This may be the author's version of a work that was submitted/accepted for publication in the following source:

**Shield, Paul**, Crouch, Stephen, Papadimos, David, & Walsh, Michael (2018)
Gata3 immunohistochemical staining is a useful marker for metastatic breast carcinoma in fine needle aspiration specimens.
*Journal of Cytology, 35*(2), pp. 90-93.

This file was downloaded from: [https://eprints.qut.edu.au/117554/](https://eprints.qut.edu.au/117554/)

**© Consult author(s) regarding copyright matters**

This work is covered by copyright. Unless the document is being made available under a Creative Commons Licence, you must assume that re-use is limited to personal use and that permission from the copyright owner must be obtained for all other uses. If the document is available under a Creative Commons License (or other specified license) then refer to the Licence for details of permitted re-use. It is a condition of access that users recognise and abide by the legal requirements associated with these rights. If you believe that this work infringes copyright please provide details by email to qut.copyright@qut.edu.au

**License:** Creative Commons: Attribution-Noncommercial-No Derivative Works 2.5

**Notice:** Please note that this document may not be the Version of Record (i.e. published version) of the work. Author manuscript versions (as Submitted for peer review or as Accepted for publication after peer review) can be identified by an absence of publisher branding and/or typeset appearance. If there is any doubt, please refer to the published source.

[https://doi.org/10.4103/JOC.JOC_132_17](https://doi.org/10.4103/JOC.JOC_132_17)
GATA3 immunohistochemical staining is a useful marker for metastatic breast carcinoma in fine needle aspiration specimens.

Abstract

**Aims:** The utility of GATA3 immunohistochemistry (IHC) as an aid to the cytological diagnosis of metastatic breast carcinoma in fine needle aspiration (FNA) specimens was investigated.

**Materials and Methods:** Cell block sections from 111 FNA cases of metastatic malignancy were stained for GATA3, including metastases from 43 breast and 44 non-mammary adenocarcinomas, 19 melanomas, 4 urothelial carcinomas and one thyroid medullary carcinoma. Sites sampled included lymph nodes (87), bone (8), liver (5), lung (6), superficial masses (4) and one pelvic mass.

**Results:** 91% (39/43) of metastatic breast carcinoma cases were positive for GATA3. All estrogen receptor (ER) positive were also GATA3 positive cases. The majority (9/14; 64%) of ER negative and 37% (3/8) of triple negative cases were positive for GATA3. All non-mammary adenocarcinoma cases were negative with the exception of one case of metastatic pancreatic adenocarcinoma. Metastatic melanoma cases were all negative but 75% (3/4) urothelial carcinomas expressed GATA3.

**Conclusions:** GATA3 IHC staining is a useful addition to immunohistochemistry panels for FNA samples in specific settings, such as distinguishing metastatic breast from lung carcinoma or melanoma.

**Keywords:** GATA3; fine needle aspiration; breast carcinoma metastatic; immunohistochemistry; cytology
Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in females worldwide.\(^1\) About 10-15% of patients will develop metastases in the first 3 years after diagnosis\(^2\) and metastases detected 10 years or more after primary diagnosis are not unknown. Common sites for breast carcinoma metastases include lung, bones, lymph nodes, liver and pleura,\(^3\) and cytology is frequently used to assess suspicious masses in these sites. Additionally, although uncommon, lymph node metastases may be the presenting symptom for occult breast carcinoma\(^4\).

Discriminating metastatic breast carcinoma from other primary or metastatic malignancies is important for determining optimal management, but may be difficult on fine needle aspiration (FNA) when based on cytomorphology alone. The diagnostic accuracy and precision of FNA in this setting can often be enhanced by adjunctive use of immunohistochemistry (IHC). Until recently, reliable IHC identification of breast as the primary site of metastatic malignancy has been limited by a lack of markers with both high sensitivity and specificity.

GATA3 (GATA binding protein 3 to DNA sequence [A/T]GATA[A/G]) is a member of the zinc finger transcription factor family. It is involved in development and differentiation in many tissues and cell types,\(^5,6\) including control of cellular proliferation and movement, and is reported to be a sensitive and specific marker for urothelial and breast carcinomas in tissue sections.\(^7\) A role for IHC detection of GATA3 has been suggested in serous effusion specimens\(^8\) and two studies have indicated that GATA3 may be a valuable aid in the identification of breast carcinoma in FNA specimens.\(^7,9\)
We evaluated GATA3 expression in FNA specimens, including samples from a range of body sites and types of metastatic malignancy where the differential diagnosis of breast carcinoma is likely.

Materials and Methods

Institutional approval for the study was obtained and a total of 111 cases reported by between 2011 and 2016 were selected for inclusion. All cases had a diagnosis of 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. Many cases, including all metastatic melanomas, also had supporting IHC results. The cases included 87 lymph node aspirates (head and neck region: 35; axilla: 42; inguinal: 6; mediastinal: 3; celiac: 1) and specimens from bone (8), liver (5), lung (6), one pelvic mass, one back and three chest masses. The 43 metastatic breast cases included 34 ductal carcinomas, 3 lobular carcinomas and in six cases the original histopathology report was unavailable. Cell blocks were prepared using an agar method as previously described. [10]

All IHC stains were performed on a Ventana Benchmark Ultra instrument (Ventana Medical Systems, Inc., Tucson, AZ). Sections for GATA3 IHC were subjected to epitope retrieval using CC1 (Ventana) for 32 min prior to incubation with GATA3 monoclonal antibody (BD Pharmingen, BD Biosciences, San Jose, CA), 1:7500 dilution for 32 min at 36°C, followed by OptiView (Ventana) detection system. TMA sections containing positive and negative breast tissues were applied to each slide.

Stained sections were scored positive if >5% of malignant cells showed nuclear staining for GATA3.
Results

Table 1 summarizes the results of GATA3 staining by primary tumor type. Ninety-one percent (39/43) of metastatic breast carcinomas and 75% (3/4) of metastatic urothelial carcinomas were positive. All other tumor types were negative with the exception of one pancreatic adenocarcinoma metastatic to liver. All positive cases showed intense nuclear staining in a majority of tumor cells (Figure 1).

All metastases from known estrogen receptor (ER) positive breast cancers were GATA3 positive. In addition, nine (64%) of fourteen cases that were ER negative were GATA3 positive. Eight cases were metastases from triple negative breast carcinomas (TNBC), and three (37%) of these were positive for GATA3.
Discussion

FNA cytology is a reliable tool for investigating suspected metastatic malignancy in a wide range of body sites. Enlarged lymph nodes in particular are a common target for FNA sampling. The diagnosis of metastatic breast carcinoma in lymph nodes using FNA has been shown to be an economical, sensitive test with high specificity.\textsuperscript{11,12} Cytological interpretation of specimens and identification of malignant cells is usually straight-forward based on recognition of cellular cohesion and nuclear atypia. Metastatic lobular carcinoma may pose some problems due to the commonly encountered dispersed pattern, small cell size and low grade nuclear changes, but FNA diagnosis is reported to be no less accurate than for metastatic ductal carcinoma.\textsuperscript{11} However, cytomorphologic discrimination of metastatic breast carcinoma from adenocarcinoma arising in other sites or from adenocarcinoma mimics, such as melanoma, may be problematic.

IHC stains that have previously been used to indicate a breast origin for metastases have been limited by poor sensitivity and variable specificity.\textsuperscript{13} Keratins 7 and 20 allow broad localization of a possible primary site but additional staining panels are required to improve tissue specificity. Gross cystic disease protein (GCDFP-15) has been reported to have high specificity for breast carcinoma, with mostly mammary tumors expressing the antigen, along with occasional sweat gland, salivary gland tumors and a small proportion of lung adenocarcinomas. However it has poor sensitivity, with staining reported in tissue samples from 41-73\% of breast carcinomas.\textsuperscript{9,14-16} A study of FNA and body fluid specimens, examining 23 primary and metastatic mammary carcinomas and 20 non-mammary tumors reported 100\% specificity but only 56\% sensitivity with GCDFP-15.\textsuperscript{17}
Mammaglobin is more sensitive than GCDFP-15 but less specific.\textsuperscript{[15]} IHC studies of tissue samples have reported mammaglobin staining in 48-72\% of breast carcinomas, 11-39\% of endometrial carcinomas, 40\% of sweat gland carcinomas, 20\% of salivary gland tumors and rare lung adenocarcinomas.\textsuperscript{[15,18,19]} Both GCDFP-15 and mammaglobin are also less likely to stain cases of triple negative breast carcinoma (TNBC). Huo et al. found GCDFP-15 and mammaglobin staining in only 21\% and 41\%, respectively, of metastatic TNBC.\textsuperscript{[20]}

Estrogen receptor (ER) and progesterone receptor (PR) have also been utilized to confirm metastatic breast carcinoma, but both receptors are commonly expressed in gynecologic malignancies\textsuperscript{3}, and pulmonary adenocarcinomas occasionally express ER.\textsuperscript{[16,21]} There are few published studies examining the performance of these breast tissue markers with FNA specimens but these appear to confirm the limitations highlighted in IHC studies of surgical specimens.\textsuperscript{[22]}

Recent IHC studies suggest that GATA3 may be a more effective marker.\textsuperscript{[7, 23, 24]} Lui et al.\textsuperscript{[7]} assessed GATA3 expression in a large number and range of tumors and tissues and reported positive staining in 94\% of breast carcinomas and 86\% of urothelial carcinomas. Other non-mammary tumors were negative except for rare endometrial carcinomas. GATA3 expression has also been reported in normal and neoplastic parathyroid cells, in T-cells and in both Hodgkin and non-Hodgkin lymphomas, however amongst epithelial tumors, GATA3 has relatively high sensitivity and specificity for urothelial and breast carcinoma, including expression in all histological sub-types of breast carcinoma.\textsuperscript{[7, 24]} Studies examining GATA3 expression in cell block preparations from serous effusion specimens confirm the superior sensitivity of GATA3 for metastatic breast carcinoma compared with mammaglobin and GCDFP-15.\textsuperscript{[8, 9]}
Two small studies have examined the utility of GATA3 staining in FNA specimens. Liu et al. reported 88% of 17 cases of primary breast carcinomas and 82% of 22 metastatic breast carcinomas tested positive for GATA3. Braxton et al. found 75% (21/28) of FNAs with metastatic breast carcinoma to be positive. This was significantly more sensitive than mammoglobin or GCDFP-15. Neither study addressed the specificity of GATA3 staining in FNA specimens. We examined GATA3 expression in a range of metastatic adenocarcinomas, melanomas and urothelial carcinoma cases.

Ninety-one percent of 43 metastatic breast carcinomas were positive in the current study, confirming the high sensitivity reported in previous cytology studies. In all positive cases a majority of cells showed strong nuclear staining. Braxton et al. found GATA3 staining of cell block specimens to be correlated with ER status. Our findings are similar, with all four of the GATA3 negative cases also ER negative. However, 64% (9/14) of the ER-negative cases and 37% (3/8) of the TNBC were positive for GATA3, indicating that it may be a useful, although less sensitive, marker in cases with negative receptor status. Previous cytology studies have found GATA3 staining in around half of TNBCs. The current study did not assess the relative sensitivity of GATA3 with other markers for breast origin but the sensitivity described is superior to that reported for previously used markers.

We found GATA3 to have high specificity amongst metastatic adenocarcinomas, with only one of 44 non-mammary metastases positive. The exception was a case of pancreatic ductal carcinoma metastatic to liver. Although Lui et al. found no staining in 28 histological cases of pancreatic adenocarcinoma, Miettinen et al. more recently reported GATA3 IHC staining in tissue from 37% of 62 pancreatic ductal carcinoma cases. IHC staining of tissue sections have also found a high proportion of urothelial carcinomas express GATA3. We
examined only a limited number of UC cases in the current study, finding staining in three of four cases. Brandler et al.\textsuperscript{[25]} reported GATA3 to be a useful marker for metastatic UC in cytological specimens, staining 78% of 32 FNA cases of metastatic urothelial carcinoma. In particular, they suggested a role for GATA3 in helping to discriminate metastatic urothelial carcinoma from squamous cell carcinoma.

The expression of GATA3 by other non-mammary carcinomas should also be considered when interpreting IHC results. Tumors most likely to be sampled by FNA that may express GATA3 include salivary gland and pancreatic ductal carcinomas, cutaneous squamous cell carcinomas and skin adnexal tumors, such as pilomatrixoma.\textsuperscript{[24]} Despite this moderately broad tissue specificity, staining for GATA3 may still have a useful role in FNA cytology in helping to resolve differential diagnoses when breast carcinoma or urothelial carcinoma are a consideration. The distinction between lung and breast adenocarcinoma, both typically CK7+ and CK20-, is a not uncommon problem in FNA cytology, and one where other described breast markers have been of limited value.\textsuperscript{[26]} We found none of the 13 metastatic lung adenocarcinomas in this study to express GATA3. IHC studies of tissue sections have also found none or only rare lung adenocarcinomas to be GATA3 positive.\textsuperscript{[7, 16, 24]} In this setting GATA3 may be a more useful addition to a panel including TTF-1 and/or NapsinA than previously used markers such as GCDFP-15, mammaglobin or hormone receptors.

Discriminating metastatic breast carcinoma from other non-glandular malignancies may also be problematic in some settings. Lymph node masses in melanoma patients are commonly sampled in our practice. The morphological similarity of melanoma to metastatic breast carcinoma in those cases presenting with a dispersed population of plasmacytoid cells with eccentric nuclei can be problematic and may lead to misdiagnoses.\textsuperscript{[27]} The problem is
exacerbated when no primary site is known or when a history of melanoma is not provided.

In addition, as many as 11% of patients with melanoma undergoing FNA will have a second malignancy.[27] Tissue studies to date have found metastatic melanoma to not express GATA3[7, 24] and in the current study all melanoma cases were negative, suggesting that in this setting the use of GATA3 with a panel of melanoma markers may be useful.

In summary, our findings indicate that, providing the multi-specificities of GATA3 are taken into account, it is a useful addition to IHC panels for FNA specimens in specific settings where metastatic breast or urothelial carcinoma are a consideration.

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 Canc. 2005;5:591-602.
3 Lee BH HJ, Pinkus JL, Pinkus GS. WT1, estrogen receptor, and progesterone receptor as markers for breast or ovarian primary sites in metastatic adenocarcinoma to body fluids. Am J Clin Pathol 2002;117:745-50.
4 Ashikari R RP, Urban JA, Senoo T. Breast cancer presenting as an axillary mass. Ann Surg 1976;183:415-7.
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 Dec;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 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.

9 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.

10 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.

11 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.

12 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 25;114:89-93.

13 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.

14 Wick MR, Lillemoe TJ, Copland GT, Swanson PE, Manivel JC, Kiang DT. Gross cystic disease fluid protein-15 as a marker for breast cancer: immunohistochemical analysis of 690 human neoplasms and comparison with alpha-lactalbumin. Hum Pathol 1989;20:281-7.

15 Bhargava R, Beriwal S, Dabbs DJ. Mammaglobin vs GCDFP-15: an immunohistological validation survey for sensitivity and specificity. Am J Clin Patho. 2007;127:103-13.

16 Yang M, Nonaka D. A study of immunohistochemical differential expression in pulmonary and mammary carcinomas. Mod Pathol 2010;23:654-61.

17 Fiel MI, Cernaianu G, Burstein DE, Batheja N. Value of GCDFP-15 (BRST-2) as a specific immunocytochemical marker for breast carcinoma in cytological specimens. Acta Cytol 1996;40:637-41.
18 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.

19 Wang Z, Spaulding B, Sienko A, Liang Y, Li H, NielsenG et al. Mammaglobin, a valuable diagnostic marker for metastatic breast carcinoma. Intl J Clin And Exp Pathol 2009;2:384.

20 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.

21 Dabbs DJ, Landreneau RJ, Liu Y, Raab SS, Maley RH, Tung MY et al. Detection of estrogen receptor by immunohistochemistry in pulmonary adenocarcinoma. Ann Thoracic Surg 2002;73:403-6.

22 Skoog L, Tani E. Immunocytochemistry: an indispensable technique in routine cytology. Cytopathol 2011;22:215-29.

23 Ordonez NG. Value of GATA3 immunostaining in tumor diagnosis: a review. Adv Anat Pathol 2013;20:352-60.

24 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. The Am J Surg Pathol 2014;38:13-22.

25 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.

26 Crapanzano JP, Saqi A. Pitfalls in pulmonary cytopathology. Diagn Cytopathol 2011;39:144-54.

27 Murali R, Doubrovsky A, Watson GF, et al. Diagnosis of metastatic melanoma by fine-needle biopsy: analysis of 2,204 cases. Am J Clin Pathol 2007;127:385-97.
Table 1. GATA3 staining results for metastatic malignancies in cell block sections from fine needle aspiration specimens. (GATA3 = GATA-binding protein 3 to DNA sequence [A/T]GATA[A/G]).

| Tumor type                      | No. cases | No. positive | (% positive) |
|---------------------------------|-----------|--------------|--------------|
| Breast carcinoma                | 43        | 39 (91%)     |              |
| Colon adenocarcinoma            | 5         | 0            |              |
| Gall Bladder adenocarcinoma     | 1         | 0            |              |
| Gastric adenocarcinoma          | 3         | 0            |              |
| Lung adenocarcinoma             | 15        | 0            |              |
| Melanoma                        | 19        | 0            |              |
| Ovarian serous carcinoma        | 4         | 0            |              |
| Pancreatic adenocarcinoma       | 1         | 1 (100%)     |              |
| Rectal adenocarcinoma           | 1         | 0            |              |
| Renal clear cell carcinoma      | 2         | 0            |              |
| Thyroid papillary carcinoma     | 12        | 0            |              |
| Thyroid medullary carcinoma     | 1         | 0            |              |
| Urothelial carcinoma            | 4         | 3 (75%)      |              |
**Figure 1:** Fine needle aspiration of metastatic breast carcinoma in an axillary lymph node. **A:** Smear. Papanicolaou stain, x400. **B:** Cell block. GATA3, x200.