More About: Prognostic Importance of Low c-erbB2 Expression in Breast Tumors

The Journal recently published correspondence reporting a debate about a particularly low expression of c-erbB2 protein in breast cancer patients, which, as in the case of very high levels of the protein, could indicate a poor prognosis *(1,2)*. That low c-erbB2 protein concentrations may have a different meaning from that of intermediate levels of the protein is not a recent finding. In 1992, by use of an enzyme-linked immunosorbent assay (ELISA) (Oncogene Science, Uniondale, NY) in breast tissue homogenates, our group *(3)* reported a nonmonotonic relationship of c-erbB2-encoded protein p185 with estrogen and progesterone receptors in breast cancer tissue. In further studies, we found a strong association between both ELISA and western blot analysis with an immunohistochemical method *(4,5)*. However, when we divided breast cancer tissue samples into three groups, both the samples with low levels of p185 and those with high levels were associated with low estrogen and progesterone receptor levels and with a high percentage of lymph node-positive tumors *(4,5)*. These findings were confirmed by other groups [*(6)* and references in *(2)*].

In a small number of tissue samples, we found that both low and high p185 concentrations indicate a similar high risk of relapse when compared with tissue samples with intermediate levels of p185 *(7)*. Three further studies [reported in *(2)*] confirmed similar prognostic behavior, while one did not *(1)*.

All of these papers *(1–7)* are, however, only preliminary studies. Although Ferrero-Poüs et al. *(1)* evaluated more patients than the number reported in previous studies, they performed only an univariate analysis, and the patients whose tissues they examined were not homogeneous with respect to lymph node status and therapies received. It is worth noting that we recently have separately evaluated groups of 100 lymph node-negative and 141 lymph node-positive breast cancer specimens, with a median follow-up of 53 months. By dividing the groups into quartiles, we found that the nonmonotonic prognostic significance (with the first and the fourth quartiles showing the same negative prognostic indication) is restricted to lymph node-positive cases (two-sided $P = .001$ by the log-rank test). Obviously, these results are also preliminary, and we are planning to enlarge the case series to evaluate separately the prognostic significance in lymph node-positive patients treated with chemotherapy and those treated with hormonal therapy. The study will be carried out by use of modeling that allows for an analysis of the concentration of p185 as a continuous variable, avoiding the need for determination of cut points. Similarly, a longer follow-up is advisable to better evaluate the new data by Koscienly et al. *(2)*, as correctly pointed out by the authors.

We believe that biochemical immunometric methods should be taken into account, at least in the design of clinical studies, for the assessment of p185 expression and its prognostic significance. They allow for quantitative and reproducible results in milligrams of tissue, which are more likely than the microgram samples assessed routinely in immunohistochemical analyses to be truly representative of the tissue. In addition, both analytic standardization and quality control are more feasible.

While the publication of results from pilot studies was certainly necessary to reveal the findings and to stimulate debate, we believe that the next step is to produce more robust studies with a sufficient number of cases in homogeneous groups of patients and possibly validated with an independent set of patients. We believe that additional provisional investigations should be discouraged to avoid the risk of disseminating misinformation about a possibly promising prognostic parameter.

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NOTES

RESPONSE

Dittadi et al. have rekindled the debate on the adverse prognostic value of low c-erbB2 expression after publication in the Journal of our results on 488 primary breast cancer patients *(1)*. Our study did not confirm the detrimental impact of low c-erbB2 expression (protein or RNA) that had been described in few studies using quantitative methods *(2–5)* or the nonmonotonic relationship between c-erbB2 and steroid receptor expression *(3,4,6,7)*. Most studies involving thousands of patients have shown that c-erbB2 gene amplification or overexpression is negatively related to steroid hormone receptors and is associated with a poor prognosis. In our updated database (June 2000, median follow-up of 10 years), the highest frequency of metastasis is still restricted to patients with high c-erbB2, both in the

Table 1. Definition and percentage of patients with "low" c-erbB2 expression in primary breast cancer*

Investigator, y (reference No.)	No. of patients	Method used (supplier)	Definition of low c-erbB2 expression	Percentage of patients with low c-erbB2 values	Adverse prognostic value of low c-erbB2
Dittadi et al., 1992 (6)	130	ELISA (Oncogene Science)	Concentrations up to the highest nonmalignant tissue level	57.8	ND
Pifanelli et al., 1996 (7)	185	EIA (Triton Diagnostic)	Concentrations up to the highest nonmalignant tissue level	44.9	ND
Dittadi et al., 1997 (3)	115	ELISA (Oncogene Science)	<First quartile	25	Yes
Koscielny et al., 1998 (2)	117†	EIA (Triton Diagnostic)	<Geometric mean minus one standard deviation	8.5	Yes
Koscielny et al., 1998 (4)	1062	EIA (Triton Diagnostic)	<Geometric mean minus one standard deviation	10.4	Yes
Ferrero-Poüs et al., 1999 (1)	488	EIA (Triton Diagnostic)	<Geometric mean minus one standard deviation	9	No
Pawlowski et al., 1999 (5)	404	Real time RT–PCR	$<0.4 \times 10^{-6}$	Not specified	Yes

*ELISA = enzyme-linked immunosorbent assay; EIA = enzyme immunoassay; ND = not determined; RT–PCR = reverse transcription–polymerase chain reaction.

†These patients are included in (4).

overall population and in subgroups defined by lymph node status, and we confirmed the strong inverse relationship between c-erbB2 and steroid receptors (not shown).

To determine whether so-called low c-erbB2 expression in the different analyses corresponds to identical subsets of patients, we have compared the published definitions of these "low" values. As shown in Table 1, the percentage of patients with low c-erbB2 values varied widely from study to study (8.5%–57.8%). In some studies, low c-erbB2 concentrations (lower than the highest level in nonmalignant tissue) were attributed to about 50% of the tumors that did not display c-erbB2 overexpression (3,6,7). In one study (3), low c-erbB2 expression was defined by values under the first quartile. In other studies (1,2,4), low levels were attributed to about 10% of the tumors with very low c-erbB2 expression, which could be qualified as c-erbB2-underexpressing tumors, but there is no consensus on whether these patients have a poorer outcome (1,4). Regarding the nonmonotonic relationship between c-erbB2 expression and hormone receptor status, failure to report certain patient characteristics sometimes hinders comparisons among studies.

Three published studies or abstracts (3–5) have examined the potential independent prognostic impact of low c-erbB2 values in multivariate analyses. Two of these reports (3,5) pooled low and high values to form a high-risk group relative to patients with intermediate values. This approach is mislead-

ing. The only valid approach is to identify patients with low expression and to determine if this variable fits the model (4). Moreover, in all cases, including our study, patient populations are heterogeneous with respect to lymph node status and adjuvant therapy.

Therefore, published studies focused on tumors with "low" c-erbB2 expression involve quite different categories of patients and are difficult to interpret. At present, the main clinical interest of c-erbB2 determination is to identify metastatic patients who will respond to anti-c-erbB2 antibodies (e.g., Herceptin) rather than to consider its prognostic value in primary breast cancer. Regarding the response to antibody-based therapy, only the highest levels of expression, corresponding to gene amplification, are considered. Quantitative biochemical methods could help to determine the optimal cutoff for this purpose in comparison with quantitative reverse transcription–polymerase chain reaction, fluorescence *in situ* hybridization, and immunohistochemistry.

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