EVALUATION OF IMMUNOHISTOCHEMICAL EXPRESSION OF EMA MARKER IN BREAST CANCER PATIENTS REFERRED TO IMAM KHOMEINI HOSPITAL IN AHVAZ 2019-2020

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Abstract

Background: Breast cancer is the most common cancer among women worldwide. The disease is quite diverse, with a wide range of prognosis for these patients. Immunohistochemistry with tumor markers is very helpful in finding the prognosis of the disease. In this study, we evaluated the immunohistochemical expression of Epithelial Membrane Antigen (EMA) in breast cancer patients.

Methods: The total number of breast cancers collected from the archives of the pathology department of Imam Khomeini Hospital in Ahvaz from 2019 to 2020. Finally, 60 suitable cases were stained with EMA marker by immunohistochemistry method, and then their histopathological characteristics were evaluated.

Result: The numbers of patients were 60 women with a mean age of 52.38 ± 1.73 years. EMA was positive in 51 (85%) of breast cancers. EMA cytoplasmic staining was observed in 41(68.3%) patients and lineal staining was observed in the remainder 10 (16.7%) and EMA was negative in 9(15%) patients, most of EMA positive had PT2 and Grade2 (P value <0/05).

Conclusion: The majority of breast cancers patients had positive EMA staining patterns was significantly related to the Age, tumor grade, and tumor size of cancer and showed the capacity for predicting the nodal stage of dissemination of breast cancers.

Keywords: breast cancer, immunohistochemistry, EMA marker, tumor markers

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Introduction

Breast cancer is the most common malignancy in women worldwide, accounting for a quarter of all newly diagnosed cancer cases in women. Each year, more than 1.5 million new cases of breast cancer are reported worldwide, with an estimated 500,000 deaths related to breast cancer(1). Early detection and accurate diagnosis are vital for effective treatment and improved patient outcomes. Recent advances have been made in both the understanding of breast cancer and the development of preventative methods. The discovery of breast cancer stem cells reveals its pathogenesis and tumor drug resistance mechanisms, and many genes related to breast cancer are discovered.(2, 3) Early detection plays a pivotal role in breast cancer management. It allows for prompt intervention, leading to better treatment

outcomes and increased chances of survival. Regular breast self-examinations, mammograms, and clinical breast examinations are essential for early detection. However, these methods may not always provide a definitive diagnosis. This is where immunohistochemical evaluation comes into play(4). Immunohistochemistry has revolutionized breast cancer diagnosis by providing valuable insights into the molecular characteristics of tumors. It helps identify the presence of hormone receptors, such as estrogen and progesterone receptors, which play a crucial role in determining the appropriate hormonal therapy. Additionally, immunohistochemistry aids in detecting the overexpression of human epidermal growth factor receptor 2 (HER2), a protein associated with aggressive breast cancer. HER2-positive breast cancers can be effectively targeted with HER2-directed therapies, leading to improved patient outcomes(5).

The epithelial membrane antigen (EMA) is a family of glycoproteins related to the milk fat globule proteins, and it is expressed by a variety of epithelial and their neoplasms(6, 7) The EMA marker is a protein expressed on the surface of epithelial cells, including breast cancer cells. Its expression has been linked to aggressive tumor behavior and poor prognosis(8). Immunohistochemical evaluation allows for the detection of EMA expression in breast cancer tissue, providing important information for diagnosis and prognosis. In the study conducted at Imam Khomeini Hospital, the researchers observed a significant association between EMA expression and tumor characteristics such as size, grade, and lymph node involvement(9).

The evaluation of the EMA marker in breast cancer diagnosis has several implications. Firstly, it can aid in distinguishing between benign and malignant breast lesions. The absence of EMA expression in a suspicious lesion would suggest a benign nature, while its presence would raise concerns for malignancy (10, 11). Additionally, EMA expression can help predict the aggressiveness of the tumor and guide treatment decisions. Patients with EMA-positive tumors may require more aggressive therapies and closer surveillance to prevent disease progression and recurrence(12). Recent studies have shown that EMA positivity is associated with tumor malignancy, estrogen receptor lymph node metastases, and survival. In addition, clinical studies have reported an association between epidermal antigen expression and poor prognosis in various malignancies such as lung cancer, gastric cancer, gallbladder, skin cancer, and hepatocellular carcinoma (13, 14). It has also been shown that EMA marker expression can be effective in the differential diagnosis of BCC and SCC skin cancers(15). The aim of this study was to evaluate the immunohistochemistry of the EMA marker in patients with breast cancer and its relationship with the determining factors in the prognosis of breast cancer.

Materials and Methods

Case selection

In this cross-sectional study with descriptive-analytical aspects, the total number (n=60) of breast cancer patients was collected from the archives of the pathology department of Imam Khomeini Hospital in Ahvaz between 2019 and 2020. Sample collection was performed non-randomly and the sample size was determined based on the census. Inclusion criteria were complete patient records, sufficient tissue, absence of necrosis or hemorrhage, and availability of invasive tumor tissue and lymph nodes. Demographic and clinical characteristics of each sample, including age and sex of the patient, tumor depth, number of lymph nodes involved and tumor grade were extracted from the patient's file.

Immunohistochemistry

Four-micrometer serial whole-tissue sections were cut from the archived formalin-fixed, paraffin-embedded tissue blocks, dewaxed, and subsequently rehydrated with xylene and graded alcohol washes. Antigen retrieval EMA was performed in EDTA (pH 9.5) for 2 min 30 s. The sections were treated with 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity and then incubated with normal goat serum for 10 min to eliminate nonspecific background staining. Thereafter, primary antibodies EMA (IgG, Clone E29, N1504 DAKO) EMA were incubated with the samples at 4 °C overnight. Antigen was sequentially detected with secondary biotin-labeled antibody and peroxidase-conjugated streptavidin. The chromogenic was 3, 3-diaminobenzidine. The sections were counterstained with hematoxylin.

All immunohistochemical markers were assessed by a light microscope. The immunohistochemical staining results were interpreted in a semiquantitative way and given a staining score from 1 to 3, as follows:

1: weak staining in less than 20% of tumor cells;

2: moderate staining in between 20% and 60%;

3: strong staining in 61% or more of the tumor cells

Positive staining was defined as a staining score of 2 or 3, while negative staining was defined as a score of 1.

Statistical analysis

Statistical analysis was performed on SPSS Statistics 23.0 (IBM Corporation, Chicago, Illinois, USA). T-test and ANOVA was performed to evaluate the relationship between EMA and histopathological characteristics.

Results

The study included a total of [60 number] breast cancer patients diagnosed and treated at Imam Khomeini Hospital during the designated timeframe. Immunohistochemical evaluation of EMA expression revealed variable patterns of staining across different subtypes of breast cancer. Additionally, the correlation between EMA expression and clinicopathological parameters was explored to identify potential associations with tumor aggressiveness and prognosis.

According to the results of Tables 1 and 2, the mean age of patients was 52.38 ± 1.73 years and included 60 women with breast cancer. Most of the tumors in these breast cancer patients were located in the External Upper site (n=19).

Primary tumor (PT) in patients 8(13.3%) PT1, 34(57.6%) PT2, 14(23.3%) PT3 and 6(9%) PT4. 30 patients (50%) had vascular invasion, 7 patients (13.3%) had perineural invasion and 33 patients (55%) had lymph node involvement. EMA marker expression was observed in 51 patients (85%). Staining intensity for EMA marker in immunohistochemistry technique was weak in 9 patients (17.6%); moderate in 37 patients (72.5%) and strong in 5 patients (9.8%).The general characteristics of the cases are shown in Table 1, 3.

Table 1- General characteristics of Clinical histopathological data in women with breast cancer

Factor	Indicator	Number (percent %)
Age	<60	24(40)
	>60	36(60)
Tumor Size	<5	28(46.7)
	>5	32(53.3)
Tumor Site	Internal Upper	14(23)
	External Upper	19(31)
	internal Lower	7(12)
	External Lower	10(16)
	Central	7(12)
	Retro areolar	3(5)
	Nipple	1(1)
Grading	1	11(18.3)
	2	43(71.7)
	3	6(10)
PT*	PT1	8(14)
	PT2	32(53)
	PT3	14(23)
	PT4	6(10)

PN*	PN0	26 (43.3)
	PN1	17 (28.3)
	PN2	11 (18.3)
	PN3	2 (3.3)
	PNx	4 (6.7)
Vascular invasion	+	30 (50)
	-	30 (50)
Neural invasion	+	7 (11.7)
	-	53 (88.3)
Marker EMA	+	51 (85)
	-	9 (15)
Chromatophilic pattern	Cytoplasmic	41 (68.3)
	Lineal	10 (16.7)
	No staining of marker EMA	9 (15)

*(PT: primary tumor, PN: lymphatic involvement)

Epithelial membrane antigen (EMA) expression was seen positive in 85% of breast cancer samples examined, of which cytoplasmic staining pattern for EMA marker (68.3%) and lineal staining pattern (16.7%). Figures of all types of staining intensity with EMA marker , Pattern staining score of cytoplasmic type breast cancer Fig1 strong, Fig2 moderate, Fig3 weak and was disordered lineal in defective tubules (Fig4). However, in most tumors diffuse EMA cytoplasmic staining, and EMA negativity was seen in Negative n=9 (15%) of which were most of them were with PT2 and Grade2. Table 3 presents the EMA staining patterns and Characteristics tumor Pattern in more detail.

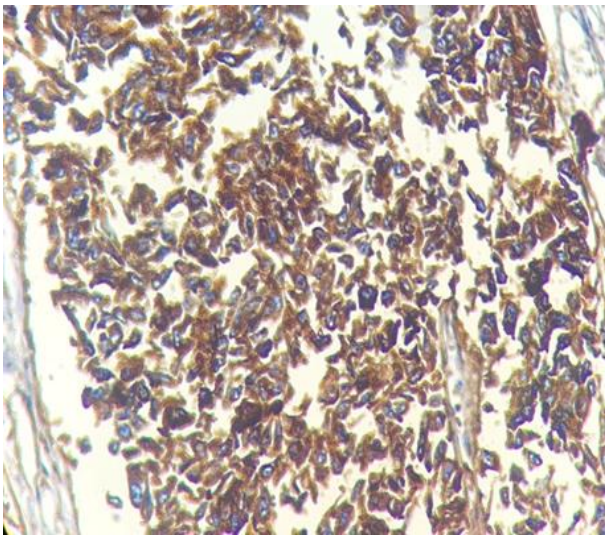

Figure 1. Pattern strong cytoplasmic staining intensity

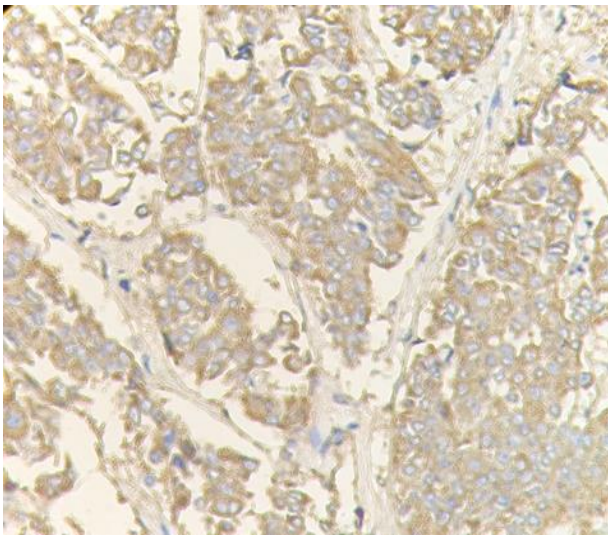

Figure 2. Pattern moderate cytoplasmic staining intensity

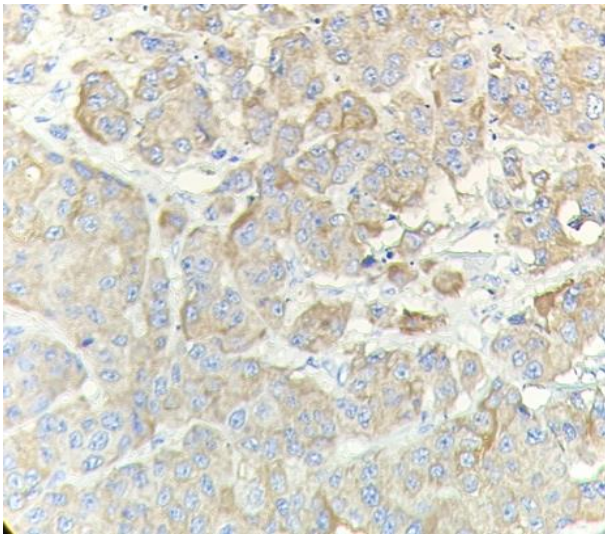

Figure 3. Pattern weak cytoplasmic staining intensity

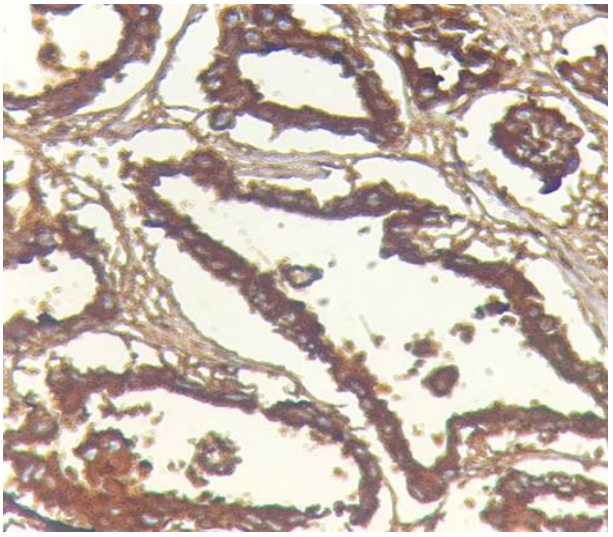

Figure 4. Pattern strong lineal staining intensity

A more significant (P value <0/05) association was found between EMA and Age, tumor grade, and tumor size, was analyzed Significance of EMA marker with tested factors (Table 2).

Table 2- Significance of EMA marker with tested factors

Factor	Mean±SD	<0/05P value
Age	52.38±1.73	**Significant**
Size	4.81±0.46	**Significant**
Site	5.6.±1.41	**Significant**
Grading	1.9±0.68	**not Significant**
PT	2.1±0.21	**not Significant**
PN	1.16±0.18	**not Significant**
Vascular invasion	0.5±0.65	**not Significant**
Neural invasion	0.11±0.41	**not Significant**
Chromatophilic pattern	**0.85±0.36**	**not Significant**

The univariate statistical study of the associations between tumor size, tumor grade, and nodal stage of dissemination in the lineal, cytoplasmic, and negative EMA groups (Table 3).

Table 3- EMA staining groups and general characteristics with which they were statistically related

Characteristics tumor Pattern	Total EMA Positive n=51(85%)	Lineal EMA Positive n=10 (16.7%)	Cytoplasmic EMA Positive n=41 (83.3%)	EMA Negative n=9 (15%)
Size pT1	**7(11.7%)**	4 (6.7%)	4 (6.7%)	**1 (1.7%)**
Size pT2	**26(43.3%)**	5 (8.3%)	27 (45%)	**6 (10%)**

Characteristics tumor Pattern	**Total EMA Positive n=51(85%)**	**Lineal EMA Positive n=10 (16.7%)**	**Cytoplasmic EMA Positive n=41 (83.3%)**	**EMA Negative n=9 (15%)**
Size pT3	**13(21.7%)**	1 (1.7%)	13 (21.7%)	**1 (1.7%)**
Size pT4	**6(10%)**	0 (0%)	6 (10%)	**0(0%)**
Grade 1	**9(15%)**	3(5%)	8(13.3%)	**1(1.7%)**
Grade 2	**37(61.7%)**	6(10%)	37(61.7%)	**6(10%)**
Grade 3	**4(6.7%)**	1(1.7%)	5(8.3%)	**2(3.3%)**
PN0	**25(41.7%)**	9(15%)	16(28.3%)	**1(1.7%)**
PN1	**16(26.7%)**	1(1.7%)	15(26.7%)	**1(1.7%)**
PN2	**6(10%)**	0(0%)	6(18.3%)	**5(8.3%)**
PN3	**0(0%)**	0(0%)	0(0%)	**2(3.3%)**
PNx	**4(6.7%)**	0(0%)	4(6.7%)	**0(0%)**
Vascular invasion +	**23(38.3%)**	2(3.3%)	21(46.7%)	**7(11.7%)**
Vascular invasion -	**28(46.7%)**	8(13.3%)	20(36.7%)	**2(3.3%)**
Neural invasion+	**5(8.3%)**	2(3.3%)	3(8.3%)	**2(3.3%)**
Neural invasion-	**46(76.7%)**	8(13.3%)	38(75%)	**7(11.7 %)**

Discussion

MUC1 glycoprotein is presented with different types used for diagnosis, staging, and therapy in certain forms of epithelial cancers. Breast cancer is one of the epithelial tumors in which the EMA marker is expressed. Breast cancer is the most frequent cancer among women all over the world. The disease is quite diverse, with a wide range of prognoses. Based on both clinical and non-clinical profiles, prognosis refers to the possibility or risk that a specific result (such as deaths, complications, quality of life, pain, or disease regression) will occur during a given period of time. Relapse-free survival (RFS) rates in breast cancer patients range from 65 to 80 percent after five years.(16, 17) Approximately 80% of breast cancer patients are above the age of 50, and in our study, people with this disease are in the age range of 52.38± 1.73 (19).

The EMA marker was employed to diagnose the prognosis of breast cancer in this investigation, and it was positive in 85 % of the samples (n = 51). MUC1 (epithelial membrane antigen (EMA)) is a large transmembrane mucin that is heavily glycosylated and expressed at the apical pole of normal glandular epithelia cells. Also expressed on epithelial cells, overexpression has been linked to a poor prognosis in a variety of malignancies. (6, 18-20). MUC1 is implicated in a number of physiological processes such as adhesion, development, and differentiation. Furthermore, MUC1 is frequently overexpressed and deregulated, with membrane circumferential or cytoplasmic expression. MUC1 has an intracellular tail that is phosphorylated and can interact with many signaling proteins and transcription factors. Cancers of the breast, colon, kidney, prostate, and gastrointestinal tract are associated with MUC1 overexpression and membrane delocalization leading to a worse prognosis and shorter survival (6, 19, 21)

Over 90% of breast cancer entities express tumor-associated MUC1, which differs greatly from its physiological form on epithelial cells, thus representing a unique target for breast cancer diagnosis and antibody-mediated immune therapy (22). EMA was expressed in %85 of breast cancer samples taken between 2019 and 2020 in Imam Khomeini Hospital in Ahvaz. EMA has traditionally a membranous reactivity in MPMs while being localized in the cytoplasm in ADCs. There is no well-defined threshold of positivity in terms of percentage of marked cells

but some authors have retained a minimum value of 10%. Cury et al. concluded that strong, diffuse, and lineal staining for EMA is a good marker of malignancy(23), In our statistical analysis, in all cases with a lineal pattern, the EMA was positive.

EMA overexpression is detected in 75% of cases (30 out of 40), and the Gleason score indicates EMA overexpression increases with grade. Patients with grade 6 prostatic adenocarcinoma showed 62.5% (n=5) overexpression of the EMA protein, patients with grade 7 prostatic adenocarcinoma showed 66.7% overexpression of the EMA protein, and patients with grade 7–10 prostatic adenocarcinoma showed 88.2% (n=15) overexpression of the EMA protein(9). In the present study, 51 out of 60 cases showed a positive EMA marker, the increased intracellular localization of EMA protein and the changes in glycosylation of this protein were related to carcinomas in poorly differentiated cases. In the present study, the most grading was 2 with 34 samples of 72%, followed by type 1 with 11 samples of 18% and then type 3 with 6 samples of 10%, but the grade did not show a significant relationship with the expression of EMA in this study. But it showed a statistically significant relationship with the age and size of the tumor and the tumor site.

The expression of EMA is usually found in epithelial tumors, but it can also be observed in some nonepithelial tumors. A strong, predominantly membranous staining pattern indicates the presence of malignant mesothelioma cells, however, when a predominantly cytoplasmic staining pattern indicates the presence of adenocarcinoma cells, it is used to distinguish between malignant mesothelioma cells and adenocarcinomas (24). In this study 41 out of 51 positive staining samples had cytoplasmic pattern.

Previous studies have shown that strong EMA staining helps exclude reactive mesothelial cells, though focal and weak positivity has been reported. Study results, however, have varied regarding the sensitivity and specificity for discriminating malignant mesothelial cells from benign ones. According to most studies, EMA was positive in the majority of effusions from patients with MM (70%-80%) but was negative in reactive mesothelial proliferations. However, other studies have shown that reactive mesothelium can be positive for EMA in up to 70% of cases (25, 26). EMA is most strongly expressed in epithelial mesothelioma and rarely in sarcomatoid subtypes. Different antibody clones can also cause inconsistent results. Saad et al. using different clones showed a significant difference in EMA positives. Some groups used paraffin-embedded specimens to investigate immunohistochemical EMA staining to distinguish between benign and malignant dermal lesions(26). EMA is a useful antibody for immunohistochemical diagnosis due to its extremely high sensitivity in pleural effusion (98.4%), peritoneal exudate (100%), AC (100%), and MM (100%) specimens (17, 27).

Conclusions

In conclusion, the evaluation of immunohistochemical expression of the EMA marker in breast cancer patients has shown promising results in aiding accurate diagnosis and guiding treatment decisions. The study conducted at Imam Khomeini Hospital in Ahvaz during the period of 2019-2020 further supports the significance of EMA as a tumor marker in breast cancer. The findings revealed a high prevalence of EMA expression in breast cancer cells and its correlation with age, tumor grade and tumor site.

With continued research and advancements in immunohistochemistry techniques, the role of EMA as a diagnostic and prognostic marker in breast cancer will likely become even more important. Healthcare professionals should consider incorporating EMA evaluation into their routine practice to enhance the accuracy of breast cancer diagnosis and improve patient outcomes.

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Ethical approval

This article does not contain any studies with human participants performed by any of the authors.

Conflict of interest

The authors declare no competing interests.

References

1. Leo CP, Hentschel B, Szucs TD, Leo C. FDA and EMA approvals of new breast cancer

drugs—A comparative regulatory analysis. Cancers. 2020;12(2):437.

2. Sun Y-S, Zhao Z, Yang Z-N, Xu F, Lu H-J, Zhu Z-Y, et al. Risk Factors and Preventions of Breast Cancer. Int J Biol Sci. 2017;13(11):1387-97.

3. DeSantis CE, Fedewa SA, Goding Sauer A, Kramer JL, Smith RA, Jemal A. Breast cancer statistics, 2015: Convergence of incidence rates between black and white women. CA Cancer J Clin. 2016;66(1):31-42.

4. Tazhibi M, Feizi A. Awareness levels about breast cancer risk factors, early warning signs, and screening and therapeutic approaches among Iranian adult women: a large population based study using latent class analysis. Biomed Res Int. 2014;2014:306352-.

5. Zaha DC. Significance of immunohistochemistry in breast cancer. World J Clin Oncol. 2014;5(3):382-92.

6. Lin W, Liu X, Cen Y. Diagnostic accuracy of epithelial membrane antigen for malignant effusions: a meta-analysis. The International Journal of Biological Markers. 2016;31(1):11-6.

7. Song Y, Sun H, Wu K, Lyu J, Zhang J, Gu F, et al. sLex expression in invasive micropapillary breast carcinoma is associated with poor prognosis and can be combined with MUC1/EMA as a supplementary diagnostic indicator. Cancer Biology & Medicine. 2021;18(2):477.

8. Luna-Moré S, Rius F, Weil B, Jimenez A, Bautista MD, Pérez-Mellado A. EMA: a differentiation antigen related to node metastatic capacity of breast carcinomas. Pathol Res Pract. 2001;197(6):419-25.

9. Ghalib R, Falah A. The role of epithelial membrane antigen (EMA) overexpression in the prognosis of prostatic adenocarcinoma. J Med Life. 2022;15(4):504-8.

10. Moriya T, Kozuka Y, Kanomata N, Tse GM, Tan PH. The role of immunohistochemistry in the differential diagnosis of breast lesions. Pathology. 2009;41(1):68-76.

11. Bhargava R, Dabbs DJ. Use of immunohistochemistry in diagnosis of breast epithelial lesions. Advances in anatomic pathology. 2007;14(2):93-107.

12. Madheswaran S. Evaluation of tumour-associated antigens to optically label cutaneous basal cell carcinoma for surgical excision. 2022.

13. Kim GJ, Rhee H, Yoo JE, Ko JE, Lee JS, Kim H, et al. Increased expression of CCN2, epithelial membrane antigen, and fibroblast activation protein in hepatocellular carcinoma with fibrous stroma showing aggressive behavior. PLoS One. 2014;9(8):e105094.

14. Kawai T, Tominaga S, Hiroi S, Ogata S, Nakanishi K, Kawahara K, et al. Peritoneal malignant mesothelioma (PMM), and primary peritoneal serous carcinoma (PPSC) and reactive mesothelial hyperplasia (RMH) of the peritoneum. Immunohistochemical and fluorescence in situ hybridisation (FISH) analyses. Journal of clinical pathology. 2016;69(8):706-12.

15. Ohsie SJ, Sarantopoulos GP, Cochran AJ, Binder SW. Immunohistochemical characteristics of melanoma. J Cutan Pathol. 2008;35(5):433-44.

16. Phung MT, Tin ST, Elwood JM. Prognostic models for breast cancer: a systematic review. BMC cancer. 2019;19(1):1-18.

17. Sharma S. Tumor markers in clinical practice: General principles and guidelines. Indian J Med Paediatr Oncol. 2009;30(1):1-8.

18. Langner C, Ratschek M, Rehak P, Schips L, Zigeuner R. Expression of MUC1 (EMA) and E-cadherin in renal cell carcinoma: a systematic immunohistochemical analysis of 188 cases. Mod Pathol. 2004;17(2):180-8.

19. Klumpp LC, Shah R, Syed N, Fonseca G, Jordan J. Invasive Lobular Breast Carcinoma Can Be a Challenging Diagnosis Without the Use of Tumor Markers. Cureus. 2020;12(5).

20. Attallah AM, El-Far M, Omran MM, Abdallah SO, El-Desouky MA, El-Dosoky I, et al. Circulating levels and clinical implications of epithelial membrane antigen and cytokeratin-1 in women with breast cancer: can their ratio improve the results? Tumor Biology. 2014;35(11):10737-45.

21. Leroy X, Buisine M-P, Leteurtre E, Aubert S, Buob D, Porchet N, et al. [MUC1 (EMA): A key molecule of carcinogenesis?]. Ann Pathol. 2006;26(4):257-66.

22. Stergiou N, Nagel J, Pektor S, Heimes A-S, Jäkel J, Brenner W, et al. Evaluation of a novel monoclonal antibody against tumor-associated MUC1 for diagnosis and prognosis of breast cancer. Int J Med Sci. 2019;16(9):1188-98.

23. Cury P, Butcher D, Corrin B, Nicholson A. The use of histological and

immunohistochemical markers to distinguish pleural malignant mesothelioma and in situ mesothelioma from reactive mesothelial hyperplasia and reactive pleural fibrosis. The Journal of pathology. 1999;189(2):251-7.

24. Morimoto A, Ito A, Hashimoto K, Nakano A, Nagasaka T, Yokoi T. New diagnostic technique for rapid fluorescence immunocytochemical staining of adenocarcinoma and mesothelial cells using liquid-based cytology. Acta Cytologica. 2014;58(5):461-8.

25. Ordóñez NG. The immunohistochemical diagnosis of mesothelioma: a comparative study of epithelioid mesothelioma and lung adenocarcinoma. Am J Surg Pathol. 2003;27(8):1031-51.

26. Saad RS, Cho P, Liu YL, Silverman JF. The value of epithelial membrane antigen expression in separating benign mesothelial proliferation from malignant mesothelioma: a comparative study. Diagnostic cytopathology. 2005;32(3):156-9.

27. Ikeda K, Tate G, Suzuki T, Kitamura T, Mitsuya T. Diagnostic usefulness of EMA, IMP3, and GLUT-1 for the immunocytochemical distinction of malignant cells from reactive mesothelial cells in effusion cytology using cytospin preparations. Diagnostic cytopathology. 2011;39(6):395-401.