Pathology & Oncology Research 2018;9:259-267. (doi: 10.1007/s12253-017-0246-y**)**

OA Green (Post print version)

Link to publisher version: https://link.springer.com/article/10.1007%2Fs12253-017-0246-y

Immunohistochemical analysis of the exression of breast markers in basal-like breast carcinomas defined as triple negative cancers expressing keratin 5

Tamás Zombori[1], Gábor Cserni[1, 2]

[1] Department of Pathology, University of Szeged, Faculty of Medicine

[2] Department of Pathology, Bács-Kiskun County Teaching Hospital

Summary

Estrogen receptor, progesteron receptor and HER2 are among the most useful immunohistochemical markers for proving breast origin of a metastatic carcinoma. These antibodies can be helpful in cases of luminal A-like, luminal B-like and HER2 subtypes but not in cases of triple negative breast cancer (TNBC) which represent approximately 15% of all breast cancers. Several immunomarkers as GATA-3, mammaglobin A and gross cystic disease fluid protein 15 (GCDFP 15) and NY-BR-1 have been studied recently to verify the breast origin in metastatic cancer.

We investigated GATA-3, Mammaglobin A, GCDFP-15, Ny-BR-1 and BCA-225 immunohistochemical staining on tissue microarrays in a series of TNBCs showing keratin 5 (CK5) expression, and therefore being consistent with a basal-like phenotype on the basis of the surrogate IHC based molecular classification.

GATA-3 staining was observed in 82 of 115 triple negative cases (71.3%) including 23 cases with >5% staining. Mammaglobin-A staining was detected in 30 cases (26.0%) including 12 cases with >5% staining. GCDFP-15 was seen in 23 cases (20.0%) including 9 cases with >5% staining. Ny-BR-1 positivity was seen in 7 cases (6.0;) including 3 patients with >5% staining. BCA-225 staining was observed in 74 cases (64.3%). We found GATA-3, mammaglobin-A and GCDFP-15 coexpression in one case (0.87%;) and GATA-3 and mammoglobin coexpression and Mammaglobin-A and GCDFP-15 coexpression in 2 and 2 cases (1.7%, 1.7%), respectively.

The expression of GATA-3, mammaglobin-A, GCDFP-15 and NY-BR-1 is lower in TNBC-s than in breast carcinomas in general. Although these markers may be positive in different other tumors, by using these markers a subset of basal-like TNBC-s can be identified. The expression of all the 4 markers was 34.7%. When comparing our result with that of others, it is shining that the positivity rates for GATA-3, and GCDFP-15 are lower in our tissue microarray based series.

Introduction

Breast cancer remains the most frequent malignant tumor among women in Europe (1DeSantis, 2Malvezzi). Although it is often mentioned as a single disease in such statistics, it is obvious that the term refers to several different diseases.

Perou and coworkers evaluated gene expression in breast cancer samples and suggested a molecular classification of the disease (3Perou). In their original classification, basal-like, Erb-B2 (human epidermal growth factor receptor 2) overexpressing (HER2+), normal-breast-like, and luminal (estrogen receptor positive, ER+) breast cancers had been identified. Gene expression profiling is the gold standard for the identification of these molecular breast cancer subtypes, but this method is not widely available. To make the classification more affordable to most pathology laboratories, immunohistochemical (IHC) profiles have been correlated to the molecular profiles. According to the IHC surrogate classification, breast carcinomas can be classified into luminal A-like (ER+ and/or progesterone receptor (PR)+ and HER2- with low proliferation), luminal B-like (ER+ and/or PR+ and HER2+ and/or highly proliferating on the basis of Ki-67 labeling), HER2+ non-luminal-like (ER-, PR- and HER2+) and triple-negative breast cancer (TNBC; ER-, PR- and HER2-). The latter group can be subclassified into basal-like TNBC (keratin (CK) 5/6+ and/or epidermal growth factor receptor, EGFR+) and non-basal-like TNBC (CK 5/6- and EGFR-) (4Nielsen).

Metastases of breast cancer develop through either the lymphatic or the blood vessels, and affect regional lymph nodes and distant organs, including the lungs, the liver, bones, and the brain. Since the lifetime risk of developing cancer is about one out of three women (5Hernandez), second primaries are not rare, and must be separated from metastases of a known breast cancer. Sometimes, metastasis is the first clinical sign of an unknown primary breast cancer. In case of metastatic carcinoma, it is essential to prove its metastatic nature and origin.

Despite their less than perfect specificity, ER, PR and HER2 are among the most useful IHC markers for suggesting breast origin. These antibodies can be helpful in cases of luminal A-like, luminal B-like and HER2+ subtypes, but not in cases of TNBC which represent approximately 15% of all breast cancers (6Huo). Without the information of a previous primary breast carcinoma, and because of its phenotypic overlap with other potential primaries, a triple negative case can easily confuse the pathologist. Several immunomarkers as GATA-3, mammaglobin, gross cystic disease fluid protein-15 (GCDFP-15) and NY-BR-1 have been studied recently to verify the breast origin in metastatic cancer.

GATA-3 is a transcription factor with role in cell proliferation and differentiation of breast luminal epithelial cells. GATA3 is involved in T-cell-specific cell regulation, in the development of the skin and its adnexal structures and in carcinomas (7Deftereos, 8Chou). Previously, GATA-3 was thought as specific marker of breast and urothelial origin, but recent studies have shown its presence in squamous carcinoma of the skin,

lung, uterine cervix, vulva, larynx and anus, salivary gland tumors, basal cell carcinoma, apocrine carcinoma, skin adnexal tumors, Brenner tumor, mesothelioma, chromophobe renal cell carcinoma, pancreatic adenocarcinoma, germ cell tumors and paraganglioma (9Esheba, 10Liu, 11Ordonez, 12Miettinen). GATA3 and ER are closely associated and involved in a positive cross-regulatory loop. This explains the positive correlation between GATA3 and ER expression in breast cancers (13Voduc, 14Hoch). Although some studies have suggested a prognostic or predictive role for GATA3 expression (15Tominaga, 16Albergaria, 17Parikh, 18Mehra), it can also be viewed as a marker to prove the mammary origin of metastatic cancer. The expression frequency of GATA-3 ranges from 47% to 100% among all breast adenocarcinomas (10Liu, 11Ordonez, 19Cimino-Matthews, 20Ciocca, 21Jacquemier, 22Demir, 13Voduc).

Mammaglobin A (MG) was described by Watson et al. in 1997 as a 10.5 kD secretory protein that shares homology with the uteroglobin family (23Watson1996). The gene of MG is located at 11q13, which is frequently amplified in breast carcinoma (24Watson1998). MG is generally positive in normal breast epithelium. Besides breast carcinomas, several tumors express MG, like endometrial carcinoma, sweat gland tumors, gastric, pulmonary, colonic and ovarian tumors and some melanomas (25Han, 26Bhargava, 27Zafrakas, 28Sasaki, 29Wang, 30Onuma). The overall expression rate among all breast carcinomas is approximately 80% (25Han, 26Bhargava, 28Sasaki, 23Watson1996, 31Al-Joudi, 32Lewis, 33Fritzsche).

Gross cystic disease fluid protein-15 (GCDFP-15 or BRST-2) was detected in breast gross cystic disease fluid by Haagensen et al. in 1977. The monomer of GCDFP-15 has a molecular weight of 15 kD (34Haagensen). Its gene region was found on chromosome 7. GCDFP-15 is normally present in apocrine metaplasia of the breast and its presence has been described in salivary and sweat gland tumors and prostatic carcinomas (35Wick). The reported expression frequency of GCDFP-15 ranges from 25% to 85% among all breast adenocarcinomas (25Han, 35Wick, 26Bhargava, 32Lewis, 33Fritzsche, 36Park).

NY-BR-1, a differentiation antigen of the mammary tissue was first described by Jäger et al in 2001(37Jager2001). Bioinformatic analysis has revealed that NY-BR1 has a DNA-binding site followed by a leucine zipper motif, therefore it could be a transcription factor. Due to its five ankyrin tandem repeats, it may have a role in protein-protein interactions, as well (37Jager2001). It has been detected in the epithelial cells of mammary ducts and lobules and in normal testis. One third of sweat gland tumors (38Jager2007) showed positivity with NY-BR1. Although NY-BR-1 positivity was demonstrated in a case of vulvar phyllodes tumor (39Giger), there is no other normal or tumor tissue which has been reported to express this protein, therefore NY-BR-1 appears to be a breast-specific protein. In invasive breast carcinomas, the range of NY-BR-1 expression has been reported between 46.6% and 70%, showing a strong association with ER and lower-grade carcinomas (38Jager2007, 40Varga, 41Theurillat, 42Seil, 43Woodard, 44Balafoutas, 45Liu).

BCA-225 is a glycoprotein with a molecular weight between 225.000-250.000 kD. It was first identified by Mesa-Tejada and coworkers in 1988 (46Mesa-Tejada). Although it was previously described as a specific immunomarker of breast carcinoma, a later study by Loy and associates concluded that BCA-225 is commonly expressed in human adenocarcinomas of different origins, and is therefore not specific for the breast (47Loy). BCA-225 expression was often present in adenocarcinomas of the breast (98%), kidney (94%), ovary (80%), lung (74%) and intermediate expression rates (36%-68%) were found in adenocarcinomas of the prostate, bile ducts, thyroid, endometrium, endocervix and pancreas (47Loy).

Although the mentioned “breast markers” have been tested in several series of breast carcinomas, only a few cases of TNBC have been assessed for them. TNBC also constitute a heterogeneous group of breast carcinomas, and basal-like carcinomas have not been specifically investigated for the expression of the above markers. The aim of the present study was to look at the IHC staining of GATA-3, MG, GCDFP-15, Ny-BR-1 and BCA-225 in a series of TNBCs showing CK5 expression, and therefore being consistent with a basal-like phenotype on the basis of the surrogate IHC based molecular classification.

Materials and methods

Case selection and tissue microarray construction

Invasive breast carcinomas operated on at the Bács-Kiskun County Teaching Hospital, Kecskemét between August 2005 and August 2015 and fulfilling the criteria of TNBC and CK5 positivity by IHC were selected for tissue microarray (TMA) construction. All of the specimens were fixed in 10% neutral buffered formalin for at least 24 hours. Only cases with more than 3 paraffin blocks available were used; otherwise the cases represent a consecutive series of such tumors, ER, PR and HER-2 IHC results were obtained from the histopathology reports.

The TMAs were constructed from archived paraffin-embedded blocks using a TMA builder device (Histopathology Ltd, Pécs, Hungary). Each TMA contained 20 tumor tissue cores, 2 mm in diameter. These were arranged in 5 rows and 4 columns and an additional row contained 2 non-mammary control tissues for orientation and identification purposes. Each carcinoma was represented in duplicate in 2 different TMAs, and the areas sampled were preferentially from the edge of the tumors. Care was taken to include minor amounts of normal paratumoral breast tissue in each TMA to serve as internal controls for the IHC reactions.

IHC for GATA-3, MG, GCDFP-15, NY-BR-1 and BCA-225 was performed using the antibodies and details listed in Table 1. All antibodies were used on both sets of TMAs, (i.e. two 2-mm-diameter cores of each tumor), except for BCA-225, where only one set of cores (and TMAs) was immunostained. The stains were assessed by the two

authors by evaluating the proportion of nuclear (GATA-3) and cytoplasmic (MG, GCDFP-15 and BCA-225) or both nuclear and cytoplasmic (NY-BR-1) labeling of tumor cells. A staining of 5% or more cells was considered a positive result.

Results

All markers could be evaluated in only 115 of the 118 tumors sampled, therefore the result are reported for these 115 cases. In 3 cases, the tissue cores were not evaluable due to necrosis or lack of tumor cells. The series included 4 recurrent tumors (including 1 with intramammary nodal recurrence only) and 10 cases treated with neoadjuvant systemic therapy with no or minimal (0-10%) regression. The basic characteristics of these tumors are summarized in Table 2.

GATA-3 labelling was characterized by intense nuclear staining in the tumor cells. In a few specimens, weak nuclear staining was noted in a very small minority of lymphocytes, but this could not be confounded with either tumor cell positivity or the staining of normal mammary epithelium. MG, GCDFP-15, NY-BR-1 and BCA-225 positivity was identified as obvious cytoplasmatic staining. Although the data sheet of NY-BR-1 suggests that occasional nuclear staining may occur with this antibody, this was not noted in tumor cells, but was present in a few normal breast epithelial cells. Examples of diffuse and focal IHC staining are presented in Figure 1, to demonstrate the range of positive reactions seen in the tumor samples.

The IHC results are displayed in Table 3 and 4, which show both the proportion of tumors demonstrating any degree of staining with a given marker and the proportion considered positive according to the 5% cut-off in this study. Taking any staining into account, GATA3 and BCA-225 labeling was seen in the majority of the cases, followed by MG and GCDFP-15, and NY-BR-1 immunoreactivity was seen in only a few tumor samples. Using the 5% cut-off, there was a marked drop in the proportion of cases showing GATA3 positivity, but reductions were seen with all markers. Only one third of the cases showed notable (at least 5%) staining with the 4 markers considered to be more specific for a breast origin if a few caveats are kept in mind. Using the frequency of labeling in this series, Figure 2 shows the hierarchical help that each of the markers can give in the assessment of a mammary origin of CK5 positive TNBC. It is clear from the figure as from overall data, that NY-BR-1 is not of great help in this context. BCA-225 which is breast specific only in its name, stained only 25/76 of the tumors negative for all 4 other markers.

Discussion

TNBCs are defined by their negativity for ER, PR and HER2. Despite this defining phenotypic character, they still represent a heterogeneous group of breast carcinomas (48Abramson). Some subsets of TNBCs can be relatively well identified using IHC, for example androgen receptor and diffuse GCDFP-15 positivity can identify apocrine carcinomas (49Vranic), and it has been suggested that tumors expressing CK5 and/or EGFR are those that best match the molecular subtype of basal-like carcinomas (50Nielsen). Basal-like TNBCs are often circumscribed (a feature shared by many metastases), predominantly solid, without much lumen-forming tendency, and they often feature necrosis, squamous metaplasia, all rendering their identification as breast carcinoma more difficult. They are aggressive tumors with a tendency to give distant metastases on the short term. Metastases to the breast are rare, but metastases from TNBCs are relatively common.

Proving the mammary origin of TNBCs may be problematic, as ER, one of the most commonly used, but not specific markers of breast origin is by definition absent in these tumors. In the present study we investigated the expression of 5 markers developed or used to support the mammary origin of cancers according to the descriptions in the data sheets, namely GATA-3, MG, GCDFP-15, NY-BR-1 and BCA-225 by IHC in a series of TNBCs deemed to be of the basal-like type on the basis of their CK5 expression.

Using the 5% cut-off which is readily detectable, BCA-225 showed the highest expression rate with about one third of the cases staining, but this was well below the 98% staining rate found for breast cancers in general. Therefore, despite not being specific for breast cancer, the marker is also not sensitive enough. Owing to these features, it was dropped from further considerations.

Overall, more than 90% of breast cancers are GATA3 positive (45Liu), whereas only about 50%, 20 to 30% and 46-70% show positivity for MG (30Onuma), GCDFP-15 and NY-BR1 (45Liu, 44Balafoutas), respectively. Most of the reported series suggest that positivity for both GATA3 and NY-BR-1 is more common in ER-positive tumors. GATA3 somatic mutations and microarray data have linked GATA3 to the estrogen signaling pathway, and therefore it is not surprising that the expression of this protein is lower in TNBCs, than in ER+ tumors or breast cancers in general, in keeping with previous results (Table 5).

Data on the staining frequency of GATA3, MG, GCDFP-15 and NY-BR-1 in TNBC are limited. Studies reporting on the expression of these markers on a relatively large number of primary or secondary TNBCs are summarized in Table 5. On the basis of these data, the sensitivity of GATA-3, Mammaglobin-A, GCDFP-15 and NY-BR-1 to suggest a mammary origin are 43.5% (95%CI 0.396-0.476), 16.4% (95%CI: 0.136-0.196), 15.1% (95%CI: 0.127-0.179), 5.4% (95%CI: 0.027-0.103), respectively.

Despite the fact that GATA-3 expression has been linked with ER expression, this was the marker to show the highest frequency of staining in CK5 expressing TNBCs. When 5% and more staining was chosen as a cut-off for positivity, the frequency of each marker decreased (Tables 3 and 4). Lower percentages of staining must be interpreted with caution as the specificity of such a low labeling is uncertain.

When our results are compared with those of others (Table 5), the positivity rates for GATA-3, and GCDFP-15 are lower in our TMA based series. A potential weakness of the present study could be the use of TMAs, because MG and GCDFP-15 often stain breast carcinomas in a patchy pattern (32Lewis), and the sensitivity of detecting these markers can be lower in TMAs than on whole slide sections. This could be one possible cause of the discrepancy between our results and those of others listed in Table 5, although two 2-mm-diameter cores are a relatively good representation of a tumor in TMA based studies. Another issue behind the difference in results might stem from the fact, that we studied a distinct subset of TNBCs.

TNBCs are heterogeneous (48Abramson), and some of them, belonging to the luminal androgen receptor positive group, are characterized by forkhead-box A1 (FOXA1) protein overexpression (51Sasahara). FOXA1 and GATA-3 are both involved in the downstream of the ER pathway. Diffuse GCDPF-15 is also a marker of apocrine differentiation in IHC studies (49Vranic, 52KőváriAPMIS). Such tumors were excluded by selecting basal-like matching carcinomas on the basis of their CK5 expression. Such differences in the subgroups analyzed may also contribute to the lower rates of positivity of GATA-3 and GCDFP-15 in this series.

Possible differences may also be attributable to the use of different antibodies/clones. But this is unlikely to be a major source of differences as 4 studies used the same antibody clones as we used for GATA3 (6Huo, 7Deftereos, 53Ordonez, 54Krings).

To our knowledge, this is the largest series of TNBC analyzed for the expression of 4 markers of mammary origin, moreover the series includes exclusively TNBC of a distinct subtype, namely tumors expressing CK5 and therefore most likely to coincide with basal-like breast carcinomas (50Nielsen). If we consider the documented lack of specificity of BCA-225 (47Loy), the remaining 4 breast markers fail to show any staining in about 15% of CK5 expressing TNBCs. A more detectable (at least 5%) expression of any of the 4 markers was seen in around one third of the cases, leaving the remaining two thirds unidentified as of mammary origin. Of the 4 markers, NY-BR-1 is rarely expressed in the tumor subset studied, and therefore its use adds practically nothing to the use of the other three.

The expression of GATA3, MG, GCDFP-15 and NY-BR-1 is lower in TNBCs than in breast carcinomas in general. Although these markers may be positive in different other tumors, by using them, a subset of basal-like TNBC-s can be identified as of mammary origin. Though the positive staining supports a breast origin, negativity for all markers does not exclude this. Therefore we suggest using GATA-3, MG and

GCDFP-15 as an IHC panel to establish breast origin when ER and PR are negative. Obviously, at the primary site, histological features such as the presence of in situ carcinoma of similar grade may also suggest the primary nature of the tumor, but this help is missing in the metastatic setting. One should be prepared to find a relatively high number of basal-like TNBCs to be negative for all the studied breast markers.

References

1. DeSantis C, Ma J, Bryan L, Jemal A. Breast cancer statistics, 2013. CA Cancer J Clin 64:52-62.

2. Malvezzi M, Carioli G, Bertuccio P*, et al.* European cancer mortality predictions for the year 2016 with focus on leukemias. Ann Oncol.

3. Perou CM, Sorlie T, Eisen MB*, et al.* Molecular portraits of human breast tumours. Nature 2000;406:747-52.

4. Nielsen TO, Perou CM. CCR 20th Anniversary Commentary: The Development of Breast Cancer Molecular Subtyping. Clin Cancer Res 21:1779-81.

5. Hernandez BY, Green MD, Cassel KD*, et al.* Preview of Hawaii Cancer Facts and Figures 2010. Hawaii Med J 69:223-4.

6. Huo L, Gong Y, Guo M*, et al.* GATA-binding protein 3 enhances the utility of gross cystic disease fluid protein-15 and mammaglobin A in triple-negative breast cancer by immunohistochemistry. Histopathology 67:245-54.

7. Deftereos G, Sanguino Ramirez AM, Silverman JF*, et al.* GATA3 immunohistochemistry expression in histologic subtypes of primary breast carcinoma and metastatic breast carcinoma cytology. Am J Surg Pathol 39:1282-9.

8. Chou J, Provot S, Werb Z. GATA3 in development and cancer differentiation: cells GATA have it! J Cell Physiol 222:42-9.

9. Esheba GE, Longacre TA, Atkins KA*, et al.* Expression of the urothelial differentiation markers GATA3 and placental S100 (S100P) in female genital tract transitional cell proliferations. Am J Surg Pathol 2009;33:347-53.

10. Liu H, Shi J, Wilkerson ML*, et al.* Immunohistochemical evaluation of GATA3 expression in tumors and normal tissues: a useful immunomarker for breast and urothelial carcinomas. Am J Clin Pathol 138:57-64.

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

12. Miettinen M, McCue PA, Sarlomo-Rikala M*, 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 38:13-22.

13. Voduc D, Cheang M, Nielsen T. GATA-3 expression in breast cancer has a strong association with estrogen receptor but lacks independent prognostic value. Cancer Epidemiol Biomarkers Prev 2008;17:365-73.

14. Hoch RV, Thompson DA, Baker RJ*, et al.* GATA-3 is expressed in association with estrogen receptor in breast cancer. Int J Cancer 1999;84:122-8.

15. Tominaga N, Naoi Y, Shimazu K*, et al.* Clinicopathological analysis of GATA3-positive breast cancers with special reference to response to neoadjuvant chemotherapy. Ann Oncol 23:3051-7.

16. Albergaria A, Paredes J, Sousa B*, et al.* Expression of FOXA1 and GATA-3 in breast cancer: the prognostic significance in hormone receptor-negative tumours. Breast Cancer Res 2009;11:R40.

17. Parikh P, Palazzo JP, Rose LJ*, et al.* GATA-3 expression as a predictor of hormone response in breast cancer. J Am Coll Surg 2005;200:705-10.

18. Mehra R, Varambally S, Ding L*, et al.* Identification of GATA3 as a breast cancer prognostic marker by global gene expression meta-analysis. Cancer Res 2005;65:11259-64.

19. Cimino-Mathews A, Subhawong AP, Illei PB*, et al.* GATA3 expression in breast carcinoma: utility in triple-negative, sarcomatoid, and metastatic carcinomas. Hum Pathol 44:1341-9.

20. Ciocca V, Daskalakis C, Ciocca RM*, et al.* The significance of GATA3 expression in breast cancer: a 10-year follow-up study. Hum Pathol 2009;40:489-95.

21. Jacquemier J, Charafe-Jauffret E, Monville F*, et al.* Association of GATA3, P53, Ki67 status and vascular peritumoral invasion are strongly prognostic in luminal breast cancer. Breast Cancer Res 2009;11:R23.

22. Demir H, Turna H, Can G*, et al.* Clinicopathologic and prognostic evaluation of invasive breast carcinoma molecular subtypes and GATA3 expression. J Buon 15:774-82.

23. Watson MA, Fleming TP. Mammaglobin, a mammary-specific member of the uteroglobin gene family, is overexpressed in human breast cancer. Cancer Res 1996;56:860-5.

24. Watson MA, Darrow C, Zimonjic DB*, et al.* Structure and transcriptional regulation of the human mammaglobin gene, a breast cancer associated member of the uteroglobin gene family localized to chromosome 11q13. Oncogene 1998;16:817-24.

25. Han JH, Kang Y, Shin HC*, et al.* Mammaglobin expression in lymph nodes is an important marker of metastatic breast carcinoma. Arch Pathol Lab Med 2003;127:1330-4.

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

27. Zafrakas M, Petschke B, Donner A*, et al.* Expression analysis of mammaglobin A (SCGB2A2) and lipophilin B (SCGB1D2) in more than 300 human tumors and matching normal tissues reveals their co-expression in gynecologic malignancies. BMC Cancer 2006;6:88.

28. Sasaki E, Tsunoda N, Hatanaka Y*, et al.* 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.

29. Wang Z, Spaulding B, Sienko A*, et al.* Mammaglobin, a valuable diagnostic marker for metastatic breast carcinoma. Int J Clin Exp Pathol 2009;2:384-9.

30. Onuma K, Dabbs DJ, Bhargava R. Mammaglobin expression in the female genital tract: immunohistochemical analysis in benign and neoplastic endocervix and endometrium. Int J Gynecol Pathol 2008;27:418-25.

31. Al-Joudi FS, Kaid FA, Ishak I*, et al.* Expression of human mammaglobin and clinicopathologic correlations in breast cancer: the findings in Malaysia. Indian J Pathol Microbiol 54:284-9.

32. Lewis GH, Subhawong AP, Nassar H*, et al.* Relationship between molecular subtype of invasive breast carcinoma and expression of gross cystic disease fluid protein 15 and mammaglobin. Am J Clin Pathol 135:587-91.

33. Fritzsche FR, Thomas A, Winzer KJ*, et al.* Co-expression and prognostic value of gross cystic disease fluid protein 15 and mammaglobin in primary breast cancer. Histol Histopathol 2007;22:1221-30.

34. Haagensen DE, Jr., Mazoujian G, Holder WD*, et al.* Evaluation of a breast cyst fluid protein detectable in the plasma of breast carcinoma patients. Ann Surg 1977;185:279-85.

35. Wick MR, Lillemoe TJ, Copland GT*, et al.* 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.

36. Park SY, Kim BH, Kim JH*, et al.* Panels of immunohistochemical markers help determine primary sites of metastatic adenocarcinoma. Arch Pathol Lab Med 2007;131:1561-7.

37. Jager D, Stockert E, Gure AO*, et al.* Identification of a tissue-specific putative transcription factor in breast tissue by serological screening of a breast cancer library. Cancer Res 2001;61:2055-61.

38. Jager D, Filonenko V, Gout I*, et al.* NY-BR-1 is a differentiation antigen of the mammary gland. Appl Immunohistochem Mol Morphol 2007;15:77-83.

39. Giger OT, Lacoste E, Honegger C*, et al.* Expression of the breast differentiation antigen NY-BR-1 in a phyllodes tumor of the vulva. Virchows Arch 2007;450:471-4.

40. Varga Z, Theurillat JP, Filonenko V*, et al.* Preferential nuclear and cytoplasmic NY-BR-1 protein expression in primary breast cancer and lymph node metastases. Clin Cancer Res 2006;12:2745-51.

41. Theurillat JP, Zurrer-Hardi U, Varga Z*, et al.* NY-BR-1 protein expression in breast carcinoma: a mammary gland differentiation antigen as target for cancer immunotherapy. Cancer Immunol Immunother 2007;56:1723-31.

42. Seil I, Frei C, Sultmann H*, et al.* The differentiation antigen NY-BR-1 is a potential target for antibody-based therapies in breast cancer. Int J Cancer 2007;120:2635-42.

43. Woodard AH, Yu J, Dabbs DJ*, et al.* NY-BR-1 and PAX8 immunoreactivity in breast, gynecologic tract, and other CK7+ carcinomas: potential use for determining site of origin. Am J Clin Pathol 136:428-35.

44. Balafoutas D, zur Hausen A, Mayer S*, et al.* Cancer testis antigens and NY-BR-1 expression in primary breast cancer: prognostic and therapeutic implications. BMC Cancer 13:271.

45. Liu H. Application of immunohistochemistry in breast pathology: a review and update. Arch Pathol Lab Med 138:1629-42.

46. Mesa-Tejada R, Palakodety RB, Leon JA*, et al.*. Immunocytochemical distribution of a breast carcinoma associated glycoprotein identified by monoclonal antibodies. Am J Pathol 1988;130:305-14.

47. Loy TS, Chapman RK, Diaz-Arias AA*, et al.* Distribution of BCA-225 in adenocarcinomas. An immunohistochemical study of 446 cases. Am J Clin Pathol 1991;96:326-9.

48. Abramson VG, Lehmann BD, Ballinger TJ*, et al.* Subtyping of triple-negative breast cancer: implications for therapy. Cancer 121:8-16.

49. Vranic S, Schmitt F, Sapino A*, et al.* Apocrine carcinoma of the breast: a comprehensive review. Histol Histopathol 2013; 28:1393-1409.

50. Nielsen TO, Hsu FD, Jensen K*, et al.* Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 2004;10:5367-74.

51. Sasahara M, Matsui A, Ichimura Y*, et al.* Overexpression of androgen receptor and forkhead-box A1 protein in apocrine breast carcinoma. Anticancer Res 34:1261-7.

52. Kővári B, Rusz O, Schally AV*, et al.* Differential immunostaining of various types of breast carcinomas for growth hormone-releasing hormone (GHRH) receptor - Apocrine epithelium and carcinomas emerging as uniformly positive. APMIS 2014; 122:824-831.

53. Ordonez NG, Sahin AA. Diagnostic utility of immunohistochemistry in distinguishing between epithelioid pleural mesotheliomas and breast carcinomas: a comparative study. Hum Pathol 45:1529-40.

54. Krings G, Nystrom M, Mehdi I*, et al.* Diagnostic utility and sensitivities of GATA3 antibodies in triple-negative breast cancer. Hum Pathol 45:2225-32.

55. David R. Braxton, Cynthia Cohen, Momin T. Siddiqui. Utility of GATA3 Immunohistochemistry for Diagnosis of Metastatic Breast Carcinoma in Cytology Specimens. Diagn. Cytopathol. 2015;43:271–277.

56. Madelyn Lew, Judy C. Pang, Xin Jing. Young Investigator Challenge: The Utility of GATA3 Immunohistochemistry in the Evaluation of Metastatic Breast Carcinomas in Malignant Effusions Cancer (Cancer Cytopathol)2015;123:576-81.

57. Nasser Rakhshani, Arash Daryakar. Are Mammaglobin and GCDFP-15 Sensitive Markers for Diagnosis of Metastatic basal-like Triple Negative breast Carcinomas? Cilt/Vol. 30, No. 1, 2014; Sayfa/Page18-22.

58. Darb-Esfahani S, von Minckwitz G, Denkert C*, et al.* Gross cystic disease fluid protein 15 (GCDFP-15) expression in breast cancer subtypes. BMC Cancer. 2014 Jul 28;14:546. doi: 10.1186/1471-2407-14-546.

59. Pala EE, Bayol Ü, Cumurcu S*, et al.* Immunohistochemical characteristics of triple negative/basal-like breast cancer. Turk Patoloji Derg. 2012;28(3):238-44. doi: 10.5146/tjpath.2012.01130.

60. Clark BZ, Beriwal S, Dabbs DJ*, et al.* Semiquantitative GATA-3 immunoreactivity in breast, bladder, gynecologic tract, and other cytokeratin 7-positive carcinomas. Am J Clin Pathol. 2014 Jul;142(1):64-71.

61. Nika C. Gloyeske, Anna H. Woodard, Esther Elishaev. Immunohistochemical Profile of Breast Cancer With Respect to Estrogen Receptor and HER2 Status, Appl Immunohistochem Mol Morphol2015;23:202–208.

Figure legends:

Figure 1.

Examples of noticeable (at least 5%) GATA-3, Mammaglobin-A, GCDFP-15, NY-BR-1 and BCA-225 staining on the left (A, C, E, G, I, respectively) as opposed to focal and weak staining with these markers (B, D, F, H, J, respectively) on the right. Arrows in B indicate the few weakly stained nuclei.

Figure 2. Hierarchical labeling of the tumors with 4 “breast specific” markers.

Table 1 Details of the antibody used for IHC

Antibody	Source	Clone / Catalog number	Dilution
GATA3	Santa Cruz, Dallas, TX	HG3-31 / sc-268	1:50
MG	Biocare, Concord, CA	1A5 / PM 269 AA, H	RTU
GCDFP-15	Cell Marque, Rocklin, CA	23A3 / CMC791	1:200
NY-BR-1	Thermo-Fisher, Rockford, IL	NY-BR1#2 / MS-1932-P0	1:300
BCA-225	Biogenex, Fremont, CA	CU18 / AM135-5M	RTU

RTU: ready to use

Table 2

Basic pathologic features of the tumors selected for TMA

Histological type	**n (%)**
No special type (ductal)	**106 (92.3%)**
Medullary-like	**7 (6.1%)**
Metaplastic (“matrix producing”) carcinoma	**1 (0.8%)**
Mixed micropapillary carcinoma	**1 (0.8%)**
Histological grade	
Grade III	**112 (97.3%)**
Grade II	**3 (2.7%)**
(y)(r)pT category of the tumors	
Tx	**1 (0.8%)**
T0*	**1 (0.8%)**
T1b or c	**57 (49.6%)**
T2	**47 (40.9%)**
T3	**3 (2.7%)**
T4	**6 (5.2%)**
Nodal status (y)(r)pN category of the tumors	
Nx	**3 (2.7%)**
N0**	**66 (57.4%)**
N1***	**33 (28.7%)**
N2	**9 (7.8%)**
N3	**4 (3.4%)**

(y)(r)pT and (y)(r)pN categories refer to the TNM classification based pT and pN categories of primary tumors (n= 101) together with those of recurrent tumors (r, n=4) and tumors after primary (i.e. neoadjuvant) systemic treatment (y, n=10). * One case with intramammary nodal recurrence; ** including 4 patients with isolated tumor cells; *** including 10 cases with micrometastasis.

Table 3. Breast marker expressions in the tumors investigated

Marker	Any positive staining (%; 95%CI) n=115	>5% positive staining (%) n=115
GATA3	82 (71.3; 0.624-0.787)	23 (20; 0.137-0.282)
MG	30 (26.0; 0.189-0.348)	12 (10.4; 0.060-0.173)
GCDFP-15	23 (20.0; 0.137-0.282)	9 (7.8; 0.041-0.142)
NY-BR-1	7 (6.0; 0.029-0.120)	3 (2.6, 0.008-0.073)
BCA-225	74 (64.3; 0.552-0.725)	40 (34.7; 0.266-0.438)
GATA3 and MG	21 (18.2; 0.122-0.263)	2 (1.7, 0.004-0.061)
MG and GCDFP-15	12 (10.4; 0.060-0.173)	2 (1.7, 0.004-0.061)
GATA-3, MG and GCDFP-15	9 (7.8; 0.041-0.142)	1 (0.87; 0.001-0.047)
NY-BR-1 and GATA-3	3 (2.6, 0.008-0.073)	0 (0; 0.000-0.031)
Ny-BR-1 and GCDFP-15	2 (1.7, 0.004-0.061)	2 (1.7, 0.004-0.061)
Any markers (without BCA-225)	97 (84.3; 0.766-0.898)	40 (34.7; 0.266-0.438)

CI: confidence interval

Table 4. Mutual breast marker expressions in the tumors investigated

Any staining	GATA3+	GATA3-	MG+	MG-	GCDFP-15+	GCDFP-15-	NY-BR-1+	NY-BR-1-
GATA3+								
GATA3-	na							
MG+	21	8						
MG-	61	25	na					
GCDFP-15+	16	6	11	11				
GCDFP-15-	66	27	18	75	na			
NY-BR-1+	2	3	0	5	1	4		
NY-BR-1-	80	30	29	81	21	89	na	
BCA225+	57	16	23	50	16	57	3	70
BCA225-	25	17	6	36	6	36	2	40

5% cut-off	GATA3+	GATA3-	MG+	MG-	GCDFP-15+	GCDFP-15-	NY-BR-1+	NY-BR-1-
GATA3+								
GATA3-	na							
MG+	2	10						
MG-	21	82	na					
GCDFP-15+	2	6	2	6				
GCDFP-15-	21	86	10	97	na			
NY-BR-1+	0	1	0	1	0	1		
NY-BR-1-	23	91	12	102	8	106	na	
BCA225+	8	31	4	35	4	35	0	39
BCA225-	15	61	8	68	4	72	1	75

Table 5. Summary of results from other series exploring the labeling of TNBCs

Author (year)	cut off	Tumor type	GATA-3	MG	GCDFP-15	NY-BR-1
Ordonez et al. (11)	any staining	metastatic TNBC	12/40 (30%)	7/40 (17.5%)	6/40 (15%)	--
Krings et al. (54)	any staining	primary TNBC	72/109 (66%)	28/107 (26.1%)	17/109 (15.5%)	--
						--
Braxton et al. (55)	>10%	metastatic TNBC	30/35 (85.7%)	9/35 (25.7%)	5/35 (14.2%)	--
Cimino-Matthews et al. (19)	>5%	primary TNBC	19/44 (43.1%)	--	--	--
		metastatic TNBC	5/9 (55.5%)	--	--	--
Lew et al. (56)	any staining	metastatic TNBC	11/13 (84.6%)	4/13 (30.7%)	1/13 (7.6%)	--
Huo et al. (6)	any staining	primary TNBC	25/62 (40.3%)	16/62 (25.8%)	9/62 (14.5%)	--
	>5%		14/62 (22.58%)	7/62 (11.2%)	4/62 (6.4%)	
	any staining	metastatic TNBC	30/68 (44.1%)	22/68 (32.3%)	11/68 (16.1%)	--
	>5%		18/68 (26.4%)	10/68 (14.7%)	6/68 (8.8%)	
Lewis et al. (32)	any staining	primary basal-like TNBC	--	5/24 (20.8%)	1/5 (20%)	--
Rakhshani et al. (57)	>10%	primary basal-like TNBC	--	6/66 (9%)	12/66 (18.1%)	--
Darb-Esfahani et al. (58)	any staining	primary TNBC	--	--	34/130 (26.1%)	--
Pala et al. (59)	>5%	primary TNBC	--	--	8/41 (19.5%)	--
Deftereos et al. (7)	H-score 99.4	primary TNBC	7/28 (25%)	2/28 (7.1%)	5/28 (17.8%)	--
Clark et al. (60)	at least 4%	primary TNBC	22/30 (73.3%)	--	--	--
Gloyeske et al. (61)	any staining				5/32 (16%)	
	>10 H-score	primary TNBC	22/30 (73%)	6/31 (19%)		5/30 (17%)
Our work	any staining	primary (or recurrent) TNBC	82/115 (71.3%)	30/115 (26.0%)	23/115 (20.0%)	7/115 (6.0%)
	>5%		23/115 (20.0%)	12/115 (10.4%)	9/115 (7.8%)	3/115 (2.6%)
Sensitivity; 95% CI*			**43.7%; 0.397-0.477**	**16.3%; 0.135-0.195**	**15,1%; 0,127-0,179**	**5.5%; 0.028-0.105**

* For calculating sensitivity values, all publications with data were considered, and whenever there were data with two staining cut-offs, the >5% data were included only.

Fig 1

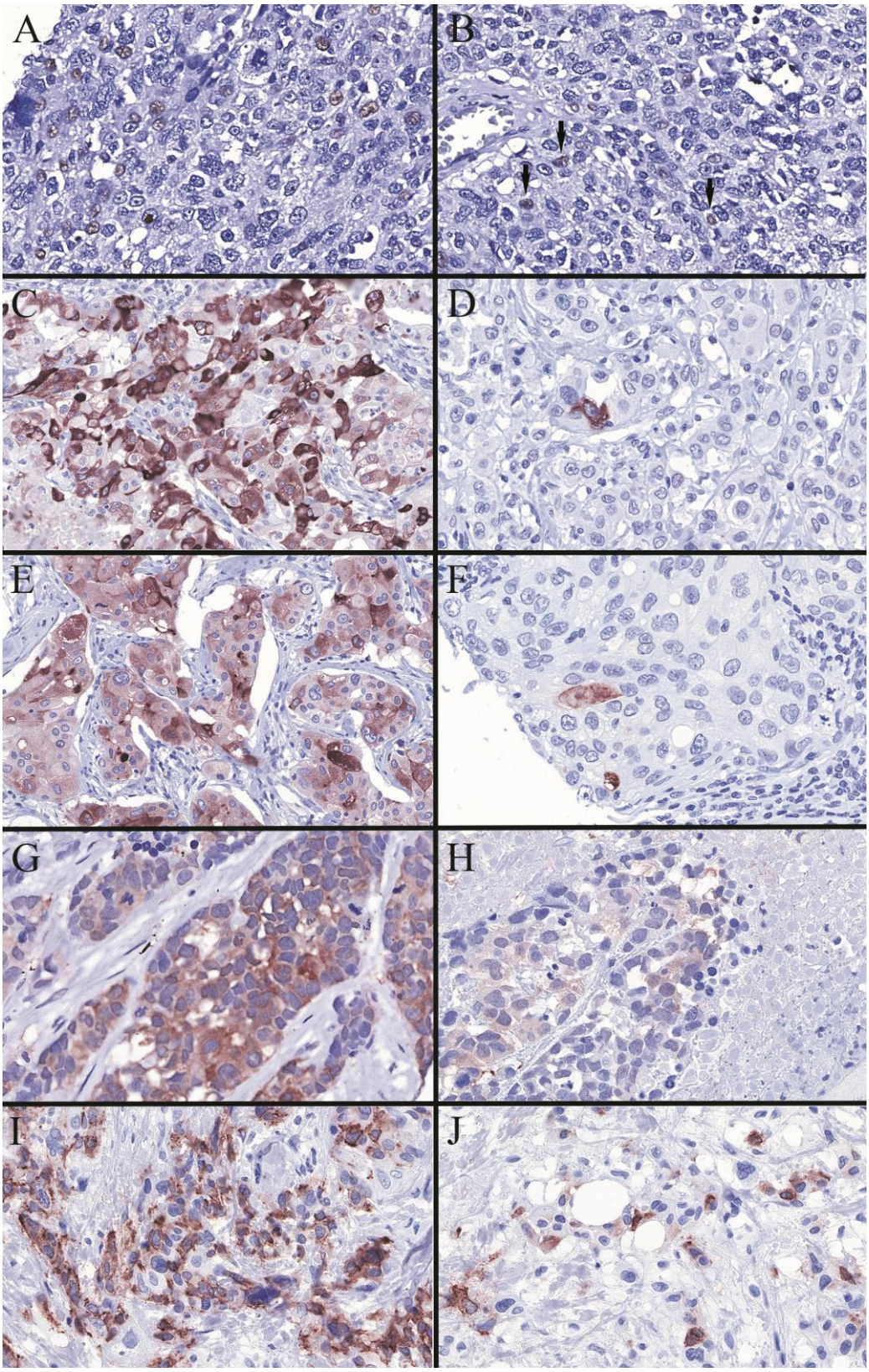
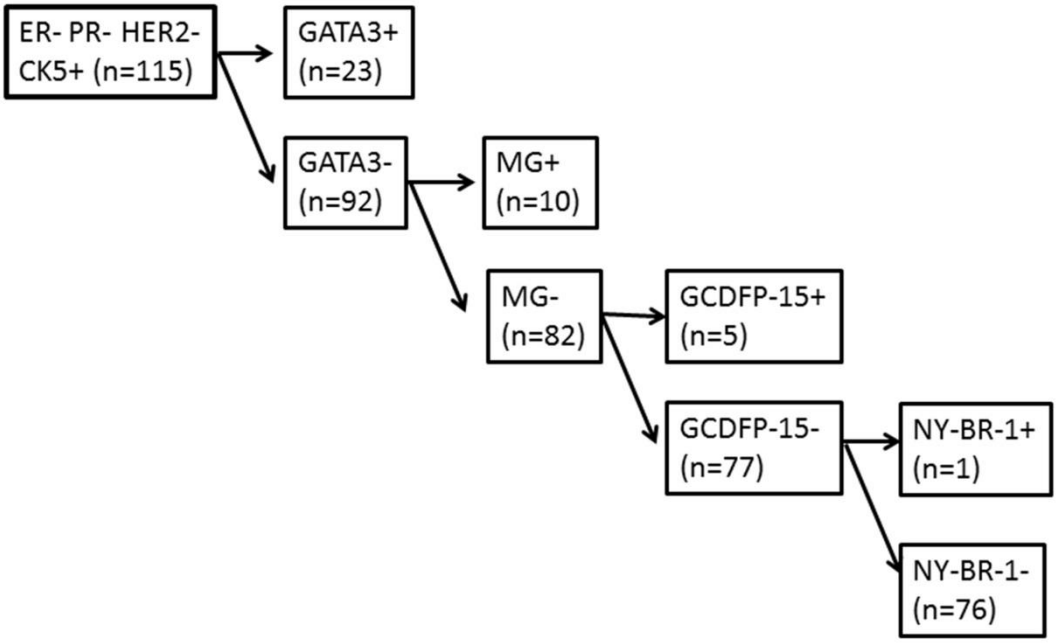


Fig 2.


ER- PR- HER2- CK5+ (n=115)
GATA3+ (n=23)
GATA3- (n=92)
MG+ (n=10)
MG- (n=82)
GCDFP-15+ (n=5)
GCDFP-15- (n=77)
NY-BR-1+ (n=1)
NY-BR-1- (n=76)