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Use of Napsin-A, TTF-1, ER, GCDFP-15, GATA-3 and GATA-3 Markers to Differentiate Breast Metastasis from Lung Adenocarcinoma

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ABSTRACT

Background and Aim: Breast carcinomas with metastasis to lungs and primary lung adenocarcinomas have significant overlap. This study aimed to investigate the differential expression of a panel of IHC markers in primary lung adenocarcinomas and invasive ductal carcinomas (IDC) of the breast.

Methods: In this cross sectional study, total of 50 specimens including 25 primary lung adenocarcinomas and 25 invasive ductal carcinomas of the breast were collected from Masih Daneshvari and Shohada-e-Tajrish hospitals. After all of the cases were stained with hematoxylin - and - eosin, the histologic diagnosis and grading of the slides were reviewed and reported by experienced pathologists based on standard classifications. The patients' medical records were reviewed for demographic data, clinical information and histopathologic reports.

Results: The median age of the patients with lung adenocarcinoma and IDC was 59 (32-78) and 50 (35-74) years, respectively. In this study, regarding lung adenocarcinomas, the most common type was acinar (56%), followed by solid (20%), mucinous (16%), lepidic (4%), and colloid (4%). Immunohistochemical expression for Napsin-A, TTF-1, ER, GCDFP-15, and GATA-3 in primary lung adenocarcinomas and invasive ductal carcinomas of the breast were different.

Conclusion: Napsin-A, TTF-1, ER, GCDFP-15, GATA-3 and GATA-3 Markers can differentiate the breast cancer from lung adenocarcinoma. Napsin-A and TTF1 only present in lung adenocarcinoma.

Highlights:

- TTF-1 and Napsin-A were merely expressed in primary lung adenocarcinomas.
- TTF-1 can be negative in some cases of lung adenocarcinomas.
- Napsin-A can be positive in some cases of breast carcinomas.
- Use a panel of multiple antibodies such as GATA-3, GCDFP-15 and Napsin-A is better to differentiate lung adenocarcinoma from breast tumor
- GATA-3 was expressed more in breast carcinomas.
- GCDFP-15 was positive in all breast carcinomas and some cases of lung adenocarcinomas.
- ER and GCDFP-15 expression was observed in breast carcinomas and lung adenocarcinomas.

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INTRODUCTION

Recent advancements in the early detection and optimal management of patients with breast cancer have led to longer survival times in these patients. Current evidence suggests that female survivors of breast cancer are more likely to develop a second primary cancer compared to the general population. Although the most frequently observed second primary cancer in these patients is breast cancer, but lung cancer is also relatively incident in this population (1, 2). Several risk factors; including radiotherapy, smoking, and genetic predisposition have been linked to this increased risk (3). Moreover, one of the most common sites of secondary tumors due to breast cancer metastasis is the lung, which is associated with a poor prognosis (4, 5). Approximately 10-20% of patients with breast cancer are at risk of developing pulmonary metastasis sometime in their life (6).

Primary lung adenocarcinomas and metastatic breast carcinomas to the lungs harbor significant histological and immunohistochemical overlap. Both of these tumors often show a positive immunostain for cytokeratin 7 (CK7) but lack CK20 expression (7).

A more detailed understanding of tumor heterogeneity or tumor marker transformation between primary and metastatic tumors can be obtained by determining the tumor biology, particularly based on the intrinsic subtype (8, 9).

Few studies have been done using different immunohistochemical markers to differentiate between lung and breast cancer such as thyroid transcription factor-1 (TTF-1), Napsin-A, estrogen receptor (ER), GCDFP-15, and GATA-3. These factors are used for tumor features diagnosis. For example, TTF1 is expressed in the thyroid and lungs. When compared to well - differentiate adenocarcinomas, TTF1 expression is lower in poorly differentiated adenocarcinomas (50%), decreasing the sensitivity of this marker to determine the pulmonary origin of a poorly differentiated adenocarcinoma. About GATA3, The identification of a breast origin in breast malignancies was suggested using GATA3. Also, 92% to 100% of ductal and lobular breast carcinomas express GATA3 (10-12).

The distinction of primary lung adenocarcinomas from metastatic breast carcinomas is extremely challenging, particularly in poorly differentiated tumors, and is also very important as their treatment and management significantly differs. Thus, misclassification of either of the tumors may result in unfavorable outcomes due to wrong treatment. Herein, this study investigated the differential expression of a panel of IHC markers in both primary lung adenocarcinomas and invasive breast ductal carcinomas involving metastasis to lungs for differentiating from each other.

METHODS

The research procedure was accepted by the ethical committee (IR.SBMU.MSP.REC.1400.061) of the Shahid Beheshti University of Medical Sciences. In this cross sectional study, a total of 50 specimens including 25 primary lung adenocarcinomas and 25 invasive ductal carcinomas of the breast were collected. The lung and breast specimens were respectively obtained from the department of pathology, Masih Daneshvari Hospital and Shohada-e-Tajrish Hospital, Tehran, Iran between 6 Jan 2019 and 11 Feb 2020. All the specimens were stained with hematoxylin - and - eosin and then, the histologic diagnosis and grading of the slides were reviewed and reported by experienced pathologists based on standard classifications (13).

The patients' medical files were reviewed for assessment of demographic data, clinical information such as the type of cancer such as acinar or solid tumors, the expression of proteins including Thyroid transcription factor 1 (TTF1), Novel aspartic proteinase of the pepsin family A (Napsin-A), estrogen receptor (ER), Gross cystic disease fluid protein 15 (GCDFP15), and GATA binding protein 3 (GATA3), and histopathologic reports including diffused of focal lesion.

Sample size

According to Yang et al.'s study (7), the minimum sample size required for each group, considering 20% dropout in the study, was equal to 25 samples (50 samples in total). Sampling was done by non-probability sequential method in such a way that since the beginning of the study, all the cases that met the inclusion criteria and did not have the exclusion criteria were selected as the sample and this continued until the final sample size was reached.

Immunohistochemistry

First, 5-µm-thick sections were cut from formalin - fixed paraffin - embedded tissue blocks by using a microtome, which were then transferred to adhesive slides coated with poly-L-lysine. After deparaffinization of the sections, antigen retrieval was achieved by microwave treatment in sodium citrate buffer (pH = 6) for 30 minutes. For blocking endogenous peroxidase activity, tissue sections were incubated with 0.3% hydrogen peroxide for 10 minutes and then incubated with blocker serum solution at room temperature for another 10 minutes. Tissue sections were incubated with primary antibodies for TTF-1, Napsin-A, ER, GCDFP-15, and GATA-3 for one night at refrigerator temperature and with secondary antibodies for one hour at room temperature the following day. The sections were then washed with phosphate-buffered saline (PBS) and were mounted on microscope slides after being treated through a series of graded alcohols and xylene (14).

Statistical analysis

All numeric data were evaluated for normal distribution using Kolmogorov-Smirnov test. When normally distributed, continuous variables were presented as mean (SD). Sensitivity and specificity of IHC markers were calculated for differentiating between the two groups. Histopathologic results were considered as the reference standard. Statistical analysis was performed using GraphPad Prism version 8.0 software. P-values less than 0.05 were considered as statistically significant.

RESULTS

The present study aimed to evaluate the efficiency and specificity of GCDFP15, ER, Napsin-A, TTF1 and GATA3 proteins in the differential diagnosis of primary lung cancer from breast cancer by immunohistochemical method. In the present study, 50 patients were studied, 25 of whom had lung cancer and 25 had aggressive breast cancer. The demographic results of the patients showed that in the lung cancer group, 10 people (40%) were female and the rest (15 people) were male. All breast cancer patients were female. The mean and standard deviation of the age of the patients in the lung cancer group was estimated as 59 ± 11.2 years (the lowest and highest age in this group was 32 and 78 years, respectively). The mean and standard deviation of the age of the patients in the breast cancer group was estimated to be 50 ± 11.5 years, and the lowest and highest ages in this group were 35 and 74 years, respectively.

All tumor tissue samples in the group of breast cancer patients were invasive ductal carcinoma.

The results of the histological examination of the tumor in the lung cancer group were shown in Figure 1. The highest frequency of adenocarcinomas was related to the acinar form (56%) and the lowest frequency was related to the lepidic (4%) and colloid (4%) forms. Solid and Mucinous forms had a prevalence of 20% and 16%, respectively.

The results of protein expression assessment are seen in Table 1. These results showed that the GATA3, ER, GCDFP15, Napsin-A, and TTF1 are significantly different between the lung cancer and breast cancer (all P-values < 0.05). TTF1 protein did not show any expression in the group of breast cancer. It was also seen that Napsin-A had no expression in the group of breast cancer.

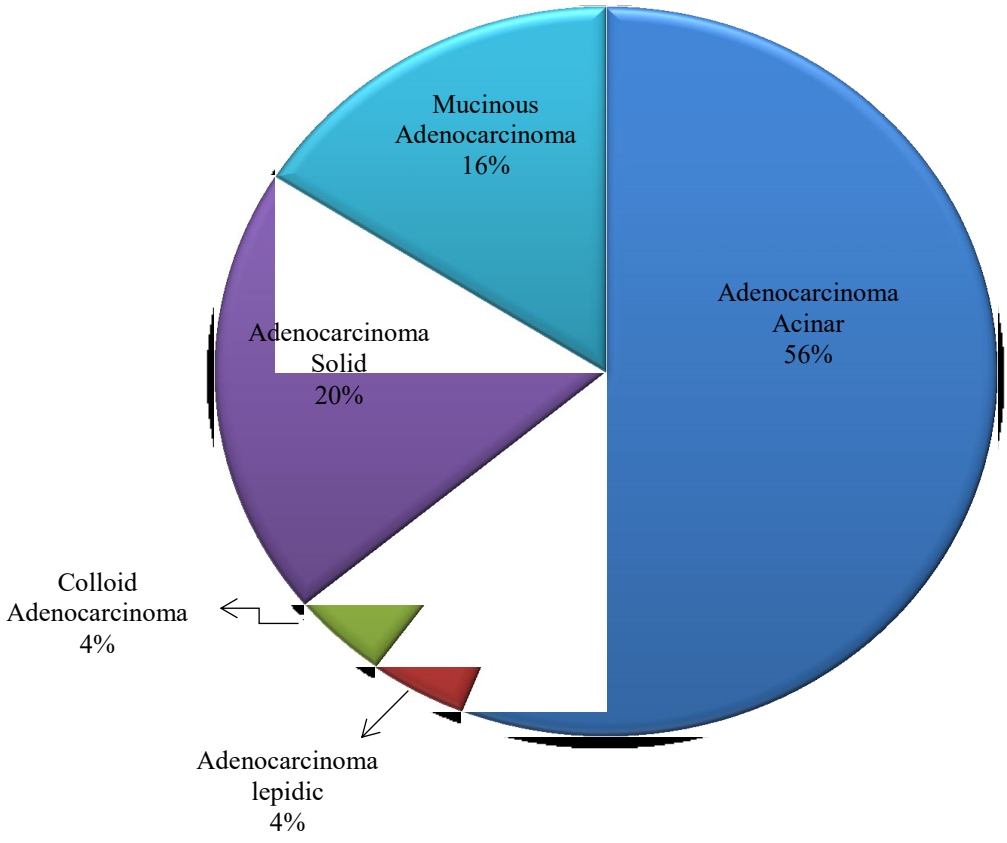

Figure 1. Frequency distribution of tumor tissue forms in lung cancer patients

Table 1. The results of protein expression assessment

Protein	Group	Expression	Pathologic feature	P-value
TTF1	Lung cancer	(92%) 23	Diffused	P < 0.0001
	Breast cancer	0 (0%)	--	
Napsin-A	Lung cancer	(%88) 22	76% diffused 12% focal	P < 0.0001
	Breast cancer	(0%) 0	--	
GCDFP15	Lung caner	(24%) 6	4% diffused 20% focal	P < 0.05
	breast cancer	(%68)17	24% diffused 44% focal	
ER	Lung caner	5 (20%)	Focal	P < 0.05
	breast cancer	17 (68%)	Diffused	
GATA3	Lung caner	(8%) 2	Focal	P < 0.0001
	breast cancer	(%100)25	4% Focal 96% diffused	

DISCUSSION

Lung adenocarcinoma and breast cancer both remain to be associated with high mortality in patients. These tumors have overlapping immunohistochemical and histological features, which makes them difficult to distinguish. The present study aimed to evaluate the efficiency of GCDFP15, ER, Napsin-A, TTF1 and GATA3 proteins in the differential diagnosis of primary lung cancer from breast cancer. In the current study, 25 patients had lung cancer and 25 had aggressive breast cancer. The mean age of the patients in the lung cancer group was 59 ± 11.2 years and the mean age of the patients in the breast cancer group was 50 ± 11.5 years. All tumor tissue samples in the group of breast cancer patients were invasive ductal carcinoma. The GATA3, ER, GCDFP15, Napsin-A, and TTF1 were significantly different between the lung cancer and breast cancer (all P-values < 0.05). TTF1 protein did not show any expression in the breast cancer. It was also seen that Napsin-A had no expression in the breast cancer. ER, GATA3, and GCDFP15 were expressed in lung cancer in 20%, 8%, and 24%, respectively.

TTF-1 is a tissue-specific homeodomain - containing transcription factor of the Nkx2 gene family that is expressed mostly in the normal respiratory epithelium and thyroid gland (15). The results of our study showed that most cases of lung adenocarcinoma diffusely expressed TTF-1, which is line with previous studies. El-Maqsoud et al. and Su et al. also reported that TTF-1 had a high expression in primary lung adenocarcinomas (with 80% and 73% of cases being positive for TTF-1, respectively) compared with secondary lung adenocarcinomas (16, 17). Except for thyroid carcinomas, TTF-1 staining has a low frequency in tumors of extrapulmonary origin (16). These finding indicates that TTF-1 may be used as a helpful marker in differentiating primary lung adenocarcinomas from other tumors that metastasize to the lung (3).

In addition, Napsin-A stained positive in 88% of lung adenocarcinomas in our study. Napsin-A, an aspartic protease, plays a role in the N- and C-terminal processing of propeptide surfactant protein B (proSP-B) in type-II pneumocytes (18). Previous studies investigating Napsin-A expression in pulmonary carcinomas similarly showed that Napsin-A is expressed in the majority of lung adenocarcinomas but not in squamous cell carcinomas (7, 16). These results point out that Napsin-A is capable of distinguishing primary lung adenocarcinoma from other metastatic carcinomas of the lung, including breast cancer.

ER expression was another protein evaluated in the current study. Our results demonstrated that ER expression in invasive ductal carcinomas of the breast had higher specificity compared to lung adenocarcinomas, in which this difference was statistically significant. These findings are in support of a study by Yang and colleagues that indicated the validity of ER for diagnosis of breast carcinomas (7).

Primary lung cancers were differentiated from breast metastatic cancers to the lung by using a model that considered the staining results for four markers: TTF-1, mammaglobin, P63 and ER (12). GCDFP-15 was another antibody that was studied. Based on our results, 68% of breast carcinomas expressed this immunohistochemical marker, while only 24% of lung adenocarcinomas stained positive. Evidence suggests that GCDFP-15 expression ranges from 41% to 73% in breast carcinomas (19). On the other hand, it has been reported that approximately 5% to 15% of lung adenocarcinomas may express GCDFP-15 (20).

Although our study found a significant differential expression of GCDFP-15 in lung adenocarcinomas vs. breast carcinomas, a positive immunostain of GCDFP-15 should be interpreted with caution.

Furthermore, GATA-3 was expressed in all invasive ductal carcinomas of the breast, while only 8% of lung adenocarcinomas showed positivity for GATA-3 staining. Similarly, other studies have previously reported that GATA-3 can be used as a diagnostic marker for distinguishing breast carcinomas metastatic to the lung from primary lung carcinomas (7, 21).

Of the limitations of this study were lack of understudied population and lack of examination with laboratory and clinical findings. In further studies, assessment of these markers that were evaluated in the current study are recommended in a large population with considering clinical and laboratory findings.

CONCLUSION

TTF-1 and Napsin-A were merely expressed in primary lung adenocarcinomas, which indicate the specificity of these two markers in the differentiation between lung adenocarcinomas and invasive ductal carcinomas of the breast. TTF-1 can be negative in some cases of lung adenocarcinomas and Napsin-A can be positive in some cases of breast carcinomas. Also, results of this study using biopsy showed that GATA-3 was expressed more in breast carcinomas showing the specificity of this protein for diagnosis of the lung adenocarcinomas and breast carcinomas. It should be noted that to differentiate lung adenocarcinoma from breast tumor that has metastasized to the lung, it is better to use a panel of multiple antibodies and not focus on one marker. If the number of tumor cells is small and there is a limitation of antibodies for any reason, GATA-3 is a specific and reliable antibody. GCDFP-15 was positive in all breast carcinomas but this protein was positive in some cases of lung adenocarcinomas. However, since ER and GCDFP-15 expression was observed in a subset of lung adenocarcinomas as well as invasive ductal carcinomas of the breast, further larger-scale studies are warranted to validate the sensitivity and specificity of these immunohistochemical markers in the differentiation between lung adenocarcinomas and breast carcinomas metastatic to the lungs. Taken together, it is important to consider a panel of antibodies for differentiation between these tumors rather than focusing on an individual IHC marker. Finally, taking more samples and checking for more markers is recommended because we had economic constraints and this was our main limitation.

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CONFLICT OF INTEREST

We do not have Conflict of interest

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REFERENCES

1. Jung HK, Park S, Kim NW, Lee JE, Kim Z, Han SW, et al.Development of second primary cancer in Korean breast cancer survivors.Annals of surgical treatment and research.2017;93(6):287-92.

2. Wei J-L, Jiang Y-Z and Shao Z-M.Survival and chemotherapy-related risk of second primary malignancy in breast cancer patients: a SEER-based study.International Journal of Clinical Oncology.2019;24(8):934-40.

3. Tennis M, Singh B, Hjerpe A, Prochazka M, Czene K, Hall P, et al.Pathological confirmation of primary lung cancer following breast cancer.Lung Cancer.2010;69(1):40-5.

4. Medeiros B and Allan AL.Molecular Mechanisms of Breast Cancer Metastasis to the Lung: Clinical and Experimental Perspectives.International Journal of Molecular Sciences.2019;20(9):2272.

5. Jin L, Han B, Siegel E, Cui Y, Giuliano A and Cui X.Breast cancer lung metastasis: Molecular biology and therapeutic implications.Cancer Biology & Therapy.2018;19(10):858-68.

6. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A.Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.CA: a cancer journal for clinicians.2018;68(6):394-424.

7. Yang M and Nonaka D.A study of immunohistochemical differential expression in pulmonary and mammary carcinomas.Modern Pathology.2010;23(5):654-61.

8. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al.Intratumor heterogeneity and branched evolution revealed by multiregion sequencing.N Engl j Med.2012;366:883-92.

9. Meng X, Song S, Jiang Z-F, Sun B, Wang T, Zhang S, et al.Receptor conversion in metastatic breast cancer: a prognosticator of survival.Oncotarget.2016;7(44):71887.

10. Clark BZ, Beriwal S, Dabbs DJ and Bhargava R.Semiquantitative GATA-3 immunoreactivity in breast, bladder, gynecologic tract, and other cytokeratin 7–positive carcinomas.American journal of clinical pathology.2014;142(1):64-71.

11. Davis DG, Siddiqui MT, Oprea-Ilies G, Stevens K, Osunkoya AO, Cohen C, et al.GATA-3 and FOXA1 expression is useful to differentiate breast carcinoma from other carcinomas.Human pathology.2016;47(1):26-31.

12. Selves J, Long-Mira E, Mathieu M-C, Rochaix P and Ilié M.Immunohistochemistry for diagnosis of metastatic carcinomas of unknown primary site.Cancers.2018;10(4):108.

13. Tavassoli FA.Pathology and genetics of tumours of the breast and female genital organs.World Hhealth Organization Classification of Tumours.2003.

14. Pradhan D, Amin M, Hooda S, Dhir R, Bastacky S and Parwani AV.Utility of the laminin immunohistochemical stain in distinguishing invasive from noninvasive urothelial carcinoma.Journal of Cancer Research and Therapeutics.2017;13(6):947.

15. Guan L, Zhao X, Tang L, Chen J, Zhao J, Guo M, et al.Thyroid transcription Factor-1: structure, expression, function and its relationship with disease.BioMed Research International.2021;2021.

16. El-Maqsoud NMRA, Tawfiek ER, Abdelmeged A, Rahman MFA and Moustafa AAE.The diagnostic utility of the triple markers Napsin A, TTF-1, and PAX8 in differentiating between primary and metastatic lung carcinomas.Tumor Biology.2016;37(3):3123-34.

17. Su Y-C, Hsu Y-C and Chai C-Y.Role Of TTF-1, CK20, And CK7 Immunohistochemistry for Diagnosis of Primary and Secondary Lung Adenocarcinoma.The Kaohsiung Journal of Medical Sciences.2006;22(1):14-19.

18. Johansson J and Curstedt T.Synthetic surfactants with SP-B and SP-C analogues to enable worldwide treatment of neonatal respiratory distress syndrome and other lung diseases.Journal of internal medicine.2019;285(2):165-86.

19. Bellizzi AM.An algorithmic immunohistochemical approach to define tumor type and assign site of origin.Advances in anatomic pathology.2020;27(3):114.

20. Enrico D, Saucedo S and Bravo I.Breast metastasis from primary lung adenocarcinoma in a young woman: A case report and literature review.World journal of clinical oncology.2019;10(7):269.

21. Lee AHS, Hodi Z, Soomro I, Sovani V, Abbas A, Rakha E, et al.Histological clues to the diagnosis of metastasis to the breast from extramammary malignancies.Histopathology.2020;77(2):303-13.