ductal versus others	1.16 (0.34–3.97)	0.06	0.79
Tumour size			
pT_1* versus pT_2-pT_3	2.52 (1.21–5.40)	5.95	0.014
Grading			
I* versus II	2.79 (0.58–13.76)	1.71	0.191
I versus III	5.02 (1.02–25.95)	3.94	0.047
ER-ICA			
−* versus +/++	0.58 (0.24–1.31)	1.76	0.18
PgR-ICA			
−* versus +/++	0.64 (0.30–1.35)	1.42	0.23

* Reference category.

Table 4. Multivariate log-logistic regression model on relapse-free survival (RFS)

Variable	RFS: Odds ratio (95% confidence intervals)	χ^2	P
Tumour size			
pT1* versus pT2	2.02 (0.92– 4.53)	3.16	0.07
Grade			
GI* versus GII	2.60 (0.52–13.92)	1.43	0.23
GI* versus GIII	3.94 (0.74–21.69)	2.67	0.10
c-*erb*B-3			
Negative * versus positive	0.60 (0.27– 1.31)	1.74	0.18
c-*erb*B-2			
Negative* versus positive	1.45 (0.62– 3.47)	0.78	0.37

* Reference category.

3 in predicting RFS is negligible, also in bivariate analyses when patients were classified in subsets on the basis of RTJ1 staining and tumour size or ER status.

DISCUSSION

Adopting an immunocytochemical assay and the novel RTJ1 monoclonal antibody developed by Rajkumar *et al.* [13], we found that 65% of the NNBC tested had varying levels of membrane expression of the c-*erb*B-3 protein, the most recently described member of the type I receptor family. However, only 13% of the carcinomas had strong and generalised over-expression, with more than 50% of membranes labelled. This frequency of positivity is similar to that recently reported by Lemoine *et al.* [12] using polyclonal antibodies. In fact, they showed that the majority of human breast cancers have "normal" immunoreactivity for the c-*erb*B-3 protein, but only 15% of their NNBC presented a staining "more intense" than in normal tissue.

While the polyclonal antibodies used by Lemoine *et al.* [12] always gave cytoplasmic staining in breast cancer, the monoclonal antibody RTJ1 gave both strong membrane-associated and cyctoplasmic staining, with low background and a clear picture of the heterogeneity of protein expression in tumour cells, as reported previously by Rajkumar *et al.* [13]. Furthermore, we observed a highly significant correlation between membrane and cytoplasmic RTJ1 staining ($P = 0.0001$). In our series, expression of c-*erb*B-3 was present in all histological types and was independent from the main clinicopathological features. It was significantly associated only with c-*erb*B-2 protein expression.

As far as c-*erb*B-2 expression is concerned, 24% of primary tumours were 21N positive, with 12% presenting strong and generalised membrane staining, with at least 50% of cells labelled. This percentage of positivity agrees with others recently reported in large series of NNBC by Allred *et al.* [6], Gusterson *et al.* [25] and Ciocca *et al.* [26] (varying from 14.3 to 17.8%).

Moreover, our results confirm the observation of Allred *et al.* [6] and Toikkanen *et al.* [27] that c-*erb*B-2 positivity is significantly higher in the ductal histotype when compared to other histological types. In the present series, only four of 36

lobular invasive carcinomas, and two of 17 of the rare histotypes were 21N positive versus 28% of the ductal invasive tumours. c-*erb*B-2 expression was also significantly more frequent in smaller tumours.

Univariate analyses showed that c-*erb*B-3 expression was not predictive for RFS in our series. Similarly, Lemoine *et al.* [12] did not demonstrate a relationship between c-*erb*B-3 positivity and patient outcome in a series of 195 cases, including both node-negative and node-positive breast cancers, even if their follow-up was much longer. c-*erb*B-2 expression did not correlate with disease outcome in the present series, in concordance with negative studies already reported in the literature (see [6] for a review).

One possible interpretation for these findings is that the small number of NNBC cases examined in the study combined with the low number of events (deaths or relapses), the relatively short follow-up time and with the low frequency of c-*erb*B-3 and c-*erb*B-2 positivity, render an effect on outcome which fails to emerge as statistically significant. Our results, however, do suggest that if there is an association between the expression of the analysed genes with relapse, it is not very strong. Thus, substantially larger series of NNBC cases would be required to reliably confirm or exclude a possible prognostic value for either c-*erb*B-3 or c-*erb*B-2 expression in this setting.

Among conventional clinicopathological features, we confirm the prognostic value of both tumour size [28] and grade [29]. In multivariate analysis, only tumour size was an independent factor, even though it did not reach a statistically significant level.

Our results, due to the lack of prognostic relevance for both the oncogenes studied, are frustrating in the search for new prognosticators, however, they may be relevant for future developments in cancer therapy. In fact, there is evidence that growth factor signalling pathways could be manipulated therapeutically [1]. Antibodies have been raised against extracellular epitopes of c-*erb*B-2, which are able to inhibit *in vitro* the proliferation of those human breast carcinomas expressing the protein [30]. In particular, the 4D5 antibody to c-*erb*B-2, enhances the antiproliferative effects of both tumour necrosis factor alpha and diamminedichloroplatinum (CDDP) on breast cancer oncogene-positive cell lines, both *in vitro* and *in vivo* [30,31]. Shepard *et al.* [32] are evaluating in phase I/II clinical trials the therapeutic efficacy, in human breast cancer, of monoclonal antibody therapy to c-*erb*B-2 in combination with CDDP. As recently reviewed [33], for better management of breast cancer patients, we need both new prognostic and predictive indicators to be able to identify those patients at high risk eligible for adjuvant therapy, and the specific therapy to which each patient is more likely to benefit. In this setting, the identification of the human breast cancers which express the receptor protein for c-*erb*B-2 and/or c-*erb*B-3, even if these markers do not possess a strong and independent prognostic value in NNBC, could be important in the future to select those patients who are more likely to obtain a response with specific forms of biological therapies (e.g. antibodies against the individual growth factor receptor).

1. Prigent SA, Lemoine NR. The type 1 (EGFR-related) family of growth factor receptors and their ligands. *Prog Growth Factor Res* 1992, **4**, 1–24.
2. Wen D, Peles E, Cupples R, *et al.* *Neu* differentiation factor: a transmembrane glycoprotein containing an EGF domain and an immunoglobulin homology unit. *Cell* 1992, **69**, 559–572.
3. Gullick WL. The role of the epidermal growth factor receptor and the c-*erb*B-2 protein in breast cancer. *Int J Cancer* 1990, Suppl. 5. 55–61.
4. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/*neu* oncogen. *Science* 1987, **235**, 177–182.
5. Levine MN, Andrulis I. The HER-2/*neu* oncogene in breast cancer: so what is new? (Editorial). *J Clin Oncol* 1992, **10**, 1034–1036.
6. Allred DC, Clark GM, Tandon AK, *et al.* HER-2/*neu* in node negative breast cancer: prognostic importance of overexpression influenced by the presence of *in situ* carcinoma. *J Clin Oncol* 1992, **10**, 599–605.
7. Elledge RM, McGuire WL, Osborne CK. Prognostic factors in breast cancer. *Sem Oncol* 1992, **19**, 244–253.
8. Gasparini G, Bevilacqua P, Pozza F, *et al.* Value of epidermal growth factor receptor status compared with growth fraction and other factors for prognosis in early breast cancer. *Br J Cancer* 1992, **66**, 970–976.
9. Nicholson S, Sainsbury JRC, Halcrow P, *et al.* Epidermal growth factor receptor (EGFr); results of a 6 year follow-up study in operable breast cancer with emphasis on the node-negative subgroup. *Br J Cancer* 1991, **63**, 146–151.
10. Kraus MH, Issing W, Miki T, Popescu NC, Aaronson SA. Isolation and characterization of ERBB3, a third member of the ERBB/epidermal growth factor receptor family: evidence for overexpression in a subset of human mammary tumors. *Proc Natl Acad Sci USA* 1989, **86**, 9193–9197.
11. Plowman GD, Whitney GS, Neubauer MG, *et al.* Molecular cloning and expression of an additional epidermal growth factor receptor-related gene. *Proc Natl Acad Sci USA* 1990, **87**, 4905–4909.
12. Lemoine NR, Barnes DM, Hollywood DP, *et al.* Expression of the *erb*B3 gene product in breast cancer. *Br J Cancer* 1992, **66**, 1116–1121.
13. Rajkumar T, Gooden CSR, Lemoine NR, Gullick WJ. Expression of the c-*erb*B-3 protein in gastrointestinal tract tumours determined by monoclonal antibody RTJ1. *J Pathol* 1993, **170**, 271–278.
14. Veronesi U, Saccozzi R, Del Vecchio M, *et al.* Comparing radical mastectomy with quadrantectomy, axillary dissection and radiotherapy in patients with small cancers of the breast. *N Engl J Med* 1981, **305**, 6–11.
15. Gasparini G, Gullick WJ, Bevilacqua P, *et al.* Human breast cancer: prognostic significance of the c-*erb*B-2 oncoprotein compared with epidermal growth factor receptor, DNA ploidy, and conventional pathologic features. *J Clin Oncol* 1992, **10**, 686–695.
16. Fisher ER, Gregorio RM, Fisher B. The pathology of invasive breast cancer. A syllabus derived from findings of the National Surgical Adjuvant Breast Project (protocol no. 4). *Cancer* 1975, **36**, 1–85.
17. Bloom HIG, Richardson WW. Histological grading and prognosis. *Br J Cancer* 1957, **11**, 359–377.
18. Andersen J, Orntoft TF, Poulsen HS. Immunohistochemical demonstration of estrogen receptors (ER) in formalin-fixed paraffin-embedded human breast cancer tissue by use of a monoclonal antibody to ER. *J Histochem Cytochem* 1988, **36**, 1553–1560.
19. Bevilacqua P, Pea M, Gasparini G. Immunocytochemical detection of progesterone receptor by monoclonal KD-68 antibody in operable breast cancer: correlations with biochemical assay, pathological features and cell proliferative rate. *Eur J Cancer Clin Oncol* 1989, **25**, 1595–1602.
20. Gullick WJ, Berger MS, Bennet PLP, Rothbard JB, Waterfield MD. Expression of the c-*erb*B-2 protein in normal and transformed cells. *Int J Cancer* 1987, **40**, 246–254.
21. Kaplan EL, Meyer P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958, **53**, 457–481.
22. Gelber RD, Goldhirsch A for the International Breast Cancer Study Group (formerly Ludwing Group). Reporting and interpreting adjuvant therapy clinical trials. *J Natl Cancer Inst Monogr* 1992, **11**, 59–69.
23. Bennet S. Log-logistic regression models for survival data. *Appl Stat* 1983, **32**, 165–171.
24. Gore SM, Pocock SJ, Kerr GR. Regression models and nonproportional hazards in the analysis of breast cancer survival. *Appl Stat* 1984, **33**, 176–195.
25. Gusterson BA, Gelber RD, Goldhirsch A, *et al.* Prognostic importance of the c-*erb*B-2 expression in breast cancer. *J Clin Oncol* 1992, **10**, 1049–1056.

26. Ciocca DR, Fujimura FK, Tandon AK, *et al.* Correlation of HER-2/neu amplification with expression and with other prognostic factors in 1103 breast cancers. *J Natl Cancer Inst* 1992, **84**, 1279–1282.
27. Toikkanen S, Helin H, Isola J, Joensuu H. Prognostic significance of HER-2 oncoprotein expression in breast cancer: a 30-year follow-up. *J Clin Oncol* 1992, **10**, 1044–1048.
28. Carter CL, Allen C, Henson DE. Relationship of tumor size, lymph nodes status, and survival in 24 740 breast cancer cases. *Cancer* 1989, **63**, 181–187.
29. Contesso G, Saccani Jotti G, Bonadonna G. Tumor grade as a prognostic factor in primary breast cancer. *Eur J Cancer Clin Oncol* 1989, 25, 403–409.
30. Hudziak RM, Lewis GD, Winget M, Fendly BM, Shepard HM, Ulrich A. Monoclonal antibody has antiproliferative effects *in vitro* and sensitizes human breast tumor cells to tumor necrosis factor. *Mol Cell Biol* 1989, **9**, 1165–1172.
31. Hancock MC, Langton BS, Chan T, *et al.* A monoclonal antibody against the c-*erb*B-2 protein enhances the cytotoxicity of *cis*-diamminedichloroplatinum against human breast and ovarian tumor cell lines. *Cancer Res* 1991, **51**, 4575–4580.
32. Shepard HM, Lewis GD, Sarup JC, *et al.* Monoclonal antibody therapy of human cancer: taking the *HER* 2 protooncogene to the clinic. *J Clin Immunol* 1991, **11**, 117–127.
33. Gasparini G, Pozza F, Harris AL. Evaluating the potential usefulness of new prognostic and predictive indicators in node-negative breast cancer patients. (Review). *J. Natl Cancer Inst* 1993, **85**, 1206–1219.

Acknowledgements—The authors wish to thank Mrs Daniela Mazzocco and Miss Lucia Regolin for the preparation of the manuscript. This study was supported in part by a grant of the Associazione Italiana per la Ricerca sul Cancro (A.I.R.C), Milan, Italy.

Pergamon

European Journal of Cancer Vol. 30A, No. 1, pp. 22–27, 1994

0959–8049/94 $6.00 + 0.00

Effect of High-dose Dexamethasone in Carcinomatous Metastatic Spinal Cord Compression Treated with Radiotherapy: a Randomised Trial

Per Soelberg Sørensen, Susanne Helweg-Larsen, Henning Mouridsen and Heine Høi Hansen

We performed a randomised single blind trial of high-dose dexamethasone as an adjunct to radiotherapy in patients with metastatic spinal cord compression from solid tumours. After stratification for primary tumour and gait function, 57 patients were allocated randomly to treatment with either high-dose dexamethasone or no steroidal treatment. Dexamethasone was administered as a bolus of 96 mg intravenously, followed by 96 mg orally for 3 days and then tapered in 10 days. A successful treatment result defined as gait function after treatment was obtained in 81% of the patients treated with dexamethasone compared to 63% of the patients receiving no dexamethasone therapy. Six months after treatment, 59% of the patients in the dexamethasone group were still ambulatory compared to 33% in the no dexamethasone group. Life table analysis of patients surviving with gait function showed a significantly better course in patients treated with dexamethasone ($P < 0.05$). Median survival was identical in the two treatment groups. Similar results were found in subgroup analysis of 34 patients with breast cancer as the primary malignancy. Significant side-effects were reported in 3 (11%) of the patients receiving glucocorticoids, 2 of whom discontinued the treatment. We conclude that high-dose glucocorticoid therapy should be given as adjunct treatment in patients with metastatic epidural spinal cord compression.
Eur J Cancer, Vol. 30A, No. 1, pp. 22–27, 1994

INTRODUCTION

COMPRESSION OF the spinal cord or cauda equina by epidural metastasis is often a devastating complication to systemic cancer. The definitive treatment is either radiation therapy, surgical decompression or a combination of the two. It is still debatable whether surgical decompression offers any advantages compared to treatment with radiotherapy alone [1–4]. A large retrospective analysis suggested a superior effect of the combination of laminectomy and radiotherapy compared to radiotherapy alone in patients who had lost gait function due to spinal cord