Mammaglobin vs GCDFP-15

An Immunohistologic Validation Survey for Sensitivity and Specificity

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Abstract

There are limited data that compare the usefulness of mammaglobin with gross cystic disease fluid protein-15 (GCDFP-15) in the identification of breast carcinomas. Whole tissue sections of 29 breast carcinomas with matched lymph node metastases and 63 breast carcinomas on tissue microarray were stained with mammaglobin cocktail and GCDFP-15 antibodies. In addition, tissue microarrays (US Biomax, Rockville, MD) containing 544 different human tumors were also stained with the mammaglobin antibody cocktail. Positive staining was seen in 67 (55.4%) of 121 breast carcinomas with mammaglobin and in 28 cases (23.1%) with GCDFP-15. In the majority of cases, the staining intensity and number of cells staining were higher with mammaglobin than with GCDFP-15. Positive mammaglobin staining was also seen in 44 (8.1%) of 544 nonbreast tumors. Mammaglobin is a more sensitive marker than GCDFP-15 for breast carcinoma; however, it lacks the specificity of GCDFP-15.

The mammaglobin gene sequence fragments were first isolated in 1994 by Watson and Fleming[1] using a modified differential display polymerase chain reaction technique. A novel full-length complementary DNA clone was isolated by the same authors in 1996, which they designated as mammaglobin-1 (*MGB1*).[2] Mammaglobin is a secretory protein that has a predicted molecular mass of 10.5 kd and shares a high degree of homology with the rat prostatic steroid-binding protein subunit C3, human Clara cell 10-kd protein, and rabbit uteroglobin.

The *MGB1* gene has been mapped to chromosome 11q12.3-q13.1 by fluorescence in situ hybridization (FISH).[3] Chromosome 11q13 is frequently amplified in breast carcinomas, but gene amplification or gross rearrangements have not been detected in breast tumors or cell lines.[3] In contrast, mammaglobin protein overexpression has been found in breast carcinomas. Watson and Fleming[2] found that 8 (23%) of 35 primary breast carcinomas overexpressed MGB1 relative to normal breast tissue specimens. Watson et al[3] also found that 5 (50%) of 10 breast carcinoma cell lines and 13 (62%) of 21 metastatic breast tumors exhibited high levels of MGB1 messenger RNA. The overexpression did not seem to correlate with histology, tumor grade, tumor stage, or hormone receptor status. Recent studies have suggested that mammaglobin is specific for breast carcinoma and expression is associated with well-differentiated, receptor-positive tumors.[4-7]

However, mammaglobin has not been extensively studied by immunohistochemical analysis in human tissues. To define the sensitivity of mammaglobin expression in breast tumors and in other malignancies, we studied mammaglobin expression by immunohistochemical analysis in a variety of human tumors. We also compared mammmaglobin sensitivity with that of gross cystic disease fluid protein-15 (GCDFP-15).

Materials and Methods

Breast Carcinoma Whole Tissue Sections

We stained 29 matched primary and metastatic (within lymph node) invasive breast carcinomas (ductal, 14; lobular, 14; mixed, 1) with a mammaglobin antibody cocktail and GCDFP-15.

Breast Carcinoma Tissue Microarray

The tissue microarray (TMA) was constructed from 64 randomly selected, well-characterized, in-house breast carcinomas. Of these 64 cases, 52 were ductal, 9 were lobular, 1 was metaplastic, and 2 were mixed ductal and lobular carcinoma. Three 0.6-mm tissue cores were obtained from 1 to 2 tissue blocks on each case. Four-micrometer TMA sections were stained with the mammaglobin antibody cocktail and GCDFP-15.

TMA Description

TMAs (US Biomax, Rockville, MD) for 12 organ systems were examined. These included 12 TMA slides prepared from formalin-fixed, paraffin-embedded normal and tumor tissues without using tape-transfer technology. Each core's diameter measured 2 mm. The slides were stained with the mammaglobin antibody cocktail.

Slide details are as follows: (1) melanoma array with 80 primary and metastatic melanomas from different sites; (2) ovarian array (80 cores): papillary serous carcinomas, 38; mucinous and clear cell carcinomas, 1 each; and benign ovary samples, 40; (3) endometrial array (63 cores): endometrioid adenocarcinoma, 59; and invasive mole, proliferative endometrium, secretory endometrium, and benign smooth muscle, 1 each; (4) uterine cervical array (80 cores): squamous cell carcinoma, 41; and benign cervical tissue samples, 39; (5) lung array (100 cores): squamous cell carcinoma, 27; adenocarcinoma, 18; and normal lung tissue samples, 55; (6) stomach array (80 cores): adenocarcinoma, 27; undifferentiated carcinoma and signet-ring cell carcinoma, 4 each; mucinous carcinoma and carcinoid tumor, 2 each; and benign stomach tissue samples, 41; (7) colon array (80 cores): adenocarcinoma, 38; signet-ring cell carcinoma, 1; and benign colonic tissue samples, 41; (8) kidney array (80 cores): clear cell carcinoma, 37; undifferentiated carcinoma, 2; B-cell lymphoma, 1; and benign kidney cortex samples, 40; (9) bladder array (80 cores): urothelial carcinoma, 37; adenocarcinoma, 2; squamous cell carcinoma, 1; and benign bladder tissue samples, 40; (10) salivary gland tumor array: adenocarcinoma not otherwise specified and mucoepidermoid carcinoma, 3 each; adenoid cystic carcinoma, basal cell adenocarcinoma, epithelial-myoepithelial carcinoma, pleomorphic adenoma, and undifferentiated carcinoma, 2 each; and acinic cell carcinoma, adenosquamous carcinoma, malignant fibrous histiocytoma, malignant myoepithelioma, mucinous adenocarcinoma, mucus adenocarcinoma, myoepithelioma, sarcomatoid carcinoma, and Warthin tumor, 1 each; (11) skin tumor array: basal cell carcinomas, 13; dermatofibrosarcoma protuberans, 9; malignant schwannoma, 3; squamous cell carcinoma, 14; fibrosarcoma, leiomyosarcoma, and sebaceous adenocarcinoma, 1 each; and sweat gland carcinoma, 10; and (12) pancreas tissue array: pancreatic adenocarcinoma, 67.

Additional Tissue Samples

Owing to the absence of endocervical adenocarcinomas in the tissue arrays, 20 cases of invasive and adenocarcinoma in situ (AIS) were also included in the study.

Immunohistochemical Analysis for Mammaglobin and GCDFP-15

Four-micrometer-thick formalin-fixed, paraffin-embedded sections were immunostained on the Benchmark XT automated stainer (Ventana Medical Systems, Tucson, AZ). The protocol consisted of a pretreatment with CC1, pH 8.0 (Ventana), followed by incubation with mammaglobin mouse (clone 304-1A5) and rabbit (clone 31A5) monoclonal cocktail (Zeta, Sierra Madre, CA) at a 1:25 dilution. In our initial validation, the mammaglobin antibody cocktail demonstrated "crisper" staining without increasing the background noise and, therefore, was preferred over a single antibody for this study. A similar pretreatment protocol was followed for the GCDFP-15 antibody (clone 23A3, Cell Marque, Hot Springs, AR). Antigen-antibody complexes were detected with an IVIEW-DAB (diaminobenzidine) detection system (Ventana). Owing to the presence of melanin in the melanoma array slide, detection was performed via Ventana's "Enhanced V-Red Detection," which is an alkaline phosphatase that uses naphthol and fast red chromogen.

Receptor Status and Grading of Breast Carcinoma

Immunohistochemical analysis for estrogen receptor was performed using the 6F11 antibody and IVIEW detection on the Benchmark XT (Ventana). Immunohistochemical analysis for the progesterone receptor was performed using the 1A6 antibody (Ventana) and IVIEW detection on the Benchmark XT. Any staining was considered as positive staining for estrogen and progesterone receptors. HER-2/neu protein was analyzed and scored using the CB11 antibody (Ventana) and basic DAB detection on the Benchmark XT. Scoring was performed similar to that in the DAKO HercepTest (DAKO, Carpinteria, CA) guidelines. FISH for the HER-2/*neu* gene was performed in 2+ cases. HER-2 positivity was defined as 3+ overexpression or amplification by FISH.

The randomly selected breast carcinomas represented on TMA were graded according to the Nottingham grading criteria. Because there was an equal mix of ductal and lobular carcinomas in the whole tissue section group, only nuclear grading was performed. Nuclear grading of tumor nuclei was performed based on nuclear pleomorphism and graded as nuclear grade 1, 2, or 3.

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Statistical Analysis

Statistical analysis was performed using the Arcus Quickstat software program (Longman Software Publishing, Cambridge, England). The breast whole tissue section group was analyzed separately from the breast TMA group for reasons detailed in the results section. Differences in percentages were analyzed by using the χ^2 test. Differences in means between different groups were analyzed by using the paired *t* test.

Results

Staining with the mammaglobin antibody is characteristically seen in the cytoplasm. The staining intensity in our series ranged from a weak blush to moderate or strong **Image 1**. The amount of cells staining with the antibody was further categorized as focal (<10%), patchy (10%-50%), and diffuse (>50%). For meaningful semiquantitative analysis, focal and/or weak staining was considered equivocal staining, and only patchy or diffuse staining with moderate or strong intensity was considered positive.

A higher percentage of breast carcinomas stained with mammaglobin and GCDFP-15 in the whole tissue section group than in the breast TMA group. Although the breast TMA was constructed with 3-fold redundancy, the often patchy pattern of mammaglobin and GCDFP-15 staining partly accounts for this difference. Moreover, the whole tissue section group contained an equal mix of ductal and lobular carcinomas, whereas the 64 TMA cases were randomly selected and contained only 9 lobular carcinomas (14%). Our study shows that mammaglobin and GCDFP-15 stain a higher percentage of cells in lobular carcinoma than in ductal carcinoma. Within the whole tissue section group, the median percentage of cellular staining with mammaglobin was 25 in ductal carcinoma and 75 in lobular carcinoma. Similarly, the median percentage of cellular staining with GCDFP-15 was 5 in ductal carcinoma and 30 in lobular carcinoma. The

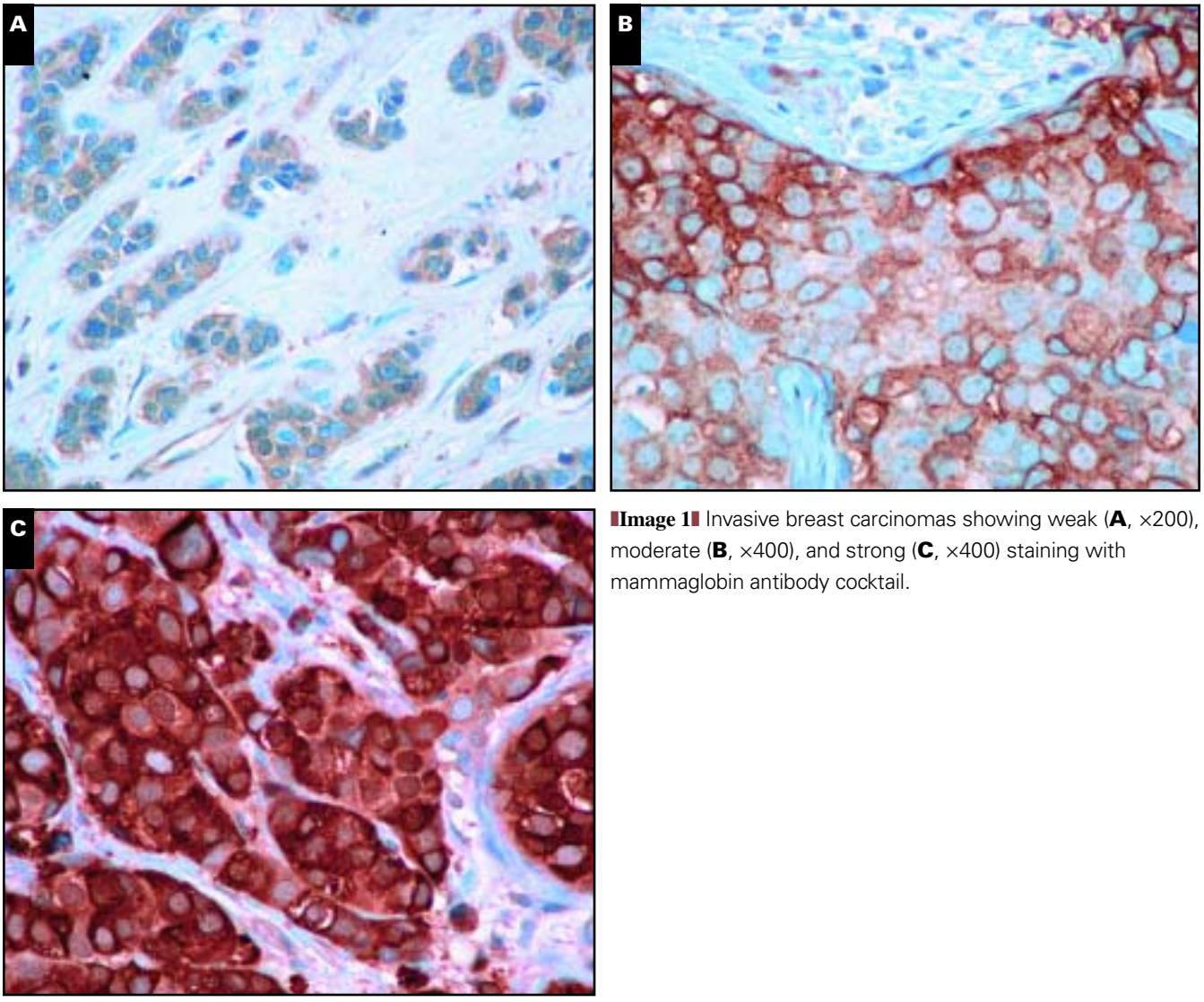

Image 1 Invasive breast carcinomas showing weak (**A**, ×200), moderate (**B**, ×400), and strong (**C**, ×400) staining with mammaglobin antibody cocktail.

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aforementioned factors are, therefore, also responsible for the apparent discrepancy within the breast whole tissue section and breast TMA groups. Therefore, the results of the whole tissue section breast carcinomas and breast TMA are reported and analyzed separately.

Mammaglobin and GCDFP-15 Staining of Primary and Metastatic Breast Carcinoma in Whole Tissue Sections

The details of the staining pattern for each case are shown in **Table 1**. In a majority of cases, the intensity of staining and proportion of cells stained was significantly

Table 1
Details for Staining Patterns in Breast Carcinomas

		Percentage of Cells Staining		Staining Intensity	
Case No./Specimen	Diagnosis	MGB	GCDFP-15	MGB	GCDFP-15
1/T	ILC	55	55	Strong	Weak
LN	ILC	55	55	Strong	Strong
2/T	ILC	55	55	Strong	Moderate
LN	ILC	55	55	Strong	Moderate
3/T	ILC	70	20	Strong	Moderate
LN	ILC	100	15	Strong	Moderate
4/T	ILC	40	60	Strong	Strong
LN	ILC	25	60	Strong	Strong
5/T	ILC	70	5	Strong	Weak
LN	ILC	100	60	Strong	Moderate
6/T	ILC	100	20	Strong	Weak
LN	ILC	100	60	Strong	Moderate
7/T	ILC	80	25	Strong	Weak
LN	ILC	80	5	Strong	Weak
8/T	ILC	80	70	Weak	Weak
LN	ILC	90	40	Strong	Moderate
9/T	ILC	90	0	Strong	Negative
LN	ILC	100	30	Strong	Moderate
10/T	ILC	85	20	Strong	Strong
LN	ILC	90	40	Strong	Strong
11/T	IDC	1	40	Weak	Moderate
LN	IDC	5	80	Weak	Moderate
12/T	IDC	5	0	Weak	Negative
LN	IDC	8	5	Weak	Strong
13/T	IDC	95	80	Strong	Weak
LN	IDC	95	80	Strong	Weak
14/T	IDC	30	10	Strong	Strong
LN	IDC	25	20	Strong	Moderate
15/T	IDC	25	5	Strong	Moderate
LN	IDC	2	1	Weak	Strong
16/T	IDC	8	8	Weak	Weak
LN	IDC	8	40	Weak	Moderate
17/T	IDC	25	5	Strong	Weak
LN	IDC	15	5	Strong	Weak
18/T	IDC	30	0	Strong	Negative
LN	IDC	30	0	Strong	Negative
19/T	IDC	60	0	Strong	Negative
LN	IDC	60	20	Strong	Moderate
20/T	IDC	100	5	Strong	Weak
LN	IDC	100	40	Strong	Moderate
21/T	IDC	80	5	Strong	Weak
LN	IDC	30	3	Strong	Weak
22/T	IDC	4	20	Weak	Weak
LN	IDC	0	0	Negative	Negative
23/T	IDC	5	1	Weak	Weak
LN	IDC	0	0	Negative	Negative
24/T	IDC	40	15	Strong	Weak
LN	IDC	60	60	Strong	Weak
25/T	ILC	55	5	Strong	Moderate
LN	ILC	80	15	Strong	Strong
26/T	ILC	3	3	Weak	Weak
LN	ILC	95	60	Strong	Strong
27/T	ILC	0	60	Weak	Moderate
LN	ILC	0	30	Negative	Moderate
28/T	ILC	5	0	Weak	Negative
LN	ILC	5	25	Weak	Weak
29/T	Mixed	60	0	Strong	Negative
LN	Mixed	55	5	Strong	Weak

GCDFP-15, gross cystic disease fluid protein-15; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; LN, lymph node; MGB, mammaglobin; T, tumor.

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higher with the mammaglobin antibody than with GCDFP-15 **Image 2** and **Image 3**. Of the 58 breast carcinomas, 40 (69%) showed a higher proportion of cells staining with mammaglobin than with GCDFP-15. In 9 cases (16%), GCDFP-15 stained more cells and in 9 cases (16%), a similar percentage of cells stained with both mammaglobin and GCDFP-15. The median percentage of cellular staining was 55% (mean, 48.7%; SD, 36.3) with mammaglobin compared with only 20% (mean, 25.9%; SD, 25.5) with GCDFP-15. This difference was statistically significant ($P < .0001$). For the staining intensity, 41 (71%) of 58 cases showed moderate to strong staining with mammaglobin compared with only 28 (48%) with GCDFP-15. This difference was also statistically significant ($P = .02$).

Based on the intensity and percentage of cells staining, unequivocal positive staining was seen in 41 (71%) of 58 cases with mammaglobin, whereas only 24 cases (41%) showed significant staining with GCDFP-15. The summary of staining patterns (positive, negative, or equivocal) with both antibodies is shown in **Table 2**. If equivocal staining is counted with positive staining, the mammaglobin sensitivity for breast carcinoma increases to 93.1% and that of GCDFP-15 approaches 84.5%.

Mammaglobin Staining in Primary vs Metastatic Breast Carcinoma in Whole Tissue Sections

Of the 29 invasive carcinomas, significant staining (patchy or diffuse with moderate to strong intensity) was seen in 20 primary tumors (69%). These included 9 ductal, 10 lobular, and 1 mixed carcinoma. A high degree of concordance was identified between primary and metastatic carcinomas. Overall 27 (93%) of 29 cases showed concordant staining when analyzed for significant staining vs negative or equivocal staining. The 2 discordant cases included 1 ductal

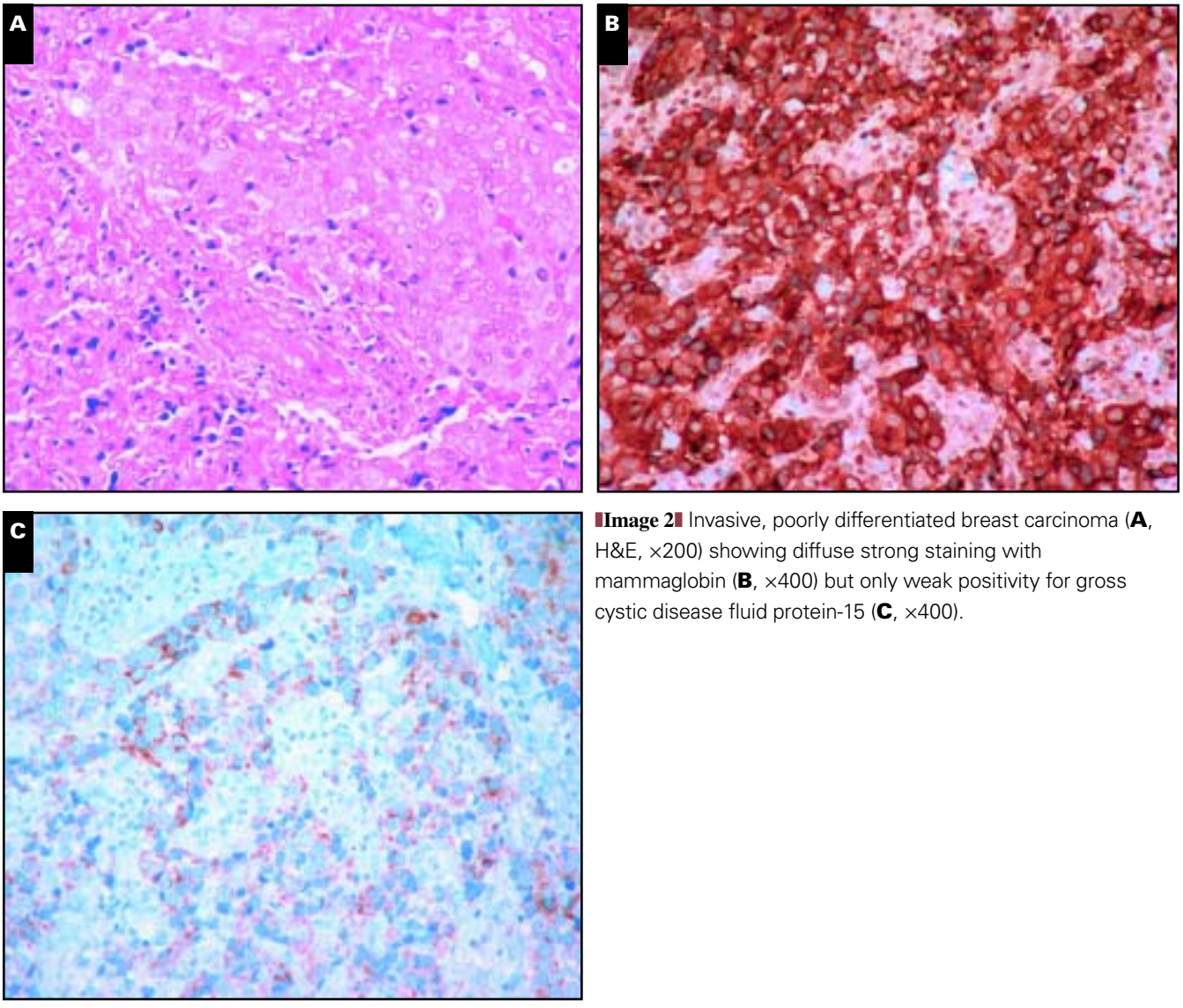

Image 2 Invasive, poorly differentiated breast carcinoma (**A**, H&E, ×200) showing diffuse strong staining with mammaglobin (**B**, ×400) but only weak positivity for gross cystic disease fluid protein-15 (**C**, ×400).

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(Table 1, case 15) and 1 lobular (Table 1, case 26) carcinoma. The primary ductal carcinoma showed patchy, strong staining, whereas the tumor in the lymph node showed only focal, weak immunoreactivity. The primary lobular carcinoma showed focal, weak staining, whereas the metastatic tumor showed diffuse, strong positivity.

Mammaglobin Staining and Nuclear Grade, Receptor Status, and HER-2/neu in Whole Tissue Sections

Mammaglobin positivity was seen in 4 (57%) of 7 grade 1, 8 (80%) of 10 grade 2, and 8 (67%) of 12 grade 3 tumors. The differences in mammaglobin staining with respect to nuclear grades (1 and 2 vs 3) were not statistically significant ($P = 1$). Because the majority of tumors in the study group were positive for estrogen and progesterone receptors, the effect of receptor status on mammaglobin staining could not be studied. Mammaglobin positivity was seen in 15 (68%) of 22 HER-2/neu– tumors and 5 (71%) of 7 HER-2/neu+ tumors. There was no statistically significant difference in mammaglobin staining with respect to HER-2 status ($P = 1$).

Table 2
Comparison of Mammaglobin and GCDFP-15 Reactivity in Breast Carcinoma*

	Mammaglobin			
GCDFP-15	**Positive**	**Negative**	**Equivocal**	**Total**
Positive	19	2	3	24
Negative	5	2	2	9
Equivocal	17	0	8	25
Total	41	4	13	58

GCDFP-15, gross cystic disease fluid protein-15.
* The equivocal category includes focal and/or weak staining, ie, focal weak, focal moderate, focal strong, patchy weak, and diffuse weak staining. Focal implies <10% of cells positive; patchy is 10%-50%; and diffuse is >50%.

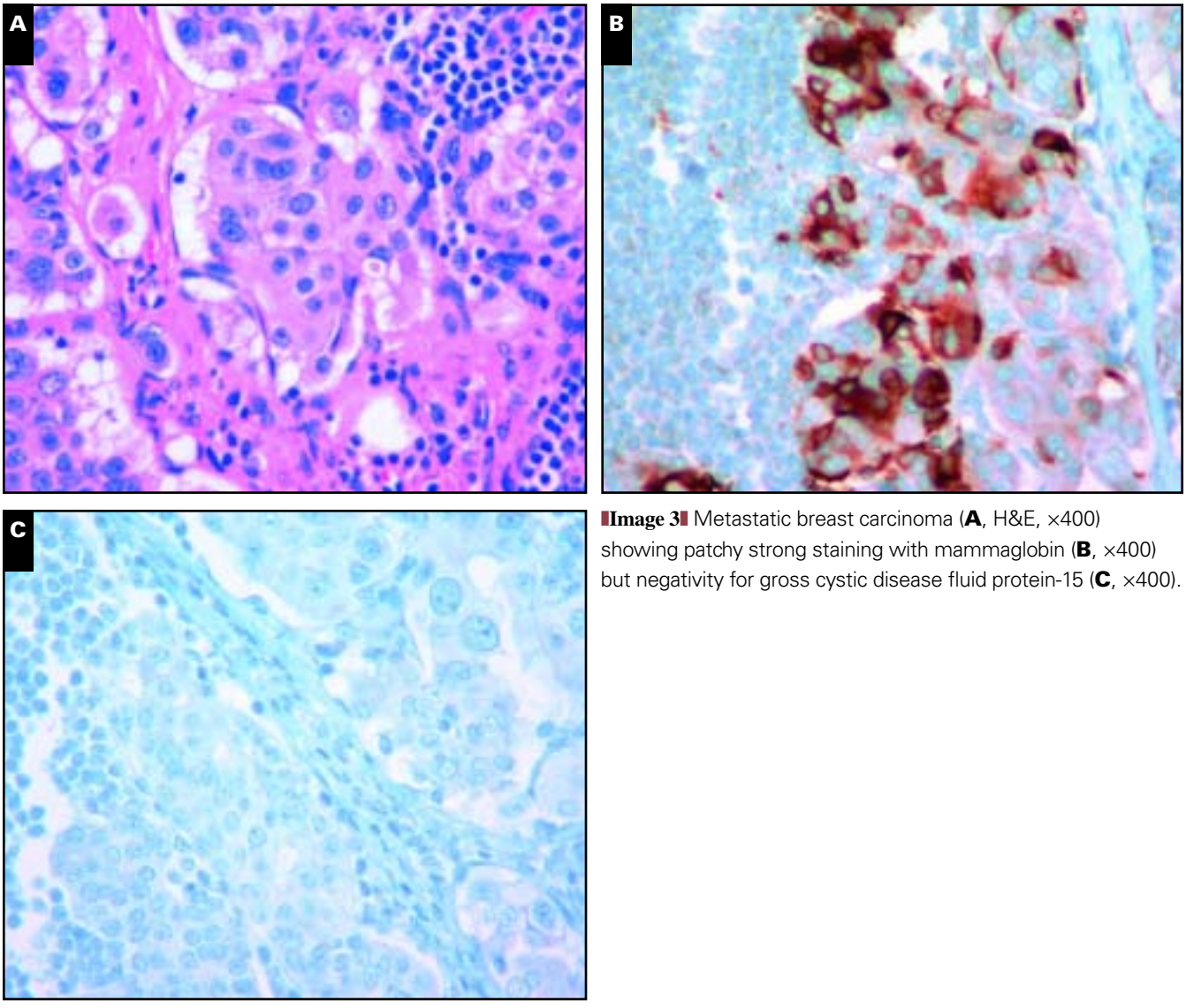

Image 3 Metastatic breast carcinoma (**A**, H&E, ×400) showing patchy strong staining with mammaglobin (**B**, ×400) but negativity for gross cystic disease fluid protein-15 (**C**, ×400).

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Mammaglobin and GCDFP-15 on Breast Carcinoma TMAs

Of 64 cases, 63 could be evaluated on TMA with mammaglobin and GCDFP-15 antibodies. The details of staining pattern for each case are shown in **Table 3**. Once again, in the majority of cases, the intensity of staining and proportion of cells stained was higher with the mammaglobin antibody than with GCDFP-15. Of the 63 breast carcinomas, 36 (57%) showed a higher proportion of cell staining with mammaglobin than GCDFP-15. In 7 cases (11%), GCDFP-15 stained more cells, and in 20 cases (32%), a similar percentage of cells stained with mammaglobin and GCDFP-15. This difference in the proportion of cellular staining was statistically significant ($P < .0001$).

Based on intensity and percentage of cells staining, unequivocal positive staining was seen in 26 (41%) of 63 cases with mammaglobin, whereas only 3 cases (5%) showed significant staining with GCDFP-15. This difference in sensitivity was statistically significant ($P = .0000001$). The summary of staining patterns (positive, negative, or equivocal) with both antibodies is shown in **Table 4**. If equivocal staining is counted with positive staining, the mammaglobin sensitivity for breast carcinoma increases to 58.7% and that of GCDFP-15 approaches 19%, and the difference was again statistically significant ($P = .000003$).

Mammaglobin Staining and Nottingham Grade and Other Clinicopathologic Variables in Breast TMA Cases

Mammaglobin positivity was seen in 10 (56%) of 18 grade 1, 8 (32%) of 25 grade 2, and 8 (40%) of 20 grade 3 tumors. The differences in mammaglobin staining with respect to Nottingham grades (1 vs 2 vs 3) were not statistically significant (grade 1 vs 2, $P = .20$; grade 2 vs 3, $P = .75$; grade 1 vs 3, $P = .51$; grade 1 vs others, $P = .16$). Mammaglobin staining with respect to other pathologic variables is summarized in **Table 5**.

Tissue Arrays

All tissue cores (tumor and normal) in the lung, stomach, colon, kidney, and bladder arrays were negative. All tumors (squamous cell carcinomas) in the uterine cervical tissue array were also negative, but 13 of 15 cores with normal endocervical glands showed weak (equivocal) to moderate staining. Positive staining was seen in endometrial carcinoma **Image 4A**, sweat gland carcinoma **Image 4B**, salivary gland, melanoma, ovarian, and pancreatic tissue arrays.

The results for nonbreast tumors are given in **Table 6**.

Discussion

Although the mammaglobin gene was discovered almost a decade ago, only a limited number of studies have discussed its clinical usefulness. In the seminal paper by Watson and Fleming,[2] mammaglobin overexpression was identified in breast carcinoma compared with normal breast tissue. Recently, mammaglobin has been identified as a breast cancer–specific gene, and its usefulness as a novel breast cancer marker has been described.[4,8,9] Nunez-Villar et al[10] suggested that elevated mammaglobin (h-mam) expression in breast cancer is associated with clinical and biologic features defining a less aggressive tumor phenotype.

A few other reports have discussed the importance of mammaglobin expression in breast tumors. Ciampa et al[11] studied mammaglobin and CrxA-01 expression in cell block material from malignant pleural effusions. Eighty percent of breast carcinomas were positive for mammaglobin and/or CrxA-01, and none of the nonbreast carcinomas were positive for mammaglobin. Because this study was performed on metastatic tumors in pleural fluid, the numbers of nonlung and nonbreast carcinomas were rather insignificant. Han et al[12] studied mammaglobin and BRST-2 (same as GCDFP-15) expression by immunohistochemical analysis in breast and nonbreast carcinomas. They concluded that mammaglobin has a superior sensitivity to that of BRST-2. Among the nonbreast carcinomas only 1 of 10 urothelial neoplasms and 1 of 10 thyroid carcinomas showed strong reactivity. Our results are very comparable to those of Han et al[12] with respect to breast carcinoma; however, because we analyzed a large number of nonbreast carcinomas, we identified some clinically significant differences.

We compared the immunohistochemical staining pattern of mammaglobin with GCDFP-15 (a sensitive and specific marker of breast carcinoma) in whole tissue sections of 29 primary mammary carcinomas with matched lymph node metastases and 63 randomly selected breast carcinomas represented on a TMA. Higher sensitivity was observed in whole tissue sections (71%) compared with TMAs (41%). Our study shows that lobular carcinomas are more diffusely and strongly stained with mammaglobin compared with ductal carcinomas, which show a more patchy staining pattern. The whole tissue section cases were an equal mix of ductal and lobular carcinomas, whereas the TMA cases were randomly selected and contained predominantly ductal carcinomas. This difference in the case types explains the lower sensitivity of mammaglobin in the breast TMA group than in the whole tissue section group. However, in either group (whole section or TMA), mammaglobin had a higher sensitivity than GCDFP-15. Moreover, the mammaglobin antibody cocktail stained the breast carcinomas more intensely than GCDFP-15, and, among the positive cases, the number of cells stained with mammaglobin is higher than with GCDFP-15.

Previous studies have suggested that mammaglobin expression is mainly seen in well-differentiated, receptor-positive breast carcinomas; however, we failed to show any correlation between mammaglobin expression and different

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Table 3
Details for Staining Patterns in Breast Carcinomas (Tissue Microarray Cases)

		Percentage of Cells Staining		Staining Intensity	
Case No.	Histologic Type	MGB	GCDFP-15	MGB	GCDFP-15
1	Ductal	20	0	Strong	Negative
2	Ductal	0	0	Negative	Negative
3	Ductal	0	1	Negative	Weak
4	Ductal	10	0	Strong	Negative
5	Mixed	25	0	Strong	Negative
6	Ductal	0	0	Negative	Negative
7	Ductal	3	0	Strong	Negative
8	Ductal	0	0	Negative	Negative
9	Ductal	5	0	Moderate	Negative
10	Ductal	90	0	Strong	Negative
11	Ductal	10	0	Strong	Negative
12	Ductal	40	0	Strong	Negative
13	Ductal	0	0	Negative	Negative
14	Ductal	50	0	Strong	Negative
15	Ductal	10	0	Strong	Negative
16	Ductal	80	0	Strong	Negative
17	Ductal	0	0	Negative	Negative
18	Ductal	90	0	Strong	Negative
19	Ductal	0	0	Negative	Negative
20	Ductal	3	0	Moderate	Negative
21	Ductal	2	0	Weak	Negative
22	Ductal	0	0	Negative	Negative
23	Ductal	50	40	Strong	Strong
24	Ductal	0	0	Negative	Negative
25	Mixed	2	0	Weak	Negative
26	Ductal	20	0	Strong	Negative
27	Ductal	0	40	Negative	Moderate
28	Ductal	10	0	Strong	Negative
29	Ductal	0	0	Negative	Negative
30	Metaplastic	0	0	Negative	Negative
31	Ductal	80	0	Strong	Negative
32	Ductal	2	0	Strong	Negative
33	Ductal	0	0	Negative	Negative
34	Lobular	95	0	Strong	Negative
35	Ductal	0	0	Negative	Negative
36	Ductal	10	0	Strong	Negative
37	Ductal	8	0	Weak	Negative
38	Lobular	5	0	Moderate	Negative
39	Ductal	0	0	Negative	Negative
40	Lobular	0	8	Negative	Weak
41	Lobular	15	0	Moderate	Negative
42	Ductal	60	0	Strong	Negative
43	Ductal	0	0	Negative	Negative
44	Ductal	0	0	Negative	Negative
45	Lobular	0	0	Negative	Negative
46	Ductal	95	0	Strong	Negative
47	Lobular	0	0	Negative	Negative
48	Ductal	0	0	Negative	Negative
49	Ductal	60	0	Strong	Negative
50	Ductal	0	1	Negative	Weak
51	Ductal	10	10	Moderate	Moderate
52	Lobular	0	1	Negative	Weak
53	Ductal	1	0	Weak	Negative
54	Lobular	50	0	Moderate	Negative
55	Lobular	90	0	Strong	Negative
56	Ductal	Lost	Lost	Lost	Lost
57	Ductal	90	0	Strong	Negative
58	Ductal	3	0	Weak	Negative
59	Ductal	20	1	Weak	Weak
60	Ductal	0	1	Negative	Weak
61	Ductal	65	5	Strong	Weak
62	Ductal	0	0	Negative	Negative
63	Ductal	0	10	Negative	Weak
64	Ductal	70	0	Strong	Negative

GCDFP-15, gross cystic disease fluid protein-15; MGB, mammaglobin.

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Table 4
Comparison of Mammaglobin and GCDFP-15 Reactivity in Breast Carcinoma (Tissue Microarray Cases)*

	Mammaglobin			
GCDFP-15	**Positive**	**Negative**	**Equivocal**	**Total**
Positive	3	1	0	4
Negative	22	19	10	51
Equivocal	1	6	1	8
Total	26	26	11	63

GCDFP-15, gross cystic disease fluid protein-15.
* The equivocal category includes focal and/or weak staining, ie, focal weak, focal moderate, focal strong, patchy weak, and diffuse weak staining. Focal implies <10% of cells positive; patchy is 10%-50%; and diffuse is >50%.

clinicopathologic variables (Table 5). Although many carcinomas would not be included in the differential diagnosis of breast carcinoma, the specificity of the mammaglobin antibody in our study set was 92%.

Among the nonbreast carcinoma group, approximately 40% of the endometrial endometrioid carcinoma showed significant staining. Our findings of mammaglobin expression in endometrial carcinoma are similar to those recently reported by Zafrakas et al.[13] These authors studied more than 300 human tumors and matched normal tissue samples by different techniques and identified significant mammaglobin expression in breast and gynecologic tissues and the absence of mammaglobin expression in prostate, kidney, colon, rectum, small intestine, stomach, pancreas, lung, and thyroid. In this regard, it is important to recognize that mammaglobin is highly related to uteroglobin, a secretory protein of the endometrium induced by progesterone.[14] This probably explains strong staining of endometrial endometrioid carcinomas.

Table 5
Mammaglobin Staining in 63 Breast Tissue Microarray Cases With Respect to Clinicopathologic Variables

Variable/Category	Mammaglobin Positivity*	*P*
Tumor size, cm (n = 62)		
≤2	18/38 (47)	.19 (NS)
>2	7/24 (29)	
Nodal status (n = 59)		
Positive	9/26 (35)	.43 (NS)
Negative	15/33 (45)	
Nottingham grade		
1	10/18 (56)	.16 (NS)
2 and 3	16/45 (36)	
Histologic type (n = 60)		
Ductal	21/51 (41)	1.0 (NS)
Lobular	4/9 (44)	
Age (y)		
≤50	7/14 (50)	.54 (NS)
>50	19/49 (39)	
Estrogen receptor status		
Positive	23/54 (43)	.72 (NS)
Negative	3/9 (33)	
Progesterone receptor status		
Positive	21/49 (43)	.76 (NS)
Negative	5/14 (36)	
HER-2 status		
Positive	4/6 (67)	.21 (NS)
Negative	22/57 (39)	

NS, not significant.
* Data are given as number/total tested (percentage).

Therefore, it is conceivable that other tumors of the reproductive tract or tumors showing progesterone receptor expression should also show mammaglobin immunoreactivity. However, only 1 of 40 ovarian carcinomas included in our study showed significant positivity, but it is important to mention that our set of ovarian carcinomas lacked endometrioid-type cancers.

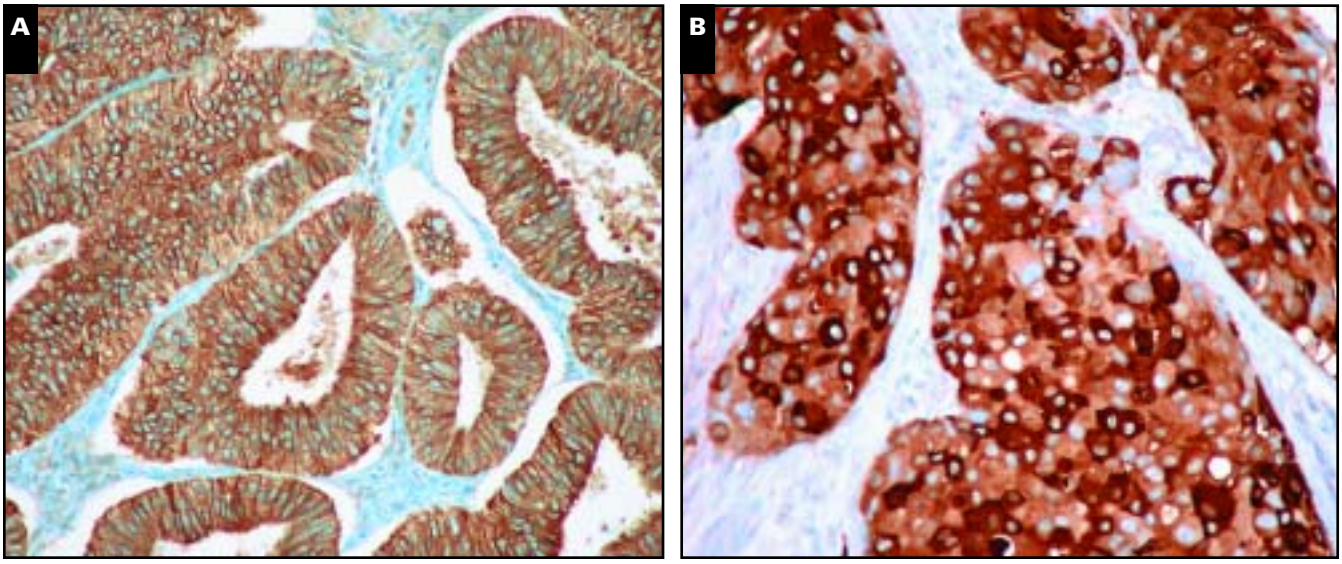

Image 4 Mammaglobin staining in nonbreast tumors. **A**, Endometrial endometrioid adenocarcinoma shows diffuse strong staining (×200). **B**, Sweat gland carcinoma showing positive mammaglobin staining (×200).

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Table 6
Mammaglobin Staining in Nonbreast Tumors

Tumor Type	Mammaglobin Staining*	Staining Pattern
Melanoma	5/80 (6)	Patchy (2 cases) or diffuse (3 cases) staining with moderate intensity
Ovarian serous carcinoma	1/40 (3)	Patchy moderate staining
Endometrioid adenocarcinoma	23/59 (39)	Patchy (8 cases) or diffuse (15 cases) staining with moderate to strong intensity
Skin sweat gland carcinoma	4/10 (40)	Patchy (2 cases) or diffuse (2 cases) staining with moderate to strong intensity
Salivary gland tumor	5/25 (20)†	Patchy staining with moderate intensity
Pancreatic adenocarcinoma	1/67 (1)	Patchy, moderate staining
Endocervical adenocarcinoma	0/9 (0)	4 cases showed equivocal staining
Endocervical adenocarcinoma in situ	5/11 (45)	Patchy, strong staining
Cervical squamous cell carcinoma	0/41 (0)	No staining
Lung carcinoma (squamous, 27; adenocarcinoma, 18)	0/45 (0)	No staining
Stomach (carcinoma, 37; carcinoids, 2)	0/39 (0)	No staining
Colonic adenocarcinoma	0/39 (0)	No staining
Kidney (clear cell, 37; undifferentiated, 2; lymphoma, 1)	0/40 (0)	No staining
Bladder (urothelial, 37; adenocarcinoma, 2; squamous cell carcinoma, 1)	0/40 (0)	No staining

* Data are given as number/total tested (percentage).
† The 5 positive tumors included 2 adenocarcinomas, 1 epithelial-myoepithelial carcinoma, 1 mucoepidermoid carcinoma, and 1 pleomorphic adenoma.

All invasive endocervical adenocarcinomas studied by whole tissue section immunohistochemical analysis were all negative or showed equivocal staining. In contrast, AIS of the cervix showed significant staining in 55% of cases, although most atypical glands were patchily stained compared with mostly diffuse staining in normal endocervical glands. A review of endocervical cases revealed that mammaglobin staining is mostly present admixed with cytoplasmic mucin. Some of the glands partially involved by AIS showed mammaglobin staining only within the normal mucinous portion of the gland **Image 5**. Similarly, in endometrioid carcinomas, mammaglobin staining is often identified within glandular secretions. This staining pattern suggests that mammaglobin is involved in some process of the cell secretory mechanism. This reduced mammaglobin expression in AIS and invasive endocervical carcinoma is worth studying in more detail.

Another interesting finding in our study was the significant staining seen in approximately 6% of melanomas. Although it is important to recognize this pitfall, this is unlikely to cause a problem in the differential diagnosis from a breast carcinoma because a panel of immunohistochemical stains is always used in this scenario. Positive mammaglobin

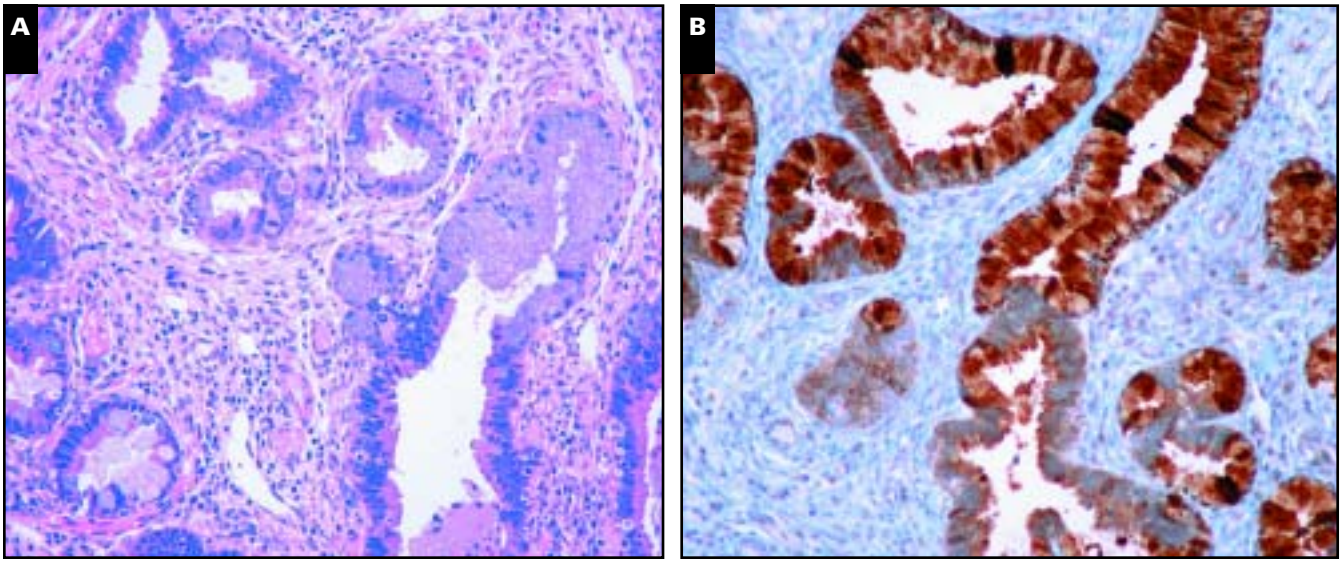

Image 5 Adenocarcinoma in situ of cervix (**A**, H&E, ×200) showing reduced mammaglobin expression (**B**, ×200). Note that intense mammaglobin staining is seen only in a normal mucin-containing gland, and the portion of gland involved by adenocarcinoma in situ demonstrates reduced mammaglobin expression.

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staining was also observed in a significant proportion of sweat gland carcinomas. This is not surprising because sweat gland carcinomas with ductal differentiation can also exhibit GCDFP-15 and estrogen receptors.[15] Mammaglobin does not seem to be a useful stain to distinguish breast from sweat gland carcinomas. Positive mammaglobin staining seen in salivary gland tumors was also expected; however, intense staining was not seen in any of the positive salivary gland tumors.

Despite some nonspecificity of the mammaglobin antibody, our data provide compelling evidence for inclusion of mammaglobin in a panel for the workup of carcinoma of an unknown primary site. For diagnosing a breast carcinoma, the sensitivity of mammaglobin is better than that of GCDFP-15 based on our present study and as reported by others.[16,17]

The mammaglobin antibody is a sensitive marker of breast carcinomas. Mammaglobin expression is not altered at the metastatic (lymph node) site. With respect to endometrial carcinoma, it may have a role in the differential diagnosis with an invasive endocervical adenocarcinoma. Mammaglobin antibody can occasionally stain a melanoma, and it is important to recognize this pitfall. Mammaglobin can help, in combination with other markers, to establish the correct diagnosis of metastatic breast carcinoma but per se does not seem to be specific.

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