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# Differentiating Breast Carcinoma with Signet-Ring Features from Gastrointestinal Signet-Ring Carcinoma: Assessment of Immunohistochemical Markers

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## Abstract

Signet-ring morphology is recognized throughout the gastrointestinal tract. However, this pattern may be observed in other primary sites giving rise to diagnostic challenges in the work-up of metastases. Relatively newer immunohistochemical markers have not been evaluated in this context. We assessed expression patterns of several common immunohistochemical markers in tumors with signet-ring morphology to delineate a pragmatic approach to this differential diagnosis.

Primary breast and gastrointestinal carcinomas showing signet-ring features were reviewed. Non-mammary and non-gastrointestinal tumors with this morphology were included for comparison. Estrogen receptor (ER), progesterone receptor (PR), e-cadherin, CK7, CK20, GCDFP-15, mammaglobin, CDX2, GATA-3, and HepPar-1 immunohistochemistry was performed. Expression patterns were compared between breast and gastrointestinal tumors as well as lobular breast and gastric tumors.

Ninety-three cases were identified: 33 breast carcinomas including 13 lobular, 50 gastrointestinal tumors including 23 gastric, and 10 from other sites. ER (Sensitivity=81.8%, Specificity=100%, Positive predictive value (PPV)=100%, Negative predictive value (NPV)=89.3%) and GATA-3 (Sensitivity=100%, Specificity=98%, PPV=96.8%, NPV=100%) expression were associated with breast origin. CK20 (Sensitivity=66.7%, Specificity=93.3%, PPV=94.1%, NPV=63.6%) and CDX2 (Sensitivity=72%, Specificity=100%, PPV=100%, NPV=68.9%) demonstrated the strongest discriminatory value for gastrointestinal origin. These markers exhibited similar discriminatory characteristics when comparing lobular and gastric signet ring carcinomas. In a limited trial on metastatic breast and gastric cases, these markers successfully discriminated between breast and gastric primary sites in 15 of 16 cases.

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ER and GATA-3 are most supportive of mammary origin and constitute an effective panel for distinguishing primary breast from primary gastrointestinal signet-ring tumors when combined with CK20 and CDX2 immunohistochemistry.

**Keywords**

Signet-ring carcinoma; lobular carcinoma; estrogen receptor; GATA-3; CDX2; immunohistochemistry

## 1. Introduction

Tumors with signet-ring morphology are most commonly recognized throughout the gastrointestinal tract. This pattern has also been observed in adenocarcinomas arising from other sites such as the breast, lung, pancreaticobiliary tract, Mullerian tract, and other less common sites. In the breast, signet-ring carcinoma is not typically recognized as a specific entity although the morphology may give rise to diagnostic challenges in certain situations [1, 2, 3].

Breast carcinoma is recognized to metastasize to the stomach with clinical and pathologic features mimicking a gastric primary [4, 5, 6]. Presently, this diagnostic issue has been explored predominantly in the form of case reports in the literature [1, 3]. In some instances, gastric metastases may be detected prior to identification of the breast primary [7]. Conversely, gastric primary signet-ring carcinomas have been reported to metastasize to the breast [8]. Tumors arising from both breast and the GI tract may metastasize to other similar locations [9]. For example, both lobular breast and gastric signet-ring carcinomas are recognized to cause peritoneal carcinomatosis and show similar patterns of infiltrative growth [10]. Given this overlap in locations and morphology, the distinction between these tumors is an important diagnostic challenge as the available management options depend on identification of the primary site.

Immunohistochemistry (IHC) is often utilized for determination of a suspected primary location. Markers such as CK20, CK7, and estrogen receptor (ER), have been employed in the differential diagnosis of signet ring tumors [11]. Chu and Weiss previously evaluated signet-ring carcinomas from the breast, stomach, and colon in a series of 60 cases for expression of a variety of markers. They found ER, MUC1 (EMA), hepatocyte paraffin 1 (HepPar-1), and CDX2 to be useful in distinguishing breast from gastric primaries while ER, CDX2, MUC2, and MUC5AC were useful for breast versus colonic primaries [12]. Relatively newer markers such as GATA-3 and mammaglobin have not been evaluated as part of a panel in this context.

We assessed staining patterns of several common immunohistochemical markers including ER, PR, e-cadherin, cytokeratin 7 (CK7), cytokeratin 20 (CK20), gross cystic disease fluid protein 15 (GCDFP-15), mammaglobin, CDX2, GATA-3, and HepPar-1 in the signet-ring component of tumors with known breast and gastric origin. We also compared expression to some tumors showing signet-ring morphology from extra-mammary and extra-

gastrointestinal sites. Using our findings, we delineate a pragmatic immunohistochemical work-up for the distinction between these tumors.

## 2. Methods

### 2.1. Case selection

With institutional review board approval, cases of primary breast and gastrointestinal carcinomas showing signet-ring features from 1/1/2002 to 2/28/2017 at Rhode Island Hospital and The Miriam Hospital were retrieved and reviewed. Primary breast cases were categorized into lobular, ductal and other histologic subtypes including mucinous carcinoma. Classification of the breast carcinomas including distinction between ductal versus lobular carcinoma was determined by morphology with confirmation through review by an expert in breast pathology (YW). The gastrointestinal cases were subcategorized by primary site. Non-mammary and non-gastrointestinal tumors with signet-ring morphology were also collected for comparison. Signet-ring tumor cells were defined by the presence of an intracytoplasmic inclusion displacing the nucleus often with discohesive single cell or linear “indian filing” growth. For gastrointestinal tumors, signet-ring cells were required to comprise at least 50% of the tumor mass on the hematoxylin and eosin-stained (H&E) section for inclusion. Tumors with fewer than four high-power fields (400X) consisting of signet-ring cells with primary sites outside of the gastrointestinal tract were excluded. These areas were identified morphologically.

### 2.2. Immunohistochemistry

Initially, immunohistochemistry for CK7, CK20, ER, PR, mammaglobin, GCDFP-15, e-cadherin, GATA-3, and CDX2 was performed on 4-micron whole tissue sections from an appropriate block showing signet-ring features. In cases with metastatic disease, tissue from the primary tumor was used for staining if available. Sections were baked at 60°C for one hour and loaded onto an Omnis Autostainer (Dako, Carpinteria, CA) for deparaffinization, antigen retrieval, antibody incubation and detection. Detection was achieved using Dako EnVision FLEX reagents (Dako). The methods of antigen retrieval and characteristics of the primary antibodies are summarized in Table 1. For CK7, CK20, mammaglobin, and GCDFP-15, positive staining was characterized by cytoplasmic staining of at least 10% of the signet-ring cells. ER, PR, GATA-3, CDX2 were considered positive if at least 10% of nuclei stained positive. Positive expression of e-cadherin required membranous or cytoplasmic staining of at least 10% of the lesional cells. Staining was only considered positive if the expected pattern was identified in the signet-ring component. Cases with expression only within the non-signet-ring component were considered negative.

### 2.3. Tissue microarray

A tissue microarray (TMA) was prepared to assess additional cases of tumors with signet-rings. For comparison, pulmonary adenocarcinomas, urothelial carcinomas, and Mullerian carcinomas showing signet-ring features were also included in the study. The tissue microarray was prepared by identifying areas enriched with tumor cells showing signet-ring morphology. For each case, three qualifying areas and one background non-tumor area were circled. Cases that did not have three qualifying signet-ring areas were excluded. The

corresponding locations on each block were then extracted using 1 mm core punches and arranged onto two TMA blocks using a manual tissue microarrayer (Beecher Instruments Inc, Sun Prairie, WI). Five-micron sections were cut from the microarray and stained for each marker on the selected panel.

### 2.4. Statistical analysis

Statistical analysis was performed using Fisher's exact test and Student's t-test using Excel software (Microsoft, Redmond, WA). Breast primary carcinomas including lobular, ductal, and mucinous tumors were compared to the gastrointestinal tumors as a group as well as all non-mammary carcinomas as a group. Further, appraisal of staining between specifically lobular and gastric carcinomas was performed. A $p$-value of 0.05 was used as the cutoff for statistical significance. The sensitivity, specificity, positive predictive value, negative predictive value, and respective 95% confidence intervals were also calculated as appropriate. These characteristics guided the composition of a proposed initial IHC panel.

### 2.5. Trial of Proposed Immunohistochemical Panel

Specimens from metastatic breast and gastric tumors from 2000–2017 were retrieved and reviewed for unequivocal signet-ring morphology. Qualifying tumors were stained with the proposed initial diagnostic panel for differentiating primary breast carcinomas from primary gastric tumors. Immunohistochemistry for the panel of markers with the best differential characteristics was applied to these cases.

## 3. Results

### 3.1. Case selection

In total, 93 cases meeting the study criteria were identified consisting of 33 primary breast adenocarcinomas and 50 gastrointestinal adenocarcinomas including 6 pancreaticobiliary tumors. Ten carcinomas with signet-ring features including 4 lung, 4 Mullerian, and 2 bladder primaries were also included. Of the 33 primary breast carcinomas, 19 were ductal, 13 lobular, and 1 was mucinous. Among the primary gastrointestinal signet ring tumors 23 were primary gastric adenocarcinomas. The remainder included 9 appendiceal, 7 colonic, 5 pancreatic, 3 esophageal, 2 small intestinal, and 1 ampullary primary carcinoma. With the exception of one case of ductal carcinoma (1/33, 3%), all of the breast tumors were from female patients. For the remaining 60 carcinomas with signet-ring features from other sites, 30 (50%) were from male patients and 30 were from female patients. For some immunostains, the tissue was either lost or the signet-ring cells were not identified on the TMA. These cases were excluded from the analysis for the respective marker. The number of cases stained from each primary site and their corresponding expression patterns for each marker are summarized in Table 2.

### 3.2. Comparison of expression in breast primary versus gastrointestinal primary tumors

CK20, ER, PR, mammaglobin, GCDFP-15, CDX2, GATA-3, and HepPar-1 all showed significant differences in staining between breast and GI tumors. The corresponding sensitivities, specificities, positive and negative predictive values, and corresponding 95% confidence intervals for these markers are tabulated in Table 3. Qualitatively, there were no

apparent differences in expression patterns for these markers. CK7 (p=0.221) and e-cadherin (p=0.813) expression was similar between the two groups. Expression of PR, mammaglobin and GCDFP-15 significantly favored breast origin. However, the sensitivity of these markers for breast tumors was low, showing 54.5%, 41.7%, and 75% sensitivity respectively. On the other hand, CK20 and Hep-Par-1 expression significantly favored gastrointestinal origin, again with low sensitivities of 66.7% and 34% respectively. Both of these markers were less sensitive compared to CDX2 (72%). ER and GATA-3 demonstrated the best characteristics for supporting breast origin with 81.8% and 100% sensitivity respectively and 100% and 98% specificity respectively.

### 3.3. Comparison of expression in lobular breast carcinomas versus gastric signet-ring carcinomas

Separate appraisal of these markers in lobular breast carcinomas versus gastric signet-ring carcinomas also showed differential expression of CK20, ER, PR, CDX2, and GATA-3. These results and corresponding sensitivities, specificities, positive and negative predictive values, and corresponding 95% confidence intervals are tabulated in Table 3. Examples of staining for lobular breast carcinoma and gastric signet-ring carcinoma are shown in Figure 1. ER, GCDFP-15, and GATA-3 had the highest sensitivities and specificities for lobular carcinoma. For gastric signet-ring carcinomas, CK20 and CDX2 were most effective while ER, GATA-3 and GCDFP-15 expression was absent in all cases. While 100% positive predictive values were found with CK20, CDX2, and HepPar-1, the negative predictive values of all three of these markers for gastric carcinoma was low. CK7 failed to differentiate between any of the compared groups. E-cadherin showed significant differences in expression only when comparing lobular breast carcinoma to gastric carcinoma (p=0.0052). Mammaglobin staining also reached statistical significance in discriminating between the lobular breast and gastric signet-ring groups. However, the differences were less pronounced compared to ER, CDX2, and GATA-3 as shown in Table 4. ER showed greater sensitivity (81.8% and 84.6%) for mammary origin compared to PR (54.5% and 38.5%) in both comparisons.

### 3.4. Unexpected expression of markers

Among the breast primaries, CK20 was positive in one case (1/33, 3%) of pleomorphic lobular carcinoma which was also CK7, ER, PR, and GATA-3 positive by IHC. One case of lobular breast carcinoma showed expression of HepPar-1. Among the GI primaries, one appendiceal signet-ring carcinoma expressed GATA-3. GATA-3 was also found in one case of primary lung adenocarcinoma. ER was negative in all non-breast tumors expressing GATA-3.

### 3.5. Trial of Proposed Immunohistochemical Panel

Eight cases of metastatic gastric signet-ring carcinoma and eight cases of metastatic breast carcinoma with signet-ring features were identified as shown in Table 5. Among the gastric tumors, 4 were metastatic to the ovary, 3 to lung, and one to liver. Of the metastatic breast cases, 3 were metastatic to the liver, 2 to bone, 2 to soft tissue, and 1 to the ovary. In all cases, the metastatic tumor exhibited unequivocal signet ring morphology. CK20 and CDX2 were both positive in 6 of the metastatic gastric tumors with absent expression of ER and

GATA-3. One metastatic gastric carcinoma exhibited only CDX2 staining while another did not express any of the four markers. In contrast, the 8 metastatic breast carcinomas with signet-ring features were positive for ER, positive for GATA-3, negative for CK20, and negative for CDX2.

## 4. Discussion

Signet-ring cells are thought to derive from cellular alterations including abrogation of cell-to-cell adhesion, enhancement of mucin production, and disruption of mucin secretion. These mechanisms are thought to contribute to signet-ring morphology in carcinomas regardless of primary site [12, 13, 14]. While their morphology may be similar from organ-to-organ, they typically retain expression of markers from their site of origin [15]. Our data demonstrate that these qualities can be leveraged to distinguish primary breast tumors with this morphology from gastrointestinal signet-ring tumors with relatively newer immunohistochemical markers.

Among the cytokeratin markers, differences in CK20 staining between breast and gastric tumors as well as breast versus all of the GI tumors as a group were statistically significant. On the other hand, CK7 did not reach statistical significance in this application. This finding is consistent with prior studies demonstrating that the utility of CK7 alone is limited to specific circumstances [11, 16]. Moreover, it suggests that for signet-ring tumors where the differential is breast versus gastrointestinal origin, CK7 could be omitted from the work-up given the appropriate context.

Differences in the membranous staining pattern with e-cadherin have been reported in some non-signet-ring micropapillary tumors. We did not observe any qualitative differences in staining observed in our signet-ring tumors. E-cadherin is implicated as a driver or participant in the development of signet-ring morphology which would suggest that this pattern of growth may show similar expression of e-cadherin regardless of primary site [13]. In our cases, no differences in e-cadherin staining were detected when comparing all breast tumors as a group to the gastrointestinal cases ($p=0.813$). However, when specifically comparing lobular breast carcinomas to gastric carcinomas, a statistically significant difference was identified ($p=0.005$) with 74% of the gastric carcinomas and 23% of lobular carcinomas showing expression. Prior to immunohistochemical staining, we classified breast carcinomas by morphologic criteria alone in accordance with consensus in the literature [17]. We also believe this approach was prudent as some e-cadherin-expressing lobular carcinomas have been reported [18]. In three cases with a consensus diagnosis of lobular carcinoma, e-cadherin expression was retained. Therefore, the significant contrast in e-cadherin staining between these two primary sites would have been more ostensible if the breast tumors retaining expression had been excluded as lobular carcinoma.

ER and PR were both absent in the gastrointestinal primary carcinomas. Aside from the breast tumors, these two markers were expressed in ovarian and endometrial carcinomas as expected. The difference in staining between the breast signet-ring tumors and primaries from other sites was statistically more significant with ER. ER also showed a greater sensitivity for breast carcinoma although its specificity was similar to PR. However, this may

reflect the higher prevalence of ER expression (82%) compared to PR expression (55%) in our breast cases. Notably, ER has been reported to be positive in some gastric carcinomas [19]. Nash et al previously reported that ER and PR had limited utility in separating metastatic breast tumors from other metastatic carcinomas to the liver. They also reported aberrant immunoreactivity for PR in some metastatic tumors from non-breast sites with ER identified only in breast primaries. [19]. In the context of our data, this confirms that ER has more favorable characteristics than PR for our differential diagnosis.

GATA-3 exhibited the strongest qualities to differentiate between the groups with high sensitivity and specificity consistent with findings from prior studies on primary breast tumors [20, 21]. Comprehensive review of the literature indicates that almost all lobular breast carcinomas express GATA-3. All 31 cases of breast carcinoma with signet ring features that were successfully stained with GATA-3 were positive in our study. Notably, Wendroth et al. previously reported a case of ER-negative signet ring breast carcinoma which lacked GATA-3 expression in their series [21]. Accordingly, it would be advisable to utilize a panel of markers that include gastric carcinoma markers for this differential diagnosis. At least 90% of carcinomas with signet-ring features arise from either the breast or GI tract [12]. Since the cases included in our study reflect this distribution with few cases arising outside of the breast and gastrointestinal tract, the utility of GATA-3 in a broader differential is less clear. In particular, GATA-3 is also expressed in urothelial carcinomas among others [20, 22]. However, all gastric signet-ring carcinomas were negative for GATA-3 lending credence to the utility of this marker when the distinction from lobular breast carcinoma is necessary.

For tumors where breast and gastrointestinal are the main primary sites suspected, our data confirm that CDX2 is an effective marker for identifying gastrointestinal-primary in the signet-ring components [23]. HepPar-1 was strongly preferentially expressed in the gastrointestinal tumors also in concordance with prior studies [12]. We identified one case of pleomorphic lobular breast carcinoma with signet rings positive for HepPar-1. To our knowledge, this is the first report of this aberrant expression in the literature. Although these markers may be effective for the differential diagnoses interrogated in this study, immunohistochemical studies for lung, bladder or other sites are still prudent in the setting of relevant past history or other strong clinical suspicion [22, 24].

Both GCDFP-15 and mammaglobin, markers commonly used to identify breast tumors, showed significant differences in staining between breast and GI or gastric tumors. Staining was considered positive only if the signet-ring component showed expression. For mammaglobin, some cases showed weak staining in the non-signet-ring components while the signet-ring cells were negative. In concordance with prior studies, our data show these markers are specific but less sensitive for breast tumors when compared to ER and GATA-3 [21, 25]. Cases found to express GCDFP15 and mammaglobin expressed GATA3 and were often ER-positive. These markers may improve sensitivity or specificity marginally. Since our goal was to propose an efficient initial diagnostic panel for this differential, our findings would support excluding these markers from the initial workup. However, given their high specificity for breast tumors, they may be useful in an expanded metastatic work-up.

The selected panel of CK20, CDX2, ER, and GATA-3 were applied to a set of metastatic breast and gastric tumors with unequivocal signet-ring morphology. In this set, gastric carcinomas were readily differentiated from primary breast carcinomas. However, metastases comprised entirely of unequivocal signet-ring cells were rare and the number of cases identified were limited. More aberrant staining patterns may be expected in a larger cohort. We detected one case of metastatic gastric carcinoma exhibiting only CDX2 staining using our panel while another expressed neither CK20 nor CDX2. However, ER and GATA-3 were negative in both tumors which could prompt a more expanded work-up should these cases present. Overall, the utilization of this panel including both gastric and breast markers reduces the likelihood of tumors that elude identification of their primary sites.

The use of whole tissue sections for staining of some cases and TMAs to assess others facilitated the examination of a larger number of cases. While the TMAs were helpful in our assessment of additional cases, some tumors with heterogeneous staining in the signet-ring component would not have been identified. Comparison of the signet-ring components to non-signet-ring components was not possible for every case. Therefore, we specifically analyzed areas exhibiting signet-ring morphology without particular assessment of other tumor components. Furthermore, direct comparison of expression in metastatic tumors and their respective primary tumors was not performed since some cases were unavailable or the material in one of the specimens was too scant for staining. Finally, our inclusion criteria required only a focal well-defined signet-ring component on the examined sections or biopsies. These may have represented a smaller proportion of the tumors compared to prior studies.

In summary, we compared expression profiles of commonly utilized immunohistochemical markers in surgical pathology practice for signet-ring components in breast and gastrointestinal tract tumors. We identified rare unexpected expression in some tumors that may constitute diagnostic pitfalls. Overall, ER, GATA-3, CK20 and CDX2 exhibited the most significant differences between the compared groups with the best discriminating characteristics for their respective tumors. Therefore, we recommend these four markers as an initial diagnostic panel for this metastatic workup. In the differential diagnosis between lobular breast carcinoma and gastric signet-ring carcinoma, a combination of ER and GATA-3 should effectively discriminate between these two entities.

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## Highlights

1. ER and GATA-3 are effective for discriminating breast from gastrointestinal signet-ring tumors
2. CK20 and CDX2 are also useful for supporting gastrointestinal origin in signet-ring tumors
3. The utility of CK7 is limited in this differential diagnosis

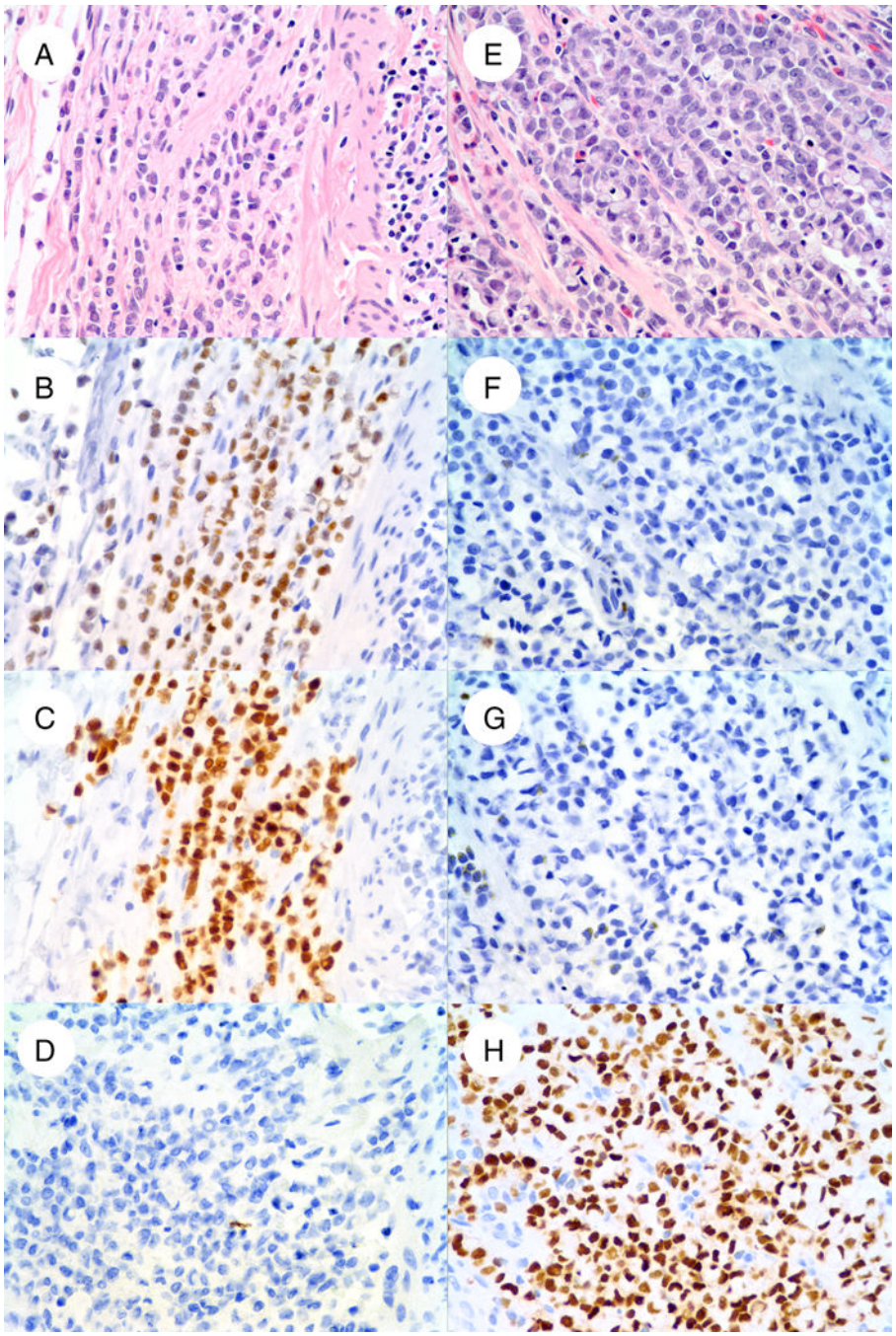


**Figure 1. Comparison of Expression between Lobular Breast Carcinoma and Gastric Signet-Ring Carcinoma–**

**[A – D]**: Metastatic lobular breast carcinoma with signet-ring features (200×) – **[A]** H&E section; **[B]** Estrogen Receptor IHC; **[C]** GATA3 IHC; **[D]** CDX2 IHC

**[E – H]**: Metastatic gastric signet ring carcinoma (200×) – **[E]** H&E section; **[F]** Estrogen Receptor IHC; **[G]** GATA3 IHC; **[H]** CDX2 IHC

**Table 1**

Characteristics of Primary Antibodies

| Antibody | Source | Host and Clone | Antigen Retrieval Buffer and Parameters | Dilution | Detection Method |
|---|---|---|---|---|---|
| CK 7 | Dako Carpinteria, CA | Mouse monoclonal OV-TL 12/30 | pH9 EDTA 97°C 30 min | Ready-to-Use | EnVision FLEX, Dako Omnis Autostainer |
| CK 20 | Dako Carpinteria, CA | Mouse monoclonal Ks20.8 | pH9 EDTA 97°C 30 min | Ready-to-Use | EnVision FLEX, Dako Omnis Autostainer |
| E-cadherin | Dako Carpinteria, CA | Mouse monoclonal NCH-38 | pH9 EDTA 97°C 30 min | Ready-to-Use | EnVision FLEX, Dako Omnis Autostainer |
| GCDFP-15 | Dako Carpinteria, CA | Mouse monoclonal 23A3 | pH9 EDTA 97°C 30 min | Ready-to-Use | EnVision FLEX, Dako Omnis Autostainer |
| Mammaglobin | Cell Marque Rocklin, CA | Mouse/Rabbit cocktail 304-1A5/31A | pH9 EDTA 97°C 30 min | 1:100 | EnVision FLEX, Dako Omnis Autostainer |
| ER | Ventana Tucson, AZ | Rabbit monoclonal SP-1 | pH9 EDTA 97°C 30 min | Ready-to-Use | EnVision FLEX, Dako Omnis Autostainer |
| PR | Ventana Tucson, AZ | Rabbit monoclonal 1E2 | pH6 Citrate 97°C 30 min | Ready-to-Use | EnVision FLEX, Dako Omnis Autostainer |
| GATA-3 | Biocare Concord, CA | Mouse monoclonal L50-823 | pH9 EDTA 97°C 30 min | 1:250 | EnVision FLEX, Dako Omnis Autostainer |
| CDX2 | Dako Carpinteria, CA | Mouse monoclonal DAK-CDX2 | pH9 EDTA 97°C 30 min | Ready-to-Use | EnVision FLEX, Dako Omnis Autostainer |
| HepPar-1 | Dako Carpinteria, CA | Mouse monoclonal OCH1E5 | pH9 EDTA 97°C 30 min | Ready-to-Use | EnVision FLEX, Dako Omnis Autostainer |

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**Table 2**

Summary of Immunohistochemical Expression

| | CK7 | CK20 | E-cadherin | GCDFP-15 | Mammagl obin | ER | PR | CDX2 | GATA-3 | HepPar-1 |
|---|---|---|---|---|---|---|---|---|---|---|
| Total Breast | 24/31 (77) | 2/30 (7) | 23/33 (70) | 9/12 (75) | 5/12 (42) | 27/33 (70) | 18/33 (55) | 0/33 (0) | 31/31 (100) | 1/30 (3) |
| Ductal | 14/19 (74) | 19/19 (100) | 19/19 (100) | 3/5 (60) | 2/5 (40) | 15/19 (79) | 12/19 (63) | 0/19 (0) | 17/17 (100) | 0/17 (0) |
| Lobular | 9/13 (69) | 1/12 (8) | 3/13 (23) | 6/7 (86) | 3/7 (43) | 11/13 (85) | 5/13 (39) | 0/13 (0) | 13/13 (100) | 1/13 (8) |
| Mucinous | 1/1 (100) | 0/1 (0) | 1/1 (100) | – | – | 1/1 (100) | 1/1 (100) | 0/1 (0) | 1/1 (100) | |
| Total GI | 31/50 (62) | 32/49 (65) | 33/50 (66) | 0/26 (0) | 0/26 (0) | 0/50 (0) | 0/50 (0) | 36/50 (72) | 1/50 (2) | 17/50 (34) |
| Esophageal | 3/3 (100) | 1/3 (33) | 3/3 (100) | 0/2 (0) | 0/2 (0) | 0/3 (0) | 0/3 (0) | 1/3 (33) | 0/3 (0) | 1/3 (33) |
| Gastric | 19/23 (82) | 15/23 (65) | 17/23 (74) | 0/11 (0) | 0/11 (0) | 0/23 (0) | 0/23 (0) | 16/23 (70) | 0/23 (0) | 12/23 (52) |
| Small intestinal | 2/2 (100) | 1/2 (50) | 0/2 (0) | – | – | 0/2 (0) | 0/2 (0) | 2/2 (100 | 0/2 (0) | 0/2 (0) |
| Ampullary | 1/1 (100) | 0/1 (0) | 1/1 (100) | 0/1 (0) | 0/1 (0) | 0/1 (0) | 0/1 (0) | 1/1 (100) | 0/1 (0) | 0/1 (0) |
| Pancreatic | 4/5 (80) | 1/4 (25) | 3/5 (60) | 0/4 (0) | 0/4 (0) | 0/5 (0) | 0/5 (0) | 0/5 (0) | 0/5 (0) | 0/5 (0) |
| Colonic | 0/7 (0) | 5/7 (71) | 5/7 (71) | 0/5 (0) | 0/5 (0) | 0/7 (0) | 0/7 (0) | 7/7 (100) | 0/7 (0) | 2/7 (29) |
| Appendiceal | 2/9 (22) | 9/9 (100) | 4/9 (44) | 0/3 (0) | 0/3 (0) | 0/9 (0) | 0/9 (0) | 9/9 (100) | 1/9 (11) | 2/9 (22) |
| Pulmonary | 4/4 (100) | 0/4 (0) | 3/4 (75) | 0/4 (0) | 0/4 (0) | 0/4 (0) | 0/4 (0) | 0/4 (0) | 1/4 (25) | 3/4 (75) |
| Urothelial | 2/2 (100) | 1/2 (50) | 2/2 (100) | 0/2 (0) | 0/2 (0) | 0/2 (0) | 0/2 (0) | 0/2 (0) | 1/2 (50) | 1/2 (50) |
| Müllerian | 4/4 (100) | 1/4 (25) | 4/4 (100) | 0/4 (0) | 0/4 (0) | 2/4 (50) | 2/4 (50) | 0/4 (0) | 0/4 (0) | 0/4 (0) |

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**Table 3**

Comparison of Immunohistochemical Expression – Breast Versus Gastrointestinal

| | | Breast (%) | GI (%) | *p* | Sensitivity Specificity | 95% CI | PPV NPV | 95% CI |
|---|---|---|---|---|---|---|---|---|
| **CK7** * | | | | | | | | |
| | **Pos** | 24 (77) | 31 (62) | 0.221 | 0.774 | 0.585–0.897 | 0.369 | 0.255–0.498 |
| | **Neg** | 7 (23) | 19 (38) | | 0.316 | 0.206–0.451 | 0.731 | 0.519–0.876 |
| **CK20**≠ | | | | | | | | |
| | **Pos** | 2 (7) | 32 (67) | 8.76E-08 | 0.667 | 0.515–0.792 | 0.941 | 0.789–0.990 |
| | **Neg** | 28 (93) | 16 (33) | | 0.933 | 0.765–0.988 | 0.636 | 0.447–0.675 |
| **E-cadherin** * | | | | | | | | |
| | **Pos** | 23 (70) | 33 (66) | 0.813 | 0.697 | 0.511–0.838 | 0.354 | 0.242–0.483 |
| | **Neg** | 10 (30) | 17 (34) | | 0.300 | 0.192–0.434 | 0.643 | 0.441–0.807 |
| **GCDFP** * | | | | | | | | |
| | **Pos** | 9 (75) | 0 | 1.35E-06 | 0.750 | 0.428–0.933 | 1.000 | 0.629–1.000 |
| | **Neg** | 3 (25) | 26 (100) | | 1.000 | 0.880–1.000 | 0.923 | 0.780–0.980 |
| **Mammaglobin** * | | | | | | | | |
| | **Pos** | 5 (42) | 0 | 0.00158 | 0.417 | 0.165–0.714 | 1.000 | 0.463–1.000 |
| | **Neg** | 7 (58) | 26 (100) | | 1.000 | 0.840–1.000 | 0.788 | 0.606–0.904 |
| **ER** * | | | | | | | | |
| | **Pos** | 27 (82) | 0 | 2.17E-16 | 0.818 | 0.639–0.924 | 1.000 | 0.845–1.000 |
| | **Neg** | 6 (18) | 50 (100) | | 1.000 | 0.911–1.000 | 0.893 | 0.775–0.956 |
| **PR** * | | | | | | | | |
| | **Pos** | 18 (55) | 0 | 1.39E-09 | 0.545 | 0.366–0.715 | 1.000 | 0.781–1.000 |
| | **Neg** | 15 (45) | 50 (100) | | 1.000 | 0.911–1.000 | 0.769 | 0.645–0.861 |
| **CDX2**≠ | | | | | | | | |
| | **Pos** | 0 | 36 (72) | 7.56E-12 | 0.720 | 0.573–0.833 | 1.000 | 0.880–1.000 |
| | **Neg** | 31 (100) | 14 (28) | | 1.000 | 0.863–1.000 | 0.689 | 0.532–0.814 |
| **GATA-3** * | | | | | | | | |
| | **Pos** | 31 (100) | 1 (2) | 1.38E-21 | 1.000 | 0.863–1.000 | 0.968 | 0.820–0.998 |
| | **Neg** | 0 | 49 (98) | | 0.980 | 0.880–0.999 | 1.000 | 0.909–1.000 |

| | | Breast (%) | GI (%) | *p* | Sensitivity | Specificity | 95% CI | PPV | NPV | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| **HepPar-1**[‡] | | | | | | | | | | |
| | **Pos** | 1 (3) | 17 (34) | 0.001 | 0.340 | | 0.216–0.489 | 0.944 | | 0.706–0.997 |
| | **Neg** | 29 (97) | 33 (66) | | 0.967 | | 0.342–0.598 | 0.468 | | 0.341–0.598 |

[*] Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and corresponding confidence intervals (CI) for breast origin.

[‡] Sensitivity, specificity, positive predictive value, negative predictive value and corresponding confidence intervals for gastrointestinal origin.

**Table 4**

Comparison of Immunohistochemical Expression – Lobular Versus Gastric

| | | Lobular (%) | Gastric (%) | *p* | Sensitivity Specificity | 95% CI | PPV NPV | 95% CI |
|---|---|---|---|---|---|---|---|---|
| **CK7** * | | | | | | | | |
| | **Pos** | 9 (69) | 19 (83) | 0.422 | 0.692 | 0.389–0.896 | 0.321 | 0.166–0.524 |
| | **Neg** | 4 (31) | 4 (17) | | 0.173 | 0.0572–0.396 | 0.500 | 0.175–0.825 |
| **CK20**≠ | | | | | | | | |
| | **Pos** | 1 (8) | 15 (65) | 0.00161 | 0.652 | 0.482–0.828 | 0.938 | 0.677–0.997 |
| | **Neg** | 11 (92) | 8 (35) | | 0.917 | 0.598–0.996 | 0.579 | 0.340–0.789 |
| **E-cadherin** * | | | | | | | | |
| | **Pos** | 3 (23) | 17 (74) | 0.00523 | 0.231 | 0.0616–0.540 | 0.150 | 0.0396–0.389 |
| | **Neg** | 10 (77) | 6 (26) | | 0.261 | 0.111–0.487 | 0.375 | 0.163–0.641 |
| **GCDFP** * | | | | | | | | |
| | **Pos** | 6 (86) | 0 | 0.00038 | 0.857 | 0.420–0.992 | 1.000 | 0.517–1.000 |
| | **Neg** | 1 (14) | 11 (100) | | 1.000 | 0.679–1.000 | 0.917 | 0.598–0.996 |
| **Mammaglobin** * | | | | | | | | |
| | **Pos** | 3 (43) | 0 | 0.043 | 0.429 | 0.118–0.798 | 1.000 | 0.310–1.000 |
| | **Neg** | 4 (57) | 11 (100) | | 1.000 | 0.679–1.000 | 0.733 | 0.448–0.911 |
| **ER** * | | | | | | | | |
| | **Pos** | 11 (85) | 0 | 1.30E-07 | 0.846 | 0.537–0.973 | 1.000 | 0.679–1.000 |
| | **Neg** | 2 (15) | 23 (100) | | 1.000 | 0.822–1.000 | 0.920 | 0.725–0.986 |
| **PR** * | | | | | | | | |
| | **Pos** | 5 (39) | 0 | 0.00341 | 0.385 | 0.151–0.677 | 1.000 | 0.463–1.000 |
| | **Neg** | 8 (62) | 23 (100) | | 1.000 | 0.822–1.000 | 0.742 | 0.551–0.875 |
| **CDX2**≠ | | | | | | | | |
| | **Pos** | 0 | 16 (70) | 0.00005 | 0.696 | 0.470–0.859 | 1.000 | 0.759–1.000 |
| | **Neg** | 13 (100) | 7 (30) | | 1.000 | 0.717–1.000 | 0.650 | 0.410–0.837 |
| **GATA-3** * | | | | | | | | |
| | **Pos** | 13 (100) | 0 | 4.33E-10 | 1.000 | 0.717–1.000 | 1.000 | 0.717–1.000 |
| | **Neg** | 0 | 23 (100) | | 1.000 | 0.822–1.000 | 1.000 | 0.822–1.000 |

| | | Lobular (%) | Gastric (%) | *p* | Sensitivity | Specificity | 95% CI | PPV | NPV | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| **HepPar-1**[≠] | | | | | | | | | | |
| | **Pos** | 1 | 12 (52) | .011 | 0.522 | | 0.311–0.726 | 0.923 | | 0.621–0.996 |
| | **Neg** | 12 (100) | 11 (48) | | 0.923 | | 0.621–0.996 | 0.522 | | 0.311–0.726 |

[*] Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and corresponding confidence intervals (CI) for lobular carcinoma.

[≠] Sensitivity, specificity, positive predictive value, negative predictive value and corresponding confidence intervals for gastric carcinoma.

**Table 5**

Trial of Proposed Immunohistochemical Panel

| Primary Site | Metastatic Site | CK20 | CDX2 | ER | GATA-3 |
|---|---|---|---|---|---|
| ***Breast*** | | | | | |
| | Ovary | Neg | Neg | Pos | Pos |
| | Soft tissue | Neg | Neg | Pos | Pos |
| | Soft tissue | Neg | Neg | Pos | Pos |
| | Liver | Neg | Neg | Pos | Pos |
| | Liver | Neg | Neg | Pos | Pos |
| | Liver | Neg | Neg | Pos | Pos |
| | Bone | Neg | Neg | Pos | Pos |
| | Bone | Neg | Neg | Pos | Pos |
| | | **0/8** | **0/8** | **8/8** | **8/8** |
| ***Gastric*** | | | | | |
| | Ovary | Pos | Pos | Neg | Neg |
| | Ovary | Pos | Pos | Neg | Neg |
| | Ovary | Pos | Pos | Neg | Neg |
| | Ovary | Pos | Pos | Neg | Neg |
| | Lung | Pos | Pos | Neg | Neg |
| | Lung | Pos | Pos | Neg | Neg |
| | Lung | Neg | Neg | Neg | Neg |
| | Liver | Neg | Pos | Neg | Neg |
| | | **6/8 (75%)** | **7/8 (87.5%)** | **0/8** | **0/8** |

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