neu determination may identify patients with a worse prognosis better than approaches using immunohistochemistry or fluorescence in situ hybridization (14).

Increased serum HER-2/neu was detected in 12% of an overall breast cancer group (15) and provided independent prognostic information (12) that was superior to that from nodal involvement or hormone receptor status (15). Preoperative HER-2/neu concentrations in sera are significantly associated with the tissue marker (16). Thus, it seemed opportune to reduce further exploratory studies on HER-2 serology to patients with HER-2/neu-overexpressing tissues. However, the main investigations were performed without selecting a subgroup of patients receiving trastuzumab therapy. We here present the first study of the longitudinal course of serologic HER-2/neu values in patients receiving trastuzumab.

The HER-2/neu values were detected using a HER-2/neu method on the Bayer Immuno 1 system. We confirmed that the data collection was fast and reproducible and could be incorporated into clinical routine easily (13, 17). Both serum and plasma are acceptable samples (18).

Retrospectively, HER-2/neu testing indicated remission or disease progression in 74% of all cases. The sensitivity increased when the patients were restricted to metastatic breast cancer patients with visceral metastases. However, increased serum concentrations have been reported in benign diseases, including liver cirrhosis and hepatitis (19). One question that remains unanswered is whether the high sensitivity in breast cancer with liver metastases reflects an acquired liver dysfunction or whether HER-2/neu-overexpressing breast tumor cells preferentially affect the liver rather than bones or the lung. Theoretically, the environment of bone and lung may induce a decrease in HER-2/neu expression. Taken together, HER-2/neu appears not to be a specific breast cancer marker (20), but because of its sensitivity, the serologic HER-2/neu determination may provide an additional tool to monitor trastuzumab therapy of breast cancer patients. Accordingly, serologic HER-2/neu determinations might be useful to improve outcome by avoiding time lost through ineffective regimens and by avoiding exacerbated side effects and unnecessary costs.

In summary, plasma HER-2/neu parallels the clinical course in patients with metastatic breast cancer, especially in patients with visceral metastases.

We thank Thérèse Tanasale for excellent technical assistance and Sibylle Fechner for preparing the graphs.

References

5. Hudziak RM, Schlessinger J, Ullrich A. Increase expression of the putative growth factor receptor p185HER-2 causes transformation and tumorigene-
carcinoembryonic antigen, which are widely used serum markers for breast cancer, can be increased in patients with most types of adenocarcinoma, especially if distant metastases are present [for a review, see Ref. (2)]. Using a modified differential display technique, Watson and Fleming (3, 4) identified a novel gene that appeared to be expressed only in breast tissue. This gene, which was designated mammaglobin (MG), was found to encode a 93-amino-acid protein with a predicted molecular mass of 10.5 kDa (4).

MG mRNA expression was reported to be increased at least 10-fold relative to nonmalignant breast tissue in 8 of 35 (23%) breast carcinomas (4), and MG protein was detected by immunohistochemistry in 81 of 100 breast cancers (5). MG was detected by quantitative reverse transcription-PCR in 31 of 42 (74%) primary breast cancers (6) and was either undetectable or present at low concentrations in small numbers of nonbreast tissues (4–6).

In 1998, Becker et al. (7) identified a new gene with marked sequence identities to MG. This new gene was named mammaglobin B (MGB) (7), and the original MG was redesignated mammaglobin A (MGA). Both MGA and MGB and several related genes [e.g., Clara cell 10-kDa protein, lipophillin A, and lipophillin B (LPB)] are localized in a dense cluster on chromosome 11q12.2 (8). Colpitts et al. (9) recently reported that MGA in breast tissue is found as a complex with LPB.

The aim of this study was to search for expression of MGA, MGB, and LPB in nopathologic and pathologic breast tissue as well as in various nonbreast tissues. In the breast carcinomas, we also correlated expression of all three species with established prognostic factors.

Details of the breast cancers used are summarized in Table 1 in the data supplement [available with the online version of this Technical Brief at Clinical Chemistry Online (http://www.clinchem.org/content/vol48/issue7)]. After pathologic assessment, tissues were snap-frozen in liquid nitrogen and then stored at −80 °C. Breast cancers were assayed for estrogen receptor (ER) and progesterone receptor (PR) by ELISA (Abbott Diagnostics). The cutoff points for ER and PR were 200 and 1000 fmol/g wet weight of tissue, respectively. Total RNA was extracted by the guanidinium thiocyanate method (10).

In a final volume of 20 μL, 1 μg of total RNA was reverse transcribed to cDNA using 200 U of Moloney murine leukemia virus reverse transcriptase (Invitrogen). cDNA was amplified using the primers previously described for MGA (11) and MGB (12). LPB primers were designed against regions in exon 1 (5′-CACCTCATTGTGTGAAGCTG-3′) and exon 3 (5′-GACAGTGGAAC-CAGGATGA-3′) of the LPB cDNA (NM_006551).

PCR was performed in 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1 g/L Triton X-100, 0.2 mM each deoxynucleotide triphosphate, 20 pmol of each primer (Genosys), 2 μL of cDNA, 1.5 mM MgCl₂, and 1 U of Taq DNA polymerase (Promega) in a final volume of 50 μL. All PCR reactions were performed in an automated thermocycler (MJ Research). The amplification conditions for each of the primer sets were as follows (11, 12): MGA: 30 cycles of 1 min at 94 °C, 1 min at 58 °C, and 1 min at 72 °C; MGB: 35 cycles of 1 min at 94 °C, 1 min at 62 °C, and 1 min at 72 °C; LPB: 30 cycles of 30 s at 94 °C, 1 min at 60 °C, and 1 min at 72 °C.

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH): 30 cycles of 1 min at 94 °C, 1 min at 62 °C, and 1 min at 72 °C.

After amplification, PCR products were visualized on ethidium bromide-stained agarose gels under ultraviolet light. As a control, PCR with primers specific for the housekeeping gene GAPDH was carried out on each sample. RNA isolated from BT-474 breast cancer cells, stimulated with phorbol-12-myristate-13-acetate for 6 h, was used as a positive control for both MGA and MGB. RNA isolated from MDA-MD-415 breast cancer cells was used as a positive control for LPB. Negative controls included (a) omission of reverse transcriptase and (b) omission of cDNA. The MGA, MGB, and LPB PCR products were confirmed by direct sequencing using the ABI Prism 310.

The distribution of MGA, MGB, and LPB mRNA positivity in nonmalignant breast tissues, fibroadenomas, and primary breast cancers is summarized in Table 1. For each mRNA species, the frequency of detectable expression was similar in nonmalignant breast tissue, fibroadenomas, and breast carcinomas. Of the 97 cancers studied for all three transcripts, 40 (41%) were positive for all three, whereas 76 (78%) were positive for MGA, MGB, or LPB. Significant agreement was observed between the expressions of MGA and LPB in the breast carcinomas (n = 96; P < 0.0001; χ² = 54.29). Detectable expression of MGB also corresponded with LPB expression (n = 96; P = 0.0007; χ² = 11.59).

No significant relationship was found between the rate of detection of any of the mRNA species and tumor size, axillary node status, or PR status (for details, see Table 2 in the data supplement). However, MGA was found more frequently in ER-positive (63 of 88; 72%) than ER-negative

---

Table 1. MGA, MGB, and LPB mRNA in nonmalignant breast tissue, fibroadenomas, and breast carcinomas.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>MGA</th>
<th>MGB</th>
<th>LPB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>No. positive</td>
<td>% positive</td>
</tr>
<tr>
<td>Nonmalignant breast</td>
<td>21</td>
<td>9</td>
<td>43</td>
</tr>
<tr>
<td>Fibroadenoma</td>
<td>32</td>
<td>19</td>
<td>59</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>142</td>
<td>86</td>
<td>61</td>
</tr>
</tbody>
</table>

---

References:
1. Watson CF, Fleming A. 2002. Modified differential display technique. Watson and Fleming (3, 4) identified a novel gene that appeared to be expressed only in breast tissue. This gene, which was designated mammaglobin (MG), was found to encode a 93-amino-acid protein with a predicted molecular mass of 10.5 kDa (4).
2. Becker et al. 1998. MGA, MGB, and LPB and several related genes were identified with marked sequence identities to MG.
3. Colpitts et al. 1998. MGA in breast tissue is found as a complex with LPB.

---

Clinical Chemistry 48, No. 8, 2002 1363
carcinomas (22 of 49; 45%; \( P < 0.01 \)). Similarly, LPB expression was found more frequently in ER-positive (43 of 53; 81.1%) than ER-negative samples (20 of 38; 52.6%; \( P = 0.007 \)). MGB, on the other hand, showed no relationship with ER status.

Of 24 nonmalignant nonbreast tissues, MGA was detected in only 1, i.e., in a sample of nonmalignant ovarian tissue. MGA was not found in any of eight nonbreast carcinomas (for details, see Tables 3 and 4 in the online data supplement). In contrast to MGA, MGB was detected in 4 of 15 nonmalignant breast tissues and in 1 of 7 nonbreast malignant samples, whereas LPB was present in 3 of 10 nonmalignant breast tissues and in 4 of 8 nonbreast malignant tissues.

In addition to sensitivity for early disease and specificity for cancer, organ specificity is a desirable property of a tumor marker. At present, prostate-specific antigen is a rare example of a marker with relative organ specificity. Our data presented here together with other reports (4–6) suggest that expression of MGA is mostly, although not exclusively, confined to breast tissue. MGA may therefore be one of the first relatively mammary-specific markers. Its potential applications include (a) the detection of breast cancer micrometastasis in lymph nodes, peripheral blood, and bone marrow; (b) identification of metastasis of unknown origin, i.e., presence in such a metastasis would suggest a likely breast origin; and (c) detection of early breast cancer. The last might be possible with the development of an ELISA for measuring MGA and its application to serum or urine.

References