Evaluation of the Diagnostic Role of Gata3 Immunohistochemistry on Cytology Specimens for Metastatic Breast Carcinoma

Eaman Suud Khalifa[1], Ayser Hameed Latif[2]

[1]Lecturer, [2]Assistant Professor/Pathology Department/College of Medicine/Mustansiriyah University/Iraq

Abstract

Objective: Distinguishing of metastatic breast carcinoma from nonmammary metastatic carcinomas by cytological examination might have some difficulties. The sensitivity of traditional tumor markers for immunostaining might be not high enough . The role of GATA3 immunohistochemistry as a novel marker of primary and metastatic stages of breast carcinoma in specimens of fine needle aspiration was evaluated.

Materials and Methods: Sections were taken from 73 cases of FNA samples of metastatic malignancies including 38 metastatic breast cancer and 35 nonmammary malignancies were stained with GATA3 immunohistochemical marker. Samples of FNA were taken from pleural effusion, ascetic fluid and lymph nodes.

Results: Eighty-nine percent (34/38) of metastatic breast carcinoma cases were regarded as GATA3 positive expression , while all nonmammary adenocarcinoma samples were GATA3 negative . Most of GATA3 positive samples showed intense nuclear staining in the majurity of the malignant cells.

Conclusions: GATA3 staining is an important addition to immunohistochemical panels for fine needle aspiration samples for distinguishing metastatic breast carcinoma from other malignancy.

Keywords*: Breast carcinoma, Cytology, Fine needle aspiration, GATA3, Immunohistochemistry*

Introduction

Breast cancer is the commonest malignancy world wide in woman(1). Nearly 10-15% .Usually the history of primary breast carcinoma is well known in the majority of patients but some cases presented with metastases of unknown origin. Lung, bones, liver and pleura are common sites for breast carcinoma metastasis. Additionally, although uncommon, lymph node metastases may be the presenting symptom for occult breast carcinoma (4). Fine needle aspiration (FNA) is commonly used to assess the masses in these sites.

Differentiation metastatic breast carcinoma from other primary or metastatic malignancies is very important for guiding optimal management. Distinguishing these masses by using FNA alone might be not enough, and more accurate diagnosis necessitate the use of immunohistochemical markers like mammoglobin , "a mammary-specific member of the uteroglobin family". Overexpression of mammoglobin in breast carcinoma is well known. Prevoius rreports stated that mammaglobin is a relatively specific and sensitive marker for breast carcinomas. other traditional marker is GCDPF-15 that is controlled by androgen receptors. This antibody is useful in the diagnosis of breast carcinoma in the metastatic stage in fluid analysis and histopathological studies but has low sensitivity and can be found in about 50% of breast cancer specimens. However, there are an wide variations in the specificity and sensitivity of these markers that used for the assessment of these carcinomas.

GATA3 is a transcription factor, in human is encoded by the GATA 3 gene many studies indicate that it has a role in controlling many genes that were biologically and clinically important for differentiation of different types of tissues and cells(5, 6) . Furthermore;

GATA3 is important for control of cellular proliferation and movement. Expression of GATA3 is mainly in breast epithelia, urothelial tissue and T-lymphocyte and it can be detected in serous effusion samples. GATA3 is useful in distinguishing primary and metastatic breast cancer from other metastatic malignancies.

Aim of the study

Our aim in this study is to evaluate GATA3 immunohistochemical staining as a usefull marker in diagnosis of metastatic breast cancer in FNA specimens.

Materials and Methods

This study was conducted in department of pathology /Gasi Alhareeri hospital/ Baghdad medical city, Baghdad, Iraq during the period between October 2020 and February 2021.

Seventy-three cases (38 metastatic breast carcinoma and 35 non mammary metastatic malignancies) were selected for inclusion in this study. FNA aspirate taken from the pleural or ascetic fluid and lymph nodes. All cases had a diagnosis of primary or metastatic malignancy reported on FNA and a cell block containing diagnostic material. The cases had a known history of a primary tumor and/or subsequent or concurrent histology.

The primary antibody to GATA3 was used in dilution of 1:500. The staining was carried out by using "Leica automated immunostainer" with the use of retrieval buffer before the addition of the primary antibody (30 min). Other markers are used for diagnosis of mammary origin like GCDFP-15,by using monoclonal antibody anti-Human Mouse Monoclonal antibody and mammaglobin, "a mammary-specific member of the uteroglobin family", mouse monoclonal antimammaglobin clone304-1A5(DAKO) was used at a1:100 dilution.

"Staining intensity and percentage of stained cells for GATA3, mammaglobin and GCDFP-15, were recorded and scored as following : 0 (no staining), 1+ (weak), 2+ (moderate), or 3+ (strong). Percentage of stained cells (0, no stained cells; 1+, 1%-10% of cells were stained; 2+, 11%-50% of cells were stained; 3+, >50% of cells were stained)"

Statistical Analysis

"Statistical significance was determined by Fisher's and Chi-square analysis for categorical variables. The level of significance was set at 0.05 or less. P value equal or less than 0.05 considers to be significant. The sensitivity, specificity, Positive predictive value, Negative predictive value were calculated".

Results

Table-1 shows GATA3 staining in different primary tumor sites . GATA3 was positive in 89.4% (34/38) of metastatic breast carcinomas. All nonmammary carcinomas specimens were negative for GATA3.

There was no statistically difference in GATA3 staining between different histological subtypes of breast carcinoma (Table 2)

Table 3 shows the score of immunocytochemical stains GATA 3, mammaglobin and GCDFP-15 distribution in positive cases of metastatic breast carcinoma. Intense nuclear staining in most of the tumour cells were seen in most of positive cases (Figure 1)

Table 1: GATA3 staining results for metastatic malignancies in cell block sections from fine needle aspiration specimens

Tumor site	NO. of cases	GATA3 positive (%)
Breast	38	34(89.4%)
Lung	11	0 (0%)
Colon	5	0 (0%)
Thyroid	4	0 (0%)
Ovary	4	0 (0%)
Endometrium	3	0 (0%)
Stomach	4	0 (0%)
Kidney	2	0 (0%)
Pancreas	1	0 (0%)
Bladder	1	0 (0%)

Table 2: GATA3 staining results in different histological subtypes of breast carcinoma

Histological subtype	Total no.	Positive GATA3
ductal	23	21 (91.3%)
Lobular	10	9 (90%)
Papillary	3	2(66%)
Mucinous	1	1(100%)
Metaplastic	1	1(100%)
Total	38	34(89.4%)

Table 3 score of Immunocytochemical stains distribution of GATA3, mammaglobin and GCDFP-15 in positive metastatic breast cases

Score	GATA3	Mammoglobin	GCDFP-15
(1+) weak	2	4	9
(2+)moderate	7	13	7
(3+)Strong	25	9	5
Total	34/74	26/74	21/74

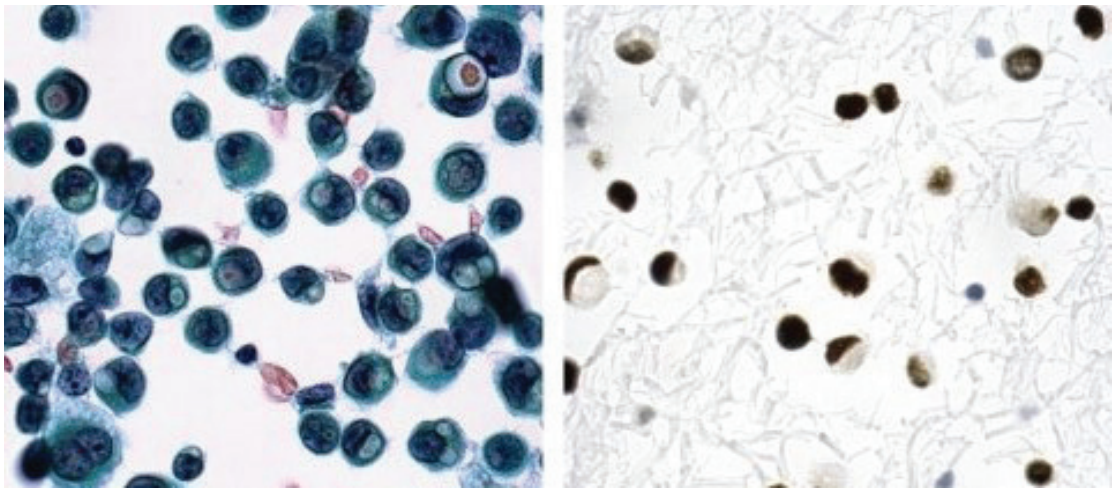

Figure 1: Fine needle aspiration of metastatic breast carcinoma in pleural fluid (a) Smear (papanicolaou stain 40x)(b)GATA 3 positive nuclear staining(cell block-40x).

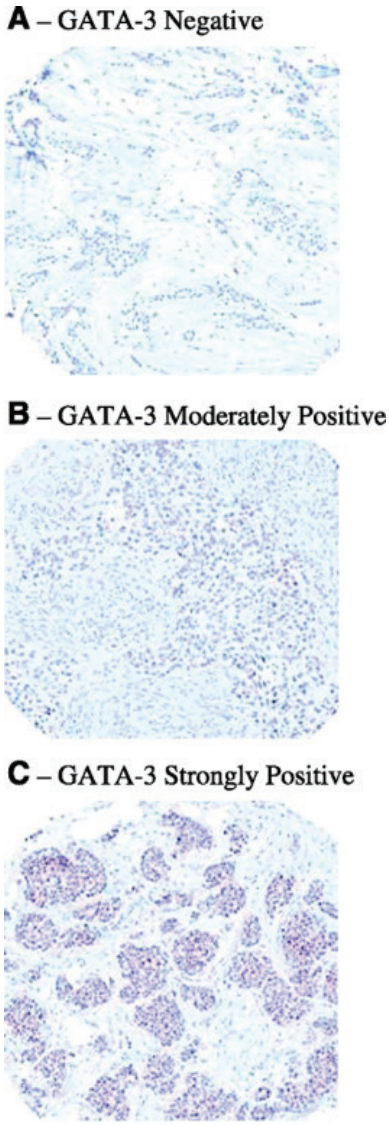

Figure 2: The intensity of GATA3 nuclear staining in the tumor cells

Discussion

The diagnosis of metastatic breast cancer using FNA has been shown to be sensitive test with a high specificity [11,12]. . Cytological assessment of specimens and recognition of malignant cells is usually straight-forward depending on the finding of sheets of cohesive cells and nuclear changes of atypia and hyperchromasia. Identification of metastatic lobular carcinoma may have some difficulties and the diagnosis is reported to be less accurate than for metastatic ductal carcinoma due to the commonly encountered single cell pattern and nuclear changes of low grade with small cell size. Previously, immunohistochemical staining used for the diagnosis of breast origin has somewhat low sensitivity and specificity although some markers like GCDFP-15 is specific for diagnosis of mammary origin but has low sensitivity reaching to 48-71%.

In our study we found that GATA3 marker as compared to GCDFP15 and mammaglobin is more sensitive. Positive results appear in 89.4% of cases (34/38) of aspirate from metastatic breast cancer.

In previous studies, the rate of positive GATA3 expression has been estimated from 75 to 100% [7- 9]. Lui et al. [7] reported that positive GATA3 expression was noted in 94% of breast carcinomas.

Leng et al., reported that positive GATA3 result was "71% of cell block sections and 89% of smear samples"[9]. In Braxton et al. [15], GATA3 expression had been seen in 75% of FNAs in metastatic breast carcinoma.

The main reason for this variation in the results might be linked to the number of samples or in the technique used for sample collection and staining.

Cell block material is regarded as better method for immunocytochemical staining similar to histopathological procedure after fixation and application of immunohistochemical stain.

The variations in GATA3 expression results between the previous studies also might be related to the scoring system that used in the study [10].

Furthermore, other causes for variation in GATA3 expression are tumor grade aand tumor molecular type in addition to methods of antigen retrieval and dilutions [11].

In our study, GATA3 was negative in all non breast carcinomas that selected in this study (lung, thyroid, gastrointestinal , female genital tract and urothelial carcinomas) which was a statistically significant ($p < 0.001$). However, our finding may be linked to limited number of samples. Deftereos et al.[10] reported in their study that "GATA3 was negative in all cases of nonmammary and non urothelial metastatic carcinomas".

Other studies reported that GATA3 expression could be seen in a relatively significant numbers of non mammary carcinoma cases [7, 8, 11, 12, 13, 14]. Miettinen et al [26] reported GATA3 staining in 37% of pancreatic ductal carcinoma cases. Other studies found a significant percentage of GATA3 expression in urothelial carcinomas [7, 22]

Therefore, these studies concluded that GATA3 marker is important when added to rhe traditional markers for the diagnosis of nonmamary carcinoma. Nevertheless, GATA3 staining should be tested in addition to clinical, radiological and morphological parameters to increase the accuracy the diagnosis. However, large number of cases might be needed to assess the role of GATA3 expression in nonmammary carcinomas.

GATA3 staining has also been studied in other malignancies like parathyroid carcinoma, Hodgkin and nonHodgkin lymphomas; however, GATA3 expression has higher sensitivity and specificity for breast carcinoma and in less extent in urothelial carcinomas.

In this study we found that no one of 11 lung adenocarcinoma express GATA3. Previous IHC studies also not or rarely reported GATA3 expression in lung adenocarcinomas [7, 16, 22]. Therefore, in this setting GATA3 may be added to a panel like mammoglobin and GCDFP-15 for differentiation between breast and lung adenocarcinoma to overcome this common problem in FNA cytology.

In the current study, no statistical difference was noted between GATA3 expression and different subtypes of breast carcinoma ($p >0.05$). This finding was in agreement with prevoius studies [7, 10].

In our study, GATA3 expression was negative for the benign cells in the specimens. Prevoius reports demonstrated a weakly positive expression of GATA3 in a small percentage of benign lymphoid cells in some specimens [12, 15]. Other studies not reported GATA3 expression in these cells [8, 12].

In current study GATA3 expression was highly sensitive in comparison to mammaglobin and GCDFP-15, and this difference was a statistically significant ($p < 0.05$). This was in agreement with previous studies [10, 11, 15] .

Seventy-three percent of positive cases showed an intense and diffused staining (strong, 3+ score) indicating that GATA3 expression was distributed equally with low staining variation within the tissue and this mean that GATA3 expression is highly dependable for highlighting malignant cells in specimens . This result was in agreement with previous studies [11, 15].

Addition of mammaglobin to GATA3 markers improved GATA3 sensitivity (94%) as it added two cases that were negative for GATA3. Meanwhile, adding GCDFP-15 to GATA3 not improve sensitivity. Therefore, using of GATA3 as a panel with the mammaglobin is important for breast carcinomas diagnosis. This result was in accordance with previous reports [12, 25].

Many previous studies stated that mamoglobin is more sensitive than GCDFP-15 but it is usually less specific [16, 19]. Studying tissue specimens have demonstrated mamoglobin staining in 48-72% of breast carcinomas. Huo et al.[24] reported that GCDFP-15 and mammaglobin expression in breast carcinoma was 21 and 41% respectively. Yan et al.[29] reported in their study that "the differences of mammoglobin and GCDFP-15 staining between breast and nonmammary carcinomas were statistically significant ($P < 0.05$) and mammoglobin is more sensitive than GCDFP-15 as a marker for metastatic breast carcinoma (87 vs. 46%)".

Conclusion

GATA3 IHC staining is an important addition to IHC panel for FNA samples for distinguishing metastatic breast carcinoma from other malignancies. Adding mammaglobin to GATA3 could improve the sensitivity.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
2. Weigelt B, Peterse JL, van't Veer LJ. Breast cancer metastasis: Markers and models. Nat Rev Cancer 2005;5:591-602.
3. Sangoi AR, Shrestha B, Yang G, Mego O, Beck AH. The novel marker GATA3 is significantly more sensitive than traditional markers mammaglobin and GCDFP15 for identifying breast cancer in surgical and cytology specimens of metastatic and matched primary tumors. Appl Immunohistochem Mol Morphol. 2016;24:229-37. 4. Ordonez NG. Value of GATA3 immunostaining in tumor diagnosis: a review. Adv Anat Pathol. 2013;20:352-360.
5. Burch JB. Regulation of GATA gene expression during vertebrate development. Semin Cell Dev Biol 2005;16:71-81.
6. Zheng R, Blobel GA. GATA Transcription Factors and Cancer. Genes Cancer 2010;1:1178-88.
7. Liu H, Shi J, Wilkerson ML, Lin F. Immunohistochemical evaluation of GATA3 expression in tumors and normal tissues: A useful immunomarker for breast and urothelial carcinomas. Am J Clin Pathol 2012;138:57-64.
8. Leng B, Guo M, Zhao J, Gong U. Utility and pitfalls of GATA3 immunocytochemistry for diagnosis of metastatic breast carcinoma and urothelial carcinoma on cytology specimens. JASC. 2017;6:73-9.
9. Dyhdalo KS, Booth CN, Brainard JA, Croyle MC, Kolosiwsky AM, Goyal A, Gildea TR, Almeida FA, Nassar A, Reynolds JP. Utility of GATA3, mammaglobin, GCDFP-15, and ER in the detection of intrathoracic metastatic breast carcinoma. JASC. 2015;4:218-24
10. Deftereos G, Sanguino Ramirez AM, Silverman JF, Krishnamurti U. GATA3 immunohistochemistry expression in histologic subtypes of primary breast carcinoma and metastatic breast carcinoma

cytology. Am J Surg Pathol. 2015;39:1282-9.

11. Lew M, Pang JC, Jing X, Fields KL, Roh MH. Young investigator challenge: The utility of GATA3 immunohistochemistry in the evaluation of metastatic in breast carcinomas in malignant effusions. Cancer Cytopathol. 2015;123:576-81.
12. Shield PW, Papadimos DJ, Walsh MD. GATA3: A promising marker for metastatic breast carcinoma in serous effusion specimens. Cancer (Cancer Cytopathol) 2014;122:307-12.
13. Cimino-Mathews A, Subhawong A P, Illei P B, Sharma R, Halushka M K, Vang R, Fetting JH, Park BH, Argani P. GATA3 expression in breast carcinoma: Utility in triple-negative, sarcomatoid, and metastatic carcinomas. Human Pathol. 2013;44:1341-9.
14. Mariotto AB, Etzioni R, Hurlbert M, PenberthyL, Mayer M. Estimation of the number of women living with metastatic breast cancer in the United States. Cancer Epidemiol Biomarkers Prev. 2017;26:809-15.
15. Braxton DR, Cohen C, Siddiqui MT. Utility of GATA3 immunohistochemistry for diagnosis of metastatic breast carcinoma in cytology specimens. Diagn Cytopathol 2015;43:271-7.
16. Shield PW, Duricic D, Truong T. Evaluation of an agar cell block method to improve cell yield in non-gynaecological cytology specimens. Aust J Med Sci 2013;34:20-3.
17. Fung AD, Collins JA, Campassi C, Ioffe OB, Staats PN. Performance characteristics of ultrasound-guided fine-needle aspiration of axillary lymph nodes for metastatic breast cancer employing rapid on-site evaluation of adequacy: Analysis of 136 cases and review of the literature. Cancer (Cancer Cytopathol) 2014;122:282-91.
18. Alkuwari E, Auger M. Accuracy of fine-needle aspiration cytology of axillary lymph nodes in breast cancer patients: A study of 115 cases with cytological-histological correlation. Cancer 2008;114:89-93.
19. Chia SY, Thike AA, Cheok PY, Tan PH. Utility of mammaglobin and gross cystic disease fluid protein-15 (GCDFP-15) in confirming a breast origin for recurrent tumors. Breast 2010;19:355-9.
20. Bhargava R, Beriwal S, Dabbs DJ. Mammaglobin vs GCDFP-15: An immunohistological validation survey for sensitivity and specificity. Am J Clin Pathol 2007;127:103-13.
21. Yang M, Nonaka D. A study of immunohistochemical differential expression in pulmonary and mammary carcinomas. Mod Pathol 2010;23:654-61.
22. Sasaki E, Tsunoda N, Hatanaka Y, Mori N, Iwata H, Yatabe Y. Breast-specific expression of MGB1/mammaglobin: An examination of 480 tumors from various organs and clinicopathological analysis of MGB1-positive breast cancers. Mod Pathol 2007;20:208-14.
23. Wang Z, Spaulding B, Sienko A, Liang Y, Li H, Nielsen G, *et al*. Mammaglobin, a valuable diagnostic marker for metastatic breast carcinoma. Intl J Clin Exp Pathol 2009;2:384.
24. Huo L, Zhang J, Gilcrease MZ, Gong Y, Wu Y, Zhang H, *et al*. Gross cystic disease fluid protein-15 and mammaglobin A expression determined by immunohistochemistry is of limited utility in triple-negative breast cancer. Histopathology 2013;62:267-74.
25. Ordonez NG. Value of GATA3 immunostaining in tumor diagnosis: A review. Adv Anat Pathol 2013;20:352-60.
26. Miettinen M, McCue PA, Sarlomo-Rikala M, Rys J, Czapiewski P, Wazny K, *et al*. 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.
27. Brandler TC, Aziz MS, Rosen LM, Bhuiya TA, Yaskiv O. Usefulness of GATA3 and p40 immunostains in the diagnosis of metastatic urothelial carcinoma in cytology specimens. Cancer (Cancer Cytopathol) 2014;122:468-73.
28. Crapanzano JP, Saqi A. Pitfalls in pulmonary cytopathology. Diagn Cytopathol 2011;39:144-54.
29. Yan Z, Gidley J, Horton D, et al. Diagnostic utility of mammaglobin and GCDFP-15 in the identification of metastatic breast carcinoma in fluid specimens. Diagn Cytopathol. 2009;37:475-478.