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Original Article

Initial panel of immunocytochemical markers for identification of primary carcinoma site for effusions and peritoneal washings from women

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Abstract: Introduction: In women, most malignant effusions are from breast and ovary primary carcinomas that have metastasized to body cavity fluids (pleural, peritoneal and pericardial). When carcinoma is diagnosed in effusions, it is not possible to identify its site of origin solely by cytology (morphology); therefore, immunocytochemistry is used as a complementary method. There are no immunocytochemical markers with 100% sensitivity and specificity for identifying carcinoma primary site. The markers most used are TTF-1 for the lung, GATA-3 for the breast, and PAX-8 for the ovary. The aim of this study was to evaluate the sensitivity and specificity of a panel including these markers for detecting the primary site of carcinoma in effusions. Methods: Samples of pleural, pericardial, and peritoneal effusions and peritoneal washings with carcinoma of known primary site from women (n = 60) and men (n = 18) were prepared by using the cell block method, and immunocytochemistry was performed to evaluate the expression of primary site markers (TTF-1, PAX-8, and GATA-3). Results: In women, the breast was the most frequent primary site of metastatic carcinoma to both pleural and pericardial cavities, followed by the lung, whereas the ovary was the most frequent primary site of carcinoma within peritoneal effusions and washings, followed by the gastrointestinal tract (stomach or intestine). The expected profiles for carcinomas of the most common primary sites were: breast (GATA-3 (+), PAX-8 (-), TTF-1 (-)), ovary (PAX-8 (+), GATA-3 (-), TTF-1 (-)), lung (TTF-1 (+), PAX-8 (-) GATA-3 (-)) and gastrointestinal tract (PAX-8 (-), GATA-3 (-), TTF-1 (-)). These were observed in 88.23% (45/51) of women's samples with carcinoma from these primary sites. By using TTF-1 as the sole primary site marker, 6.25% of carcinomas of primary site other than the lung would have been misdiagnosed. Conclusion: An initial panel of markers including GATA-3, PAX-8, and TTF-1 allows, with high sensitivity and specificity, the identification or exclusion of frequent primary sites of carcinoma in effusions from women. Our results highlight the importance of using a panel of markers to avoid misidentification of the primary site of tumor.

Keywords: Cytology, immunocytochemistry, effusion, peritoneal washing, pleural, pericardial, peritoneal, carcinoma, TTF-1, GATA-3, RE, PAX-8, HBME

Introduction

Pleural, peritoneal, and pericardial effusion analysis for cancer is a diagnostic challenge. Effusions may be the first sign of the diagnosis of a carcinoma of unknown primary site since only approximately 31% effusion specimens with positivity for malignancy have known primary sites [1]. In addition, the presence of carcinoma in effusions of patients with a previous history of carcinoma may either correspond to metastasis of this known primary or to the appearance of a second primary carcinoma [2-4].

Metastases from breast or ovarian carcinoma are the most common etiology for malignant effusions in female patients [5]. Breast carcinomas are more common in pleural fluid and ovarian carcinomas in peritoneal fluid, but metastasis to an unexpected serous cavity may occur [5]. Most effusions associated with metastatic

carcinoma of ovarian origin are synchronous, that is, they are detected simultaneously with the primary tumor, while most effusions of breast carcinoma origin are metachronous, diagnosed months after the primary tumor [6].

When carcinoma is diagnosed in effusions, it is not possible to identify the site of origin of the carcinoma solely by cytologic morphology [7-9]. Immunocytochemistry represents the most commonly employed study in effusions, complementary to cytomorphology, but other ancillary methods also have specific and relevant applications [7-9]. For instance, flow cytometry is well established as a valuable technique in the diagnosis of lymphoproliferative disorders. The use of fluorescence *in situ* hybridization (FISH) in effusion cytology is currently focused on the diagnosis of mesothelioma (detection of homozygous deletion of p16/CDKN2A) and for detection of ALK gene rearrangements, while PCR-based assays have been used for EGFR mutations. Multiplexed genetic sequencing panels that detect multiple types of alterations using a single platform, such as next-generation sequencing (NGS), have emerged as a preferred testing platform, especially for tumor types that require more comprehensive molecular profiling.

Due to its lower cost, ease of use, availability of its reagents and equipment, high accuracy, and its different applications, immunocytochemistry is still the ancillary method of choice in anatomic pathology laboratories [7-9]. Specific applications of immunocytochemistry in the routine diagnosis of effusion and peritoneal washing samples are 1) resolving the mesothelial or epithelial origin of isolated atypical cells and cell clusters; 2) identifying the primary site of malignancy in a patient with an unknown primary site or with a history of multiple malignancies; and 3) establishing the expression of therapeutic response markers. The most sensitive and specific markers for the differentiation between mesothelial and epithelial origin of atypical cells in effusions and peritoneal washing are Claudin-4 and EpCAM (detected by clone MOC-31) [10, 11]. For women, the immunocytochemical panel used to identify the primary site of the carcinoma is different from the panel used for men, since markers for the primary site in breast and ovary must be included. The most commonly used markers are TTF-1 for the lung, GATA-3 for the breast, and PAX-8 for the ovary; nonetheless, there are no immunocytochemical markers with 100% specificity and sensitivity [12, 13]. Thus, the aim of this study was to evaluate the sensitivity and specificity of a panel including these markers for detecting the primary site of carcinoma in effusions and peritoneal washings.

Materials and methods

This was an observational and cross-sectional study approved by the Human Ethics Review Committee of Brasilia University under the number CAAE: 34150314.9.0000.5558. All samples of effusion and peritoneal washing were analysed at the Pathological Anatomy Unit of University Hospital of Brasilia between 2013 and 2020. Only samples diagnosed with carcinoma by cytology and immunocytochemistry (positivity for clone MOC-31 of Epcam and Claudin-4) and with known primary sites were included. The cell block adequacy was assessed by the presence of a minimum of five carcinoma cell groups [14]. Most carcinomas in the present study were classified as adenocarcinomas by histologic type, with the exception of one small cell carcinoma of the lung and one squamous cell uterine cervix carcinoma.

No fixative solution was used, and cell block preparation was performed as previously described using the plasma-thromboplastin or agar method [15]. In the plasma-thromboplastin method, cytologic samples were centrifuged, and 100 µl of plasma and 100 µl of thromboplastin (Stago®, Asnières sur Seine, France) were added to the cell pellet. For samples with a large amount of sediment, cell pellets were homogenized with agar 1-5%. The clot/gel was formalin-fixed, submitted to usual histologic processing, and sections mounted on previously silanized slides. These were stained with hematoxylin-eosin and used for immunocytochemistry.

Prior to exposure to the primary antibodies, samples were submitted to antigen retrieval with citrate buffer pH 6.0 in a water bath at 95-99°C for 45 min. For blockade of endogenous tissue peroxide, the slides were immersed in 3% H_2O_2 solution at room temperature for 30 min and thoroughly washed with phos-

Table 1. Antibodies used for immunocytochemistry

Antibody	Manufacturer	Clone	Dilution
GATA-3	CELL MARQUE	L50-823	1:300
PAX-8	MEDAYSIS	1491&1492	1:100
TTF-1	ABCAM	BP1 1584Y	1:50

phate buffered saline (PBS). Incubation with primary antibodies (**Table 1**) was performed overnight at 4°C. After a 30-min incubation with secondary antibody at room temperature, positive cells were marked with the streptavidin-peroxidase complex (Kit REVEAL-Biotin-Free Polyvalent DAB - Spring Bioscience®, CA, USA), and the reactions were developed using a diaminobenzidine chromogen solution (kit REVEAL-Biotin-Free Polyvalent DAB-Spring Bioscience®, California, USA). Counterstaining was performed with Harris hematoxylin. Positive and negative controls were used for each primary antibody according to the manufacturer's recommendation. An additive scoring system that ranges from 0 to 8 was used: proportion score (0 = no nuclear staining; 1≤1% nuclei staining; 2 = 1-10% nuclei staining; 3 = 11-33% nuclei staining; 4 = 34-66% nuclei staining and 5 = 67-100% nuclei staining) and intensity score (0 = no staining; 1 = weak staining; 2 = moderate staining and 3 = strong staining) [16].

Results

The expression of GATA-3, PAX-8, and TTF-1 markers was analyzed in 78 samples with carcinoma: pleural fluid, n = 40; ascitic fluid, n = 24; pericardial fluid, n = 7; and peritoneal washing, n = 7. Of the total samples, 76.92% (60/78) were from female patients. Female patient samples comprised 65% (26/40), 87.5% (21/24), 85.71% (6/7) and 100% (7/7) of pleural fluid, ascitic fluid, pericardial fluid and peritoneal washings, respectively.

The frequency of the carcinoma origin sites according to the sample type (pleural, ascitic, pericardial and peritoneal washing) is shown in **Table 2**. In women, the most frequent carcinoma primary site was the breast in pleural and pericardial fluids and the ovary in ascitic fluid and peritoneal washings. Breast carcinomas accounted for 50% (13/26) of carcinomas in pleural fluid and 66.66% (4/6) of carcinomas in pericardial fluid in women, while ovarian carcinomas accounted for 52.38% (11/21) of ascitic fluid carcinomas and 71.42% (5/7) of peritoneal washing carcinomas in women. Samples with carcinoma from the breast and ovary together accounted for 55% (33/60) of samples with carcinoma in effusions/peritoneal washing from female patients. In women, the second most frequent carcinoma sites in pleural fluid and ascitic fluid were the lung and gastrointestinal tract (stomach and intestine), respectively.

The marker profile expression frequency according to the carcinoma primary site in effusions/peritoneal washings in women is shown in **Table 3**. All samples showed a positivity score ≥ 3.

The sensitivity and specificity of the GATA-3 (+), PAX-8 (-), and TTF-1 (-) profile for breast carcinoma detection were 94.11% (16/17) and 100% (43/43), respectively. One of the breast carcinoma samples was GATA-3 (+), PAX-8 (-), and TTF-1 (+). The sensitivity and specificity of the PAX-8 (+), GATA-3 (-), and TTF-1 (-) profile for ovarian carcinoma detection were 87.50% (14/16) and 86.36% (38/44), respectively. One ovarian carcinoma sample was PAX-8 (-), GATA-3 (-), TTF-1 (-) and another was PAX-8 (+), GATA-3 (-), TTF-1 (+). The sensitivity and specificity of the TTF-1 (+) and PAX-8 (-) GATA-3 (-) profile for the detection of lung carcinoma were 83.33% (10/12) and 97.91% (47/48), respectively. Two lung carcinoma samples were PAX-8 (-), GATA-3 (-), and TTF-1 (-). The sensitivity and specificity of the PAX-8 (-), GATA-3 (-), and TTF-1 (-) profile for gastrointestinal tract (stomach and intestine) carcinoma detection were 83.33% (5/6) and 88.88% (48/54), respectively. One sample of stomach carcinoma was TTF-1 (+), PAX-8 (-), and GATA-3 (-). The expected profile for carcinomas of the most common primary sites, breast (GATA-3 (+), PAX-8 (-), TTF-1 (-)), ovary (PAX-8 (+), GATA-3 (-), TTF-1 (-)), lung (TTF-1 (+), PAX-8 (-) GATA-3 (-)) and gastrointestinal tract (PAX-8 (-), GATA-3 (-), TTF-1 (-)), was observed in 88.23% (45/51) of women's samples with carcinoma from these primary sites. By using TTF-1 as the sole primary site marker, 6.25% (3/48) of carcinomas of primary sites other than the lung would have been misdiagnosed: breast (n = 1), ovary (n = 1) and stomach (n = 1). The expression of TFF-1 was intense and diffuse in breast and gastric

Table 2. Frequency of primary sites of carcinoma according to type of effusion or peritoneal washing

	Pleural fluid n = 40		Ascitic fluid n = 24		Pericardial fluid n = 7		Peritoneal washing n = 7	
	M (n = 14)	F (n = 26)	M (n = 3)	F (n = 21)	M (n = 1)	F (n = 6)	M (n = 0)	F (n = 7)
Lung n = 27	13	11	2	0	0	1	0	0
Breast n = 17	0	13	0	0	0	4	0	0
Ovary n = 16	0	0	0	11	0	0	0	5
Stomach n = 7	1	1	1	2	1	0	0	1
Peritoneum n = 3	0	0	0	3	0	0	0	0
Cervix n = 3	0	0	0	2	0	1	0	0
Colon n = 2	0	0	0	2	0	0	0	0
Bile ducts n = 1	0	1	0	0	0	0	0	0
Pancreas n = 1	0	0	0	1	0	0	0	0
Kidney n = 1	0	0	0	0	0	0	0	1

M: male; F: female.

Table 3. Frequency of marker profiles in primary carcinoma sites

Marker panel/Primary site	GATA-3 (+) PAX-8 (-) TTF-1 (-) n	PAX-8 (+) GATA-3 (-) TTF-1 (-) n	TTF-1 (+) GATA-3 (-) PAX-8 (-) n	TTF-1 (-) GATA-3 (-) PAX8 (-) n
Lung n = 12	0	0	10	2
Breast n = 17	16	0	0	0
Ovary n = 16	0	14	0	1
Stomach n = 4	0	0	1	3
Peritoneal n = 3	0	3	0	0
Uterine cervix n = 3	0	2	0	1
Colon n = 2	0	0	0	2
Biliary tract n = 1	0	0	0	1
Pancreas n = 1	0	0	0	1
Kidney n = 1	0	1	0	0

carcinomas (**Figure 1**) and focal in ovarian carcinoma.

Discussion

Use of the most appropriate panel of markers makes it possible to suggest the probable primary site of carcinoma in effusions and to exclude other possible sites, thus reducing immunocytochemistry cost. The marker choice should consider the patient's gender, the frequency of primary sites, the effusion type (pleural, peritoneal or pericardial fluid), the markers' sensitivity and specificity, and clinical findings.

In the present study, the majority (76.92%) of all samples, including pleural, peritoneal, and pericardial effusions and peritoneal washings with carcinoma, were obtained from female patients. The high frequency of effusion and peritoneal washing samples with carcinoma diagnosed in women can be explained by the fact that breast and ovarian carcinomas are types of cancer that most often spread to body cavity metastases. Accordingly, here, the samples of both breast and ovarian carcinomas combined accounted for the majority (55%) of the effusion/peritoneal washing samples with carcinoma in female patients. Thus, the immunohistochemical markers that constitute the initial panel for identifying the carcinoma primary site in effusions/peritoneal washings are different in women's samples and should include markers for breast and ovary primary tumor. Breast, ovary and lung were, respectively, the most frequent carcinoma primary sites corresponding to 28.33%, 26.66% and 20% of the total carcinoma samples in effusions/peritoneal washings of women. This result justifies the use of markers in an initial panel to identify primary site.

The most frequent type of carcinoma-containing effusion in women was pleural fluid (43.33%), followed by ascitic fluid (35%) and pericardial fluid (10%). The distribution of primary sites according to effusion types/peritoneal washings in female samples herein was similar to that observed in previous studies [17-

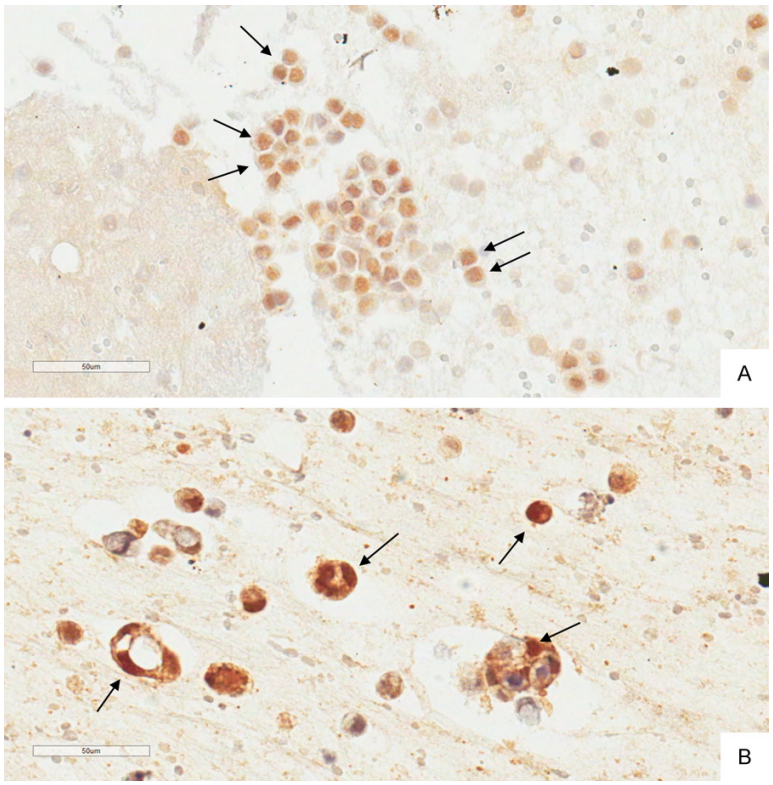

Figure 1. Immunocytochemistry expression of TTF-1 (arrows) in breast (A) and gastric (B) carcinoma effusions.

19]. Breast was the most frequent primary site, followed by lung in pleural fluid, while ovary was the most frequent primary site, followed by the gastrointestinal tract (stomach and intestine), in ascitic fluid. In pericardial fluid, the most frequent primary site was breast and, in peritoneal washings, ovary. The possibility of carcinoma dissemination to a cavity other than the one where the site of its origin is located highlights the importance of using a panel of markers rather than a single marker. In the present study, lung carcinoma was identified in ascitic and pericardial fluid, stomach carcinoma in pleural and pericardial fluid, and cervical carcinoma in pericardial fluid.

The choice of GATA-3 and PAX-8 as markers for breast and ovary primary sites, respectively, was based on the high sensitivity and high positivity score of these markers in relation to other markers, such as mammoglobin, GCDFP-15, estrogen receptor, and WT1 [14, 20-22]. Regarding the best choice for lung primary site, no significant difference was reported in positivity of napsin A and TTF-1 as a single marker in metastatic pulmonary adenocarcinomas [23]. Napsin-A and TTF-1 are both useful markers in distinguishing pulmonary and extrapulmonary adenocarcinoma in effusions; however, the nuclear staining of TTF-1 is much easier to interpret than Napsin-A cytoplasmic staining [24]. For TTF-1, there are a variety of antibody clones with different sensitivities and specificities; for example, the SPT24 clone is more sensitive but less specific for the detection of all lung adenocarcinomas in comparison to the 8G7G3/1 clone [25, 26]. No prior study evaluated TTF-1 expression of metastatic lung adenocarcinoma in effusions by using the same antibody used herein. However, its sensitivity in the present study was greater than that observed in a previous study for the TTF-1 clone (8G7G3/1) and Napsin-A [24]. Another important aspect is specificity: although the markers used in the initial panel (GATA-3, PAX-8, TTF-1) can be expressed in mesotheliomas, they are usually not expressed in normal mesothelial cells, which are usually found in effusions and peritoneal washings and are morphologically confused with carcinoma cells [27-33]. Although considered a sensitive marker for ovarian carcinoma primary site, WT1, for example, can be expressed both in normal mesothelial cells and mesothelioma [34].

The GATA-3 (+), PAX-8 (-), and TTF-1 (-) profile for the detection of breast carcinoma showed high sensitivity (94.11%) and 100% specificity. All carcinomas with a primary site in the breast were positive for GATA-3. This result is in agreement with previous studies in which GATA-3 expression ranged from 89% to 93.5% in breast carcinoma effusions [33, 35, 36].

Breast carcinomas were identified in the pleural and pericardial fluid herein. In pleural fluid, the breast was the most common primary site, followed by the lung. Thus, in pleural fluid, markers were used to allow for differential diagnosis between breast and lung. In the peri-

cardium, TTF-1 expression was found in one breast carcinoma sample, resulting in GATA-3 (+), PAX-8 (-), and TTF-1 (+) marker profile. Therefore, the sensitivity of the GATA-3 (+), PAX-8 (-), and TTF-1 (-) profile was not 100%. In a previous study with 546 histopathologic breast carcinoma samples, 2.4% showed the expression of TTF-1 [37]. The predominant histologic type in these TTF-1-positive breast carcinoma samples was the ductal type, a finding similar to the one observed here. In the present study, the specificity of the GATA-3 (+), PAX-8 (-), and TTF-1 (-) profile was 100%, as there was no expression of GATA-3 in carcinomas of other sites. However, in a previous study in which a large number of surgical specimens (n = 2500) with carcinoma of different primary sites were analysed, GATA-3 expression was observed in carcinoma primary sites frequently found in effusions such as lung (8%), ovary (6%), and stomach (5%) [38]. This result indicates that the presence of GATA-3 immunoreactivity cannot by itself be used to exclude the possibility of carcinomas of additional primary sites, especially in carcinomas from effusion. Thus, a panel including GATA-3, PAX-8 and TTF-1 should be applied, as it increases specificity while excluding carcinomas from those primary sites.

The PAX-8 (+), GATA-3 (-), and TTF-1 (-) profile showed high sensitivity (87.50%) and specificity (86.36%) for detecting primary ovarian carcinoma. The sensitivity of this profile is in agreement with results from previous studies in which PAX-8 expression ranged from 70% to 100% of ovarian carcinoma in effusions [39-41].

Gastrointestinal tract carcinoma, the second most frequent carcinoma in ascitic fluid samples of women, is the main differential diagnosis from ovarian carcinomas. Importantly, PAX-8 expression has not been observed in studies with large numbers of samples using a tissue microarray (TMA) or whole-tissue sections of colon and gastric carcinoma [42, 43].

The PAX-8 (+), GATA-3 (-), TTF-1 (-) profile was not observed in 2 samples from ovary primary site; in one of them there was no expression of PAX-8, resulting in a PAX-8 (-), GATA-3 (-), TTF-1 (-) profile; and in another there was expression of TTF-1, in addition to the expression of PAX-8, resulting in a PAX-8 (+), GATA-3 (-), TTF-1 (+) profile. In carcinomas with PAX-8 (-), GATA-3 (-), and TTF-1 (-) profile, adding markers such as estrogen receptor and HBME would be useful to suggest the possibility of a primary site in the ovary. In the present study, ER expression was observed in 37.5% of the ovarian carcinoma samples and in none of the gastrointestinal carcinoma samples, while HBME expression was observed in 87.5% of the ovarian carcinoma samples and in 16.66% of the gastrointestinal carcinoma samples (data not shown). The expression of TTF-1 was detected in 17.7% and 3.2% of ovarian carcinomas using SPT24 and 8G7G3/1 antibody clones, respectively, in a previous study [44].

PAX-8 expression was not observed in primary sites of breast and lung carcinoma in this study. However, in previous studies, a significant fraction of breast carcinoma showed immunoreactivity for PAX-8, and positivity rates seemed to be antibody-dependent [45, 46]. A recent meta-analysis indicated that primary lung cancers showed PAX-8 expression in rare cases regardless of tumor subtype [47]. Thus, the presence of PAX-8 immunoreactivity alone cannot exclude mammary or pulmonary origin.

As expected, the PAX-8 (+), GATA-3 (-), and TTF-1 (-) profile is not specific for ovarian carcinoma due to the high expression of PAX-8 in carcinomas from other female genital organs and kidneys [39, 42, 43]. In the present study, PAX-8 expression was observed in 2 adenocarcinomas of cervical origin and in 1 renal carcinoma. Carcinomas originating from these organs are not frequent in effusions, but appropriate markers should be included for differential diagnoses.

The TTF-1 (+), PAX-8 (-) and GATA-3 (-) profile showed high sensitivity (83.33%) and specificity (97.91%) for detecting lung carcinoma. Similar results were observed in a meta-analysis that evaluated TTF-1 expression in metastatic lung carcinoma in effusions in 20 studies, in which the sensitivity was 74.95% and the specificity was 99% [48]. TTF-1 was found in adenocarcinoma and small cell lung carcinoma with high frequencies of 96% and 89%, respectively, and was not detected in squamous cell carcinoma and large cell carcinoma [49]. In the present study, most lung carcinomas were adenocarcinoma except for one small cell carcinoma.

The TTF-1 (-) PAX-8 (-), GATA-3 (-) profile was observed in two lung carcinoma samples, and in these cases, the panel for lung carcinoma detection should be complemented with the use of napsin A. The combined use of napsin A and TTF-1 results in improved sensitivity and specificity for identifying pulmonary adenocarcinoma in a metastatic setting [50].

The PAX-8 (-), GATA-3 (-), and TTF-1 (-) profile showed high sensitivity (83.33%) and specificity (88.88%) for identifying carcinomas of the gastrointestinal tract; in all samples, CK20 was expressed (data not shown).

In peritoneal effusion/washing samples from female patients, the main differential diagnosis of carcinomas of the gastrointestinal tract is ovarian carcinoma, especially mucinous ovarian tumors, which are positive for PAX-8 in only 32% of samples, are negative for WT1, and can be positive for CK20 and CDX-2 [51, 52]. The expressions of CK20 and CDX2 in paraffin-embedded tissue microarrays of carcinoma primary sites were as follows: colon 88.6% and 93.3%; stomach 24.6% and 46.9%; lung, 5.6% and 2.8%; serous ovary, 8.5% and 4.4%; mucinous ovary 50% and 66.7%; and breast 2.7% and 0% [53], respectively, all of which are often observed in effusions.

One stomach carcinoma sample was TTF-1 (+), PAX-8 (-) and GATA-3 (-). In a previous study using the most sensitive TTF-1 antibody clone (SPT24), TTF-1 was positive in 25% of all gastric adenocarcinomas, and of the TTF-1-positive cases by SPT24, 31% also showed focal to diffusely positive granular cytoplasmic staining for Napsin A [54]. In the same study, by using the 8G7G3/1 clone, only 1 SPT24-positive case showed positive nuclear staining [42]. TTF-1 and Napsin A positivity, therefore, cannot be used as conclusive evidence of pulmonary origin, and the TTF-1 antibody clone should be considered [54].

By using tissue microarrays from resected primary lung cancer and pulmonary metastases, TTF-1 was found to be expressed in 90% of primary lung adenocarcinomas, napsin A in 84%, CDX2 in 7%, and CK20 in 2%, while 83%, 99%, and 4% of colorectal cancer pulmonary metastases were positive for CK20, CDX2, and TTF-1, respectively, and no napsin expression was observed [55].

Thus, within the TTF-1 (-), PAX-8 (-) and GATA-3 (-) profile, positivity for CK20 and/or CDX2 favors the gastrointestinal tract as the primary site in relation to other common primary sites (breast, ovary and lung). However, this pattern can be observed in carcinomas of sites that are less frequently observed in cavities such as the pancreatic and biliary tracts, which, in some clinical settings, should be further investigated.

The expected profile for carcinomas of breast (GATA-3 (+), PAX-8 (-), TTF-1 (-)), ovary (PAX-8 (+), GATA-3 (-), TTF-1 (-)), lung (TTF-1 (+), PAX-8 (-) GATA-3 (-)) and gastrointestinal tract (PAX-8 (-), GATA-3 (-), TTF-1 (-)) was observed in most female samples (88.23%) that had carcinoma from these primary sites. Thus, by using the initial panel, it is possible to define the probable primary site and exclude the other common sites with high sensitivity and specificity. Nonetheless, one should consider that carcinomas in effusions and peritoneal washings could still arise from less frequent primary sites, such as the uterus and pancreas/bile duct, which may have overlapping profiles with ovary and gastrointestinal tract carcinomas, respectively. Importantly, 6.25% of carcinomas from other primary sites would be diagnosed as lung carcinoma if TTF-1 were used as the sole marker. It is noteworthy that TTF-1 expression was intense and diffuse in carcinoma samples of these primary sites other than the lung.

In conclusion, an initial panel including GATA-3, PAX-8 and TTF-1 allows, with high sensitivity and specificity, the identification of the probable carcinoma primary site and the exclusion of the other most frequent sites in effusions of women. Our results highlight the importance of using a panel of markers to avoid misidentification of the primary sites of metastatic carcinoma in effusions.

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Disclosure of conflict of interest

None.

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References

[1] Dermawan JKT and Policarpio-Nicolas ML. Malignancies in pleural, peritoneal, and pericardial effusions. Arch Pathol Lab Med 2020; 144: 1086-1091.

[2] Awadallah SF, Bowling MR, Sharma N and Mohan A. Malignant pleural effusion and cancer of unknown primary site: a review of literature. Ann Transl Med 2019; 7: 353-353.

[3] Imazio M, Colopi M and De Ferrari GM. Pericardial diseases in patients with cancer: contemporary prevalence, management and outcomes. Heart 2020; 106: 569-574.

[4] Ford CE, Werner B, Hacker NF and Warton K. The untapped potential of ascites in ovarian cancer research and treatment. Br J Cancer 2020; 123: 9-16.

[5] Lepus CM and Vivero M. Updates in effusion cytology. Surg Pathol Clin 2018; 11: 523-544.

[6] Porcel JM, Solé C, Salud A and Bielsa S. Prognosis of cancer with synchronous or metachronous malignant pleural effusion. Lung 2017; 195: 775-779.

[7] Yu GH, Glaser LJ and Gustafson KS. Role of ancillary techniques in fluid cytology. Acta Cytol 2020; 64: 52-62.

[8] Sundling KE and Cibas ES. Ancillary studies in pleural, pericardial, and peritoneal effusion cytology. Cancer Cytopathol 2018; 126: 590-598.

[9] Li M, Zhao L, Zhou X, Zhang K, Yin P, Liu S, Zou Y and Li Q. Detection of carcinoma in serous effusions: a review. Am J Cancer Res 2021; 11: 43-60.

[10] Carneiro FP, Muniz-Junqueira MI, De Vasconcelos Carneiro M, De Araújo Oliveira Í, Soares AC, De Vargas Haar N, Takano GHS, De Sousa Vianna LM, De Carvalho Caldas G, Vieira DLM, Frutuoso LL, Brito LMR, De Siqueira RVM, Parente AM, De Castro TMML, Peres I, Mendes LMS, Dos Santos Borges TK, Ferreira VM and Motoyama AB. Anti-EpCAM antibodies for detection of metastatic carcinoma in effusions and peritoneal wash. Oncol Lett 2019; 18: 2019-2024.

[11] Oda T, Ogata S, Kawaguchi S, Minabe S, Dokyu M, Takahashi H, Kumazawa F, Shimazaki H, Takano M, Hase K, Ozeki Y, Kanoh S and Nakanishi K. Immunocytochemical utility of claudin-4 versus those of Ber-EP4 and MOC-31 in effusion cytology. Diagn Cytopathol 2016; 44: 499-504.

[12] Doxtader EE and Chute DJ. Evaluation of carcinoma of unknown primary on cytologic specimens. Surg Pathol Clin 2018; 11: 545-562.

[13] Kandalaft PL and Gown AM. Practical applications in immunohistochemistry: carcinomas of unknown primary site. Arch Pathol Lab Med 2016; 140: 508-523.

[14] Braxton DR, Cohen C and Siddiqui MT. Utility of GATA3 immunohistochemistry for diagnosis of metastatic breast carcinoma in cytology specimens. Diagn Cytopathol 2015; 43: 271-277.

[15] Carneiro FP, Amorim RF, de Vasconcelos Carneiro M, de Castro TMML, de Souza Vianna LM, Takano GHS, Daros AC, Peres I, Kuckelhaus SAS and Motoyama AB. P16 as a marker of carcinoma in effusions and peritoneal washing. BMC Cancer 2020; 20: 225.

[16] Leake R, Walker R, Barnes D, Pinder S, Ellis I, Anderson L, Anderson T, Adamson R, Rhodes T and Miller K. Immunohistochemical detection of steroid receptors in breast cancer: a working protocol. J Clin Pathol 2000; 53: 634-635.

[17] Lobo C, Costa J, Petronilho S, Monteiro P, Leça L and Schmitt F. Cytohistological correlation in serous effusions using the newly proposed international system for reporting serous fluid cytopathology: experience of an oncological center. Diagn Cytopathol 2021; 49: 596-605.

[18] Cibas ES. Pleural, pericardial, and peritoneal fluids. Cytology 2009; 12: 590-598.

[19] Saab J, Hoda RS, Narula N, Hoda SA, Geraghty BE, Nasar A, Alperstein SA, Port JL and Giorgadze T. Diagnostic yield of cytopathology in evaluating pericardial effusions: clinicopathologic analysis of 419 specimens. Cancer Cytopathol 2017; 125: 128-137.

[20] Deftereos G, Sanguino Ramirez AM, Silverman JF and Krishnamurti U. GATA3 immunohistochemistry expression in histologic subtypes of primary breast carcinoma and metastatic breast carcinoma cytology. Am J Surg Pathol 2015; 39: 1282-1289.

[21] Zhao L, Guo M, Sneige N and Gong Y. Value of PAX8 and WT1 immunostaining in confirming the ovarian origin of metastatic carcinoma in serous effusion specimens. Am J Clin Pathol 2012; 137: 304-309.

[22] El Hag MI, Hag AM, Ha JP and Michael CW. Comparison of GATA-3, mammaglobin, GCDFP-15 expression in breast carcinoma in serous effusions: a cell-block micro-array study. Pleura Peritoneum 2017; 2: 143-148.

[23] Kim MY, Go H, Koh J, Lee K, Min HS, Kim MA, Jeon YK, Lee HS, Moon KC, Park SY, Kim WH and Chung DH. Napsin A is a useful marker for

metastatic adenocarcinomas of pulmonary origin. Histopathology 2014; 65: 195-206.
[24] El Hag M, Schmidt L, Roh M and Michael CW. Utility of TTF-1 and Napsin-A in the work-up of malignant effusions. Diagn Cytopathol 2016; 44: 299-304.
[25] Gown AM. Diagnostic Immunohistochemistry: what can go wrong and how to prevent it. Arch Pathol Lab Med 2016; 140: 893-898.
[26] Vidarsdottir H, Tran L, Nodin B, Jirström K, Planck M, Mattsson JSM, Botling J, Micke P, Jönsson P and Brunnström H. Comparison of three different TTF-1 clones in resected primary lung cancer and epithelial pulmonary metastases. Am J Clin Pathol 2018; 150: 533-544.
[27] Dejmek A, Naucler P, Smedjeback A, Kato H, Maeda M, Yashima K, Maeda J and Hirano T. Napsin A (TA02) is a useful alternative to thyroid transcription factor-1 (TTF-1) for the identification of pulmonary adenocarcinoma cells in pleural effusions. Diagn Cytopathol 2007; 35: 493-497.
[28] Ordóñez NG. Value of thyroid transcription factor-1 immunostaining in tumor diagnosis. Appl Immunohistochem Mol Morphol 2012; 20: 429-444.
[29] Husain AN, Colby T, Ordonez N, Krausz T, Attanoos R, Beasley MB, Borczuk AC, Butnor K, Cagle PT, Chirieac LR, Churg A, Dacic S, Fraire A, Galateau-Salle F, Gibbs A, Gown A, Hammar S, Litzky L, Marchevsky AM, Nicholson AG, Roggli V, Travis WD and Wick M. Guidelines for pathologic diagnosis of malignant mesothelioma: 2012 update of the consensus statement from the international mesothelioma interest group. Arch Pathol Lab Med 2013; 137: 647-667.
[30] Klebe S, Swalling A, Jonavicius L and Henderson DW. An immunohistochemical comparison of two TTF-1 monoclonal antibodies in atypical squamous lesions and sarcomatoid carcinoma of the lung, and pleural malignant mesothelioma. J Clin Pathol 2016; 69: 136-141.
[31] Ordonez NG and Sahin AA. Diagnostic utility of immunohistochemistry in distinguishing between epithelioid pleural mesotheliomas and breast carcinomas: a comparative study. Hum Pathol 2014; 45: 1529-1540.
[32] Xing D, Banet N, Sharma R, Vang R, Ronnett BM and Illei PB. Aberrant Pax-8 expression in well-differentiated papillary mesothelioma and malignant mesothelioma of the peritoneum: a clinicopathologic study. Hum Pathol 2018; 72: 160-166.
[33] Shield P, Crouch S, Papadimos D and Walsh M. Gata3 immunohistochemical staining is a useful marker for metastatic breast carcinoma in fine needle aspiration specimens. J Cytol 2018; 35: 90-93.
[34] McKnight R, Cohen C and Siddiqui MT. Utility of paired box gene 8 (PAX8) expression in fluid and fine-needle aspiration cytology: an immunohistochemical study of metastatic ovarian serous carcinoma. Cancer Cytopathol 2010; 118: 298-302.
[35] Lew M, Pang JC, Jing X, Fields KL and Roh MH. Young investigator challenge: the utility of GATA3 immunohistochemistry in the evaluation of metastatic breast carcinomas in malignant effusions. Cancer Cytopathol 2015; 123: 576-581.
[36] El Hag MI, Ha J, Farag R, El Hag AM and Michael CW. Utility of GATA-3 in the work-up of breast adenocarcinoma and its differential diagnosis in serous effusions. Diagn Cytopathol 2016; 44: 731-736.
[37] Robens J, Goldstein L, Gown AM and Schnitt SJ. Thyroid transcription factor-1 expression in breast carcinomas. Am J Surg Pathol 2010; 34: 1881-1885.
[38] Miettinen M, McCue PA, Sarlomo-Rikala M, Rys J, Czapiewski P, Wazny K, Langfort R, Waloszczyk P, Biernat W, Lasota J and Wang Z. GATA3: a multispecific but potentially useful marker in surgical pathology: a systematic analysis of 2500 epithelial and nonepithelial tumors. Am J Surg Pathol 2014; 38: 13-22.
[39] Tong GX, Devaraj K, Hamele-Bena D, Yu WM, Turk A, Chen X, Wright JD and Greenebaum E. Pax8: a marker for carcinoma of Müllerian origin in serous effusions. Diagn Cytopathol 2011; 39: 562-566.
[40] Waters L, Crumley S, Truong L, Mody D and Coffey D. PAX2 and PAX8: useful markers for metastatic effusions. Acta Cytol 2014; 58: 60-66.
[41] Wiseman W, Michael CW and Roh MH. Diagnostic utility of PAX8 and PAX2 immunohistochemistry in the identification of metastatic Müllerian carcinoma in effusions. Diagn Cytopathol 2011; 39: 651-656.
[42] Laury AR, Perets R, Piao H, Krane JF, Barletta JA, French C, Chirieac LR, Lis R, Loda M, Hornick JL, Drapkin R and Hirsch MS. A comprehensive analysis of PAX8 expression in human epithelial tumors. Am J Surg Pathol 2011; 35: 816-826.
[43] Tacha D, Zhou D and Cheng L. Expression of PAX8 in normal and neoplastic tissues. Appl Immunohistochem Mol Morphol 2011; 19: 293-299.
[44] Hakim SA and Youssef NS. Diagnostic utility of thyroid transcription factor-1 in ovarian carcinoma and its relationship with clinicopathologic prognostic parameters. Appl Immunohistochem Mol Morphol 2017; 25: 237-243.
[45] Lu S, Yakirevich E, Hart J, Wang L and Wang Y. PAX8 expression in breast cancer. Appl Immu-

nohistochem Mol Morphol 2021; 29: 293-298.
[46] Kilgore MR, Bosch DE, Adamson KH, Swanson PE, Dintzis SM and Rendi MH. Unexpected PAX8 immunoreactivity in metastatic high-grade breast cancer. Appl Immunohistochem Mol Morphol 2019; 27: 637-643.
[47] Jeong JH, Kim NY and Pyo JS. Analysis of PAX8 immunohistochemistry in lung cancers: a meta-analysis. J Pathol Transl Med 2020; 54: 300-309.
[48] Shen Y, Pang C, Shen K, Wu Y, Li D, Wan C, Liao Z, Yang T, Chen L and Wen F. Diagnostic value of thyroid transcription factor-1 for pleural or other serous metastases of pulmonary adenocarcinoma: a meta-analysis. Sci Rep 2016; 6: 19785.
[49] Nakamura N, Miyagi E, Murata Si, Kawaoi A and Katoh R. Expression of thyroid transcription factor-1 in normal and neoplastic lung tissues. Mod Pathol 2002; 15: 1058-1067.
[50] Ye J, Findeis-Hosey JJ, Yang Q, McMahon LA, Yao JL, Li F and Xu H. Combination of napsin A and TTF-1 immunohistochemistry helps in differentiating primary lung adenocarcinoma from metastatic carcinoma in the lung. Appl Immunohistochem Mol Morphol 2011; 19: 313-317.
[51] Aldaoud N, Erashdi M, AlKhatib S, Abdo N, Al-Mohtaseb A and Graboski-Bauer A. The utility of PAX8 and SATB2 immunohistochemical stains in distinguishing ovarian mucinous neoplasms from colonic and appendiceal mucinous neoplasm. BMC Res Notes 2019; 12: 770-770.
[52] Acs G, Pasha T and Zhang PJ. WT1 is differentially expressed in serous, endometrioid, clear cell, and mucinous carcinomas of the peritoneum, fallopian tube, ovary, and endometrium. Int J Gynecol Pathol 2004; 23: 110-118.
[53] Altree-Tacha D, Tyrrell J and Haas T. CDH17 is a more sensitive marker for gastric adenocarcinoma than CK20 and CDX2. Arch Pathol Lab Med 2017; 141: 144-150.
[54] Choi SM, Furth EE and Zhang PJ. Unexpected TTF-1 positivity in a subset of gastric adenocarcinomas. Appl Immunohistochem Mol Morphol 2016; 24: 603-607.
[55] Vidarsdottir H, Tran L, Nodin B, Jirström K, Planck M, Jönsson P, Mattsson JSM, Botling J, Micke P and Brunnström H. Immunohistochemical profiles in primary lung cancers and epithelial pulmonary metastases. Hum Pathol 2019; 84: 221-230.