

Paired Immunohistochemical Evaluation of TRPS1 and GATA3 in Triple-Negative Breast Carcinoma: A Multi-Institutional Series from Iraq

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ABSTRACT

Background: TRPS1 is an emerging nuclear marker of breast lineage that may retain expression in triple-negative breast carcinoma (TNBC), in which GATA3 expression can be absent. This study compared TRPS1 and GATA3 expression within the same TNBC specimens and examined their associations with clinicopathologic characteristics. **Methods:** This retrospective cross-sectional study included 30 formalin-fixed, paraffin-embedded TNBC specimens selected from 40 cases screened at three pathology units in Karbala, Iraq, between January 2023 and December 2024. Triple-negative status was obtained from the original diagnostic pathology records and was required for eligibility. Whole-tissue sections were immunostained for TRPS1 (clone EP392) and GATA3 (clone L50-823). Nuclear staining extent and intensity were multiplied to generate an immunoreactivity score, with a score ≥ 2 considered positive. Wilson 95% confidence intervals (CIs), the two-sided exact McNemar test, Fisher's exact test, and the Benjamini–Hochberg correction were applied. **Results:** TRPS1 was positive in 28 of 30 tumors (93.3%; 95% CI, 78.7%–98.2%), whereas GATA3 was positive in 22 of 30 tumors (73.3%; 95% CI, 55.6%–85.8%). Twenty-one tumors were positive for both markers, seven were TRPS1-positive/GATA3-negative, one was TRPS1-negative/GATA3-positive, and one was negative for both markers. The observed positivity rate was 20.0 percentage points higher for TRPS1 than for GATA3; however, the exact paired comparison did not reach the conventional threshold for statistical significance ($P = 0.070$). At least one marker was positive in 29 of 30 tumors (96.7%; 95% CI, 83.3%–99.4%). High expression was observed in 22 tumors for TRPS1 and 14 tumors for GATA3. No association with clinicopathologic characteristics remained statistically significant after adjustment for multiple comparisons. **Conclusions:** TRPS1 showed a higher observed positivity rate than GATA3 and identified seven of the eight GATA3-negative TNBCs. The single GATA3-positive/TRPS1-negative case suggests that these markers may be complementary rather than interchangeable. In diagnostically challenging TNBC cases, TRPS1 may strengthen a breast-lineage immunohistochemical panel when interpreted alongside morphology, clinical context, and site-directed markers.

Keywords: TRPS1; GATA3; Triple-negative breast carcinoma; Immunohistochemistry; Breast lineage; diagnostic pathology.

Article Information

Received: July 09, 2026; Revised: August 14, 2026; Online: September 2026y

INTRODUCTION

Breast cancer continues to be the most common malignancy in females globally and one of the leading causes of cancer-related mortality (1). Current classification is based on morphology, histologic grade, stage, and biomarkers, with estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) driving both nomenclature and management (2, 3, 4). Triple-negative breast carcinoma (TNBC), characterized by the lack of clinically actionable ER, PR, and HER2 expression or amplification, is a heterogeneous group rather than a unique entity (5, 6). Its heterogeneity is seen in molecular subtypes, immune profiles, morphology, treatment response, and clinical behavior.

Demonstration of breast origin is challenging in poorly differentiated TNBC, small core-needle biopsies, or distant lesions without the presence of an in situ component. Traditional breast markers such as mammaglobin and gross cystic disease fluid protein 15 are focal or absent in high-grade and triple-negative lesions. GATA binding protein 3 (GATA3), a nuclear transcription factor of luminal breast differentiation, is widely used due to technical reliability and frequent positivity in breast carcinoma. However, it has limited sensitivity in certain subsets of TNBC and metaplastic carcinoma and has been detected in urothelial, cutaneous, salivary, and other tumors (7, 8). Therefore, there has been a trend towards broad testing of breast lineage panels rather than reliance on a single marker.

Trichorhinophalangeal syndrome type 1 protein (TRPS1) is an atypical GATA family transcriptional regulator and a part of the pathway responsible for mammary epithelial differentiation. Large initial studies showed strong nuclear expression of TRPS1 in breast carcinoma, including many cases of TNBC negative for GATA3 (9, 10). Later studies of metaplastic carcinoma, metastatic samples,

cytology, and various histological subtypes confirmed high positivity of TNBCs, although the rate of positivity varied depending on clone, platform, specimen type, and positivity threshold (11, 12). Thus, the marker addresses a diagnostic challenge, especially when traditional breast markers are negative.

A high sensitivity does not mean that TRPS1 is a breast-specific marker. Broader studies have found TRPS1 expression in subsets of gynecologic, prostatic, urothelial, cutaneous, salivary, and mesenchymal tumors (13, 14, 15, 16, 17). Also, apocrine breast carcinomas may show decreased TRPS1 expression while remaining GATA3 positive. Additionally, series based on effusion samples have not always revealed the same hierarchy of TRPS1 and GATA3 expression found in resection specimens (14, 16, 18, 19). Thus, TRPS1 should be interpreted in the context of tumor morphology, the differential diagnosis, and an appropriate panel including GATA3, SOX10, keratins, and organ-specific markers (14, 18, 20).

When the markers are studied in the same tumors, the main statistical question becomes paired: how often does one marker detect a case that is negative for the other marker? Comparison of two marginal frequencies with an unpaired chi-square test does not take into account within-case correlation and does not analyze discordant cases. Reporting all four paired frequencies and using an exact McNemar test provides a clinically understandable analysis of incremental detection. This approach is particularly appropriate in small series, when the asymptotic assumption is not valid and the number of discordant tumors defines the accuracy of the study.

The current study examined the application of TRPS1 and GATA3 to paired whole sections of 30 TNBCs obtained from three pathology laboratories in Karbala, Iraq. The main goal was to compare case-level

positivity of the markers and quantify discordance. We also aimed to provide study-specific categories of expression and analyze the association with selected clinicopathologic characteristics. The current cohort consisted of known TNBCs; no non-breast mimics or outcome data were

MATERIALS AND METHODS

Study design and case selection

This study is a retrospective cross-sectional analysis of archival tissue collected from January 2023 to December 2024 at the Histopathology Laboratory of Al-Hussein Medical City and the Pathology Department of Al-Warith and Al-Kafeel hospitals in Karbala, Iraq. A total of 40 cases with invasive breast carcinoma were identified. The exclusion criteria excluded 10 cases that either did not satisfy the predefined eligibility criteria or had low-quality pathologic samples. Finally, the sample consisted of 30 cases, each represented by one formalin-fixed, paraffin-embedded block. Cases satisfying the predefined criteria had a previous diagnosis of invasive breast carcinoma based on H&E staining and were clinically diagnosed as triple-negative via routine immunohistochemistry tests. Information on triple negativity was retrieved from the original pathology reports and served as inclusion criteria. Data collected included patient age, sample type/site, histologic type, lymphovascular invasion, Nottingham grade, tumor category, nodal category, distant metastasis, and stage. Oncology records of Al-Hussein Medical City and Al-Warith Hospital provided data on stage. Missing data were kept as such without being assigned to any favorable category. Sampled tissue included 12 cases of mastectomy, 8 cases of wide local excision, 8 cases of breast core biopsies, 1 case of liver core biopsy, and 1 case of brain metastasis excision. Of these cases, 28 samples corresponded to primary tumors and 2 to metastatic sites. Photomicrographs taken

included; thus, the study design was focused on within-cohort detection and complementarity.

from one block were not considered as independent observations.

Histopathologic review and immunohistochemistry

Four-micrometer sections were made from each block, and three of them were stained for pathological evaluation with H&E and used for IHC. The deparaffinization and rehydration process started after incubation at 60°C, while antigen retrieval was performed using heat with citrate buffer (pH 6.0) at 95°C for 30 minutes. Prior to incubation with the primary antibodies, endogenous peroxidase activity was blocked.

Prediluted TRPS1 rabbit monoclonal antibody (clone EP392; PathnSitu, USA) and GATA3 mouse monoclonal antibody (clone L50-823; PathnSitu, USA) were used separately for 60 minutes. For detection, PolyExcel horseradish peroxidase/3,3'-diaminobenzidine was used, followed by Mayer hematoxylin counterstaining, dehydration, and mounting. In each staining process, normal breast tissue was used as a positive control, and omission of the primary antibody was used as a negative control. Nuclear staining was recorded only in invasive carcinoma cells. Representative fields were captured using an Olympus DP72 microscope camera. In each staining process, normal breast tissue was used as a positive control, and omission of the primary antibody was used as a negative control (Figure 1A,B).

Immunoreactivity scoring

Each marker was scored separately with respect to nuclear staining according to the percentage of stained tumor nuclei: 0 = <1%, 1 = 1% to 10%, 2 = 11% to 50%, or 3 = 51% to 100%. Intensity of staining was scored as absent = 0, weak = 1, moderate = 2, or strong

= 3. A product score of 0, 1, 2, 3, 4, 6, or 9 was calculated for each of the two markers. The samples were scored as follows: 0–1 = negative, 2 = low positive, 3–4 = intermediate positive, or 6 or 9 = high positive. Any score ≥ 2 was defined as positive for the primary binary analysis.

Statistical analysis

Continuous variables were described by the mean, standard deviation, and range, while categorical variables were described as number of cases and percentage with variable-specific denominators. Wilson score 95% confidence intervals were computed for the marker positivity in each case and positivity of both markers combined. The primary matched-pair analysis utilized a two-sided exact McNemar test with discordant pairs as its basis. The matched-pair odds ratio was calculated as an effect measure, together with an exact confidence interval calculated using the conditional binomial distribution.

The associations of binary marker positivity with age (below 50 vs above 50), lymphovascular invasion, Nottingham grade, grouped T classification (T1-2 vs T3-4), grouped N classification (N0-1 vs N2-3), distant metastasis, and grouped TNM stage (I-II vs III-IV) were assessed using two-sided

Fisher's exact tests. To minimize erroneous inferences in interpreting the 14 marker-feature associations, the Benjamini-Hochberg adjusted q-values were computed. This study was purely exploratory, and therefore no multivariable model was fitted as there were only two tumors that were TRPS1-negative. The original data were analyzed using SPSS version 24.0, while the exact tests, confidence intervals, and multiplicity adjustments were independently calculated using Python 3.12 and SciPy 1.17.0. A two-sided nominal P-value of less than 0.05 was used.

RESULTS

Clinicopathologic characteristics

The mean age of the 30 subjects was 52.33 (SD: 13.12; range: 27–76 years), of whom 17 (56.7%) were ≥ 50 years of age. In total, 29 lesions were invasive breast carcinomas of no special type, while one lesion was a metaplastic carcinoma. Lymphovascular invasion was detected in 15 out of 30 tumors. Grade II and III lesions were noted in 13 and 17 tumors, respectively. T stage was available in 29 subjects, while N stage was available in 27 subjects **Table 1**.

Table 1: Clinicopathologic characteristics of the study cohort.

Characteristics	Category	Value
Age, years	Mean (SD)	52.33 (13.12)
	Range	27-76
Age group, years	<50	13 (43.3)
	≥ 50	17 (56.7)
Specimen	Mastectomy	12 (40.0)
	Wide local excision	8 (26.7)
	Breast core biopsy	8 (26.7)
	Liver core biopsy	1 (3.3)
	Brain metastasis excision	1 (3.3)
Histologic type	Invasive carcinoma NST	29 (96.7)
	Metaplastic carcinoma	1 (3.3)
Lymphovascular invasion	Present	15 (50.0)
	Absent	15 (50.0)
Nottingham grade	II	13 (43.3)
	III	17 (56.7)

Characteristics	Category	Value
T category (n=29)	T1	6 (20.7)
	T2	14 (48.3)
	T3	7 (24.1)
	T4	2 (6.9)
N category (n=27)	N0	12 (44.4)
	N1	7 (25.9)
	N2	5 (18.5)
	N3	3 (11.1)
M category (n=27)	M0	21 (77.8)
	M1	6 (22.2)
Stage (n=27)	I	2 (7.4)
	II	12 (44.4)
	III	7 (25.9)
	IV	6 (22.2)
Data are presented as n (%) unless otherwise indicated. Percentages were calculated using the denominator specified for each characteristic. SD, standard deviation; NST, no special type.		

Paired TRPS1 and GATA3 expression

TRPS1 was positive in 28/30 tumors (93.3%; 95% confidence interval [CI], 78.7% to 98.2%), and GATA3 was positive in 22/30 tumors (73.3%; 95% CI, 55.6% to 85.8%). Twenty-one tumors had expression of both markers, and one tumor had no expression of either marker. Of the eight tumors that were negative for GATA3, seven were positive for TRPS1. Of the two tumors that were negative for TRPS1, one was positive for GATA3, the reciprocal TRPS1-negative/GATA3-positive staining pattern is illustrated in Figure 4A–C. Therefore, at least one of the markers was

positive in 29/30 tumors (96.7%; 95% CI, 83.3% to 99.4%).

There was an absolute difference of 20.0 percentage points in favor of TRPS1. The exact McNemar test based on 7 TRPS1-positive/GATA3-negative tumors and 1 TRPS1-negative/GATA3-positive tumor yielded $P = 0.0703$. The odds ratio of discordant pairs was 7.0 (wide exact 95% CI, 0.90 to 315.48), **Table 2**. Representative TRPS1-positive/GATA3-negative primary tumors are shown in Figures 1C–F and 2A–E, while a metastatic TNBC involving the brain with the same immunophenotype is shown in Figure 3A–D.

Table 2: Paired case-level positivity of TRPS1 and GATA3 (N=30)

TRPS1 status	GATA3 positive, n	GATA3 negative, n	Total, n (%)
Positive	21	7	28 (93.3)
Negative	1	1	2 (6.7)
Total	22 (73.3)	8 (26.7)	30 (100.0)
Exact two-sided McNemar test: $P = 0.070$; matched-pairs odds ratio = 7.00 (exact 95% CI, 0.90–315.48). Wilson 95% CIs for positivity were 78.7%–98.2% for TRPS1 (93.3%), 55.6%–85.8% for GATA3 (73.3%), and 83.3%–99.4% for either marker (96.7%).			

Immunoreactivity categories

High immunoreactivity scores (6 or 9) were recorded in 22/30 TRPS1-stained tumors and 14/30 GATA3-stained tumors. Intermediate expression was recorded in 1 and 5 tumors, respectively; low expression in

5 and 3; and negative staining in 2 and 8, **Table 3**. These are marginal distributions for each stain. A patient-level joint ordinal matrix was not available; therefore, no paired inferential test of the four-level expression categories was performed.

Table 3: Distribution of study-specific nuclear immunoreactivity categories for TRPS1 and GATA3 (N=30)

Score category	TRPS1, n (%)	GATA3, n (%)
High (score 6 or 9)	22 (73.3)	14 (46.7)
Intermediate (score 3 or 4)	1 (3.3)	5 (16.7)
Low (score 2)	5 (16.7)	3 (10.0)
Negative (score 0 or 1)	2 (6.7)	8 (26.7)
Total	30 (100.0)	30 (100.0)

Values are n (%); percentages may not total 100.0 because of rounding. The study-specific composite immunoreactivity score was calculated as the product of the proportion score (0-3) and nuclear staining intensity score (0-3). Scores were classified as negative (0-1), low (2), intermediate (3-4), or high (6 or 9). For the primary binary analysis, a score ≥ 2 was classified as positive.

Exploratory clinicopathologic associations

Neither marker showed a statistically significant association with age, lymphovascular invasion, grade, grouped T category, grouped nodal category, distant metastasis category, or grouped stage. The smallest nominal P-values were observed for TRPS1 with grouped nodal category (P=0.080) and grouped T category (P=0.089), and for GATA3 with age and

grade (both P=0.092). After Benjamini-Hochberg correction, all q-values were ≥ 0.323 , **Table 4**.

Among the 27 tumors with recorded stage, high TRPS1 expression was observed in 9/14 stage I–II tumors (64.3%) and 11/13 stage III–IV tumors (84.6%); the two-sided Fisher's exact P-value was 0.385. This descriptive difference was not statistically significant.

Table 4: Associations of clinic-pathological parameters with GATA3 and TRPS1 expression among study patients

Feature	Comparison	TRPS1		GATA3	
		Positive/total	P (q)	Positive/total	P (q)
Age	<50 vs ≥ 50 years	13/13 vs 15/17	0.492 (0.861)	12/13 vs 10/17	0.092 (0.323)
Lymphovascular invasion	Present vs absent	13/15 vs 15/15	0.483 (0.861)	12/15 vs 10/15	0.682 (0.954)
Nottingham grade	II vs III	12/13 vs 16/17	1.000 (1.000)	12/13 vs 10/17	0.092 (0.323)
T category	T1-T2 vs T3-T4	20/20 vs 7/9	0.089 (0.323)	15/20 vs 7/9	1.000 (1.000)
N category	N0-N1 vs N2-N3	19/19 vs 6/8	0.080 (0.323)	15/19 vs 5/8	0.633 (0.954)

Feature	Comparison	TRPS1		GATA3	
		Positive/total	P (q)	Positive/total	P (q)
M category	M0 vs M1	21/21 vs 5/6	0.222 (0.622)	15/21 vs 4/6	1.000 (1.000)
Stage	I-II vs III-IV	14/14 12/13	vs 0.481 (0.861)	10/14 vs 9/13	1.000 (1.000)

Values are positive/total within each comparison group. P values are from two-sided Fisher exact tests. q values are Benjamini-Hochberg false-discovery-rate adjusted across all 14 marker-feature comparisons. All analyses were exploratory.

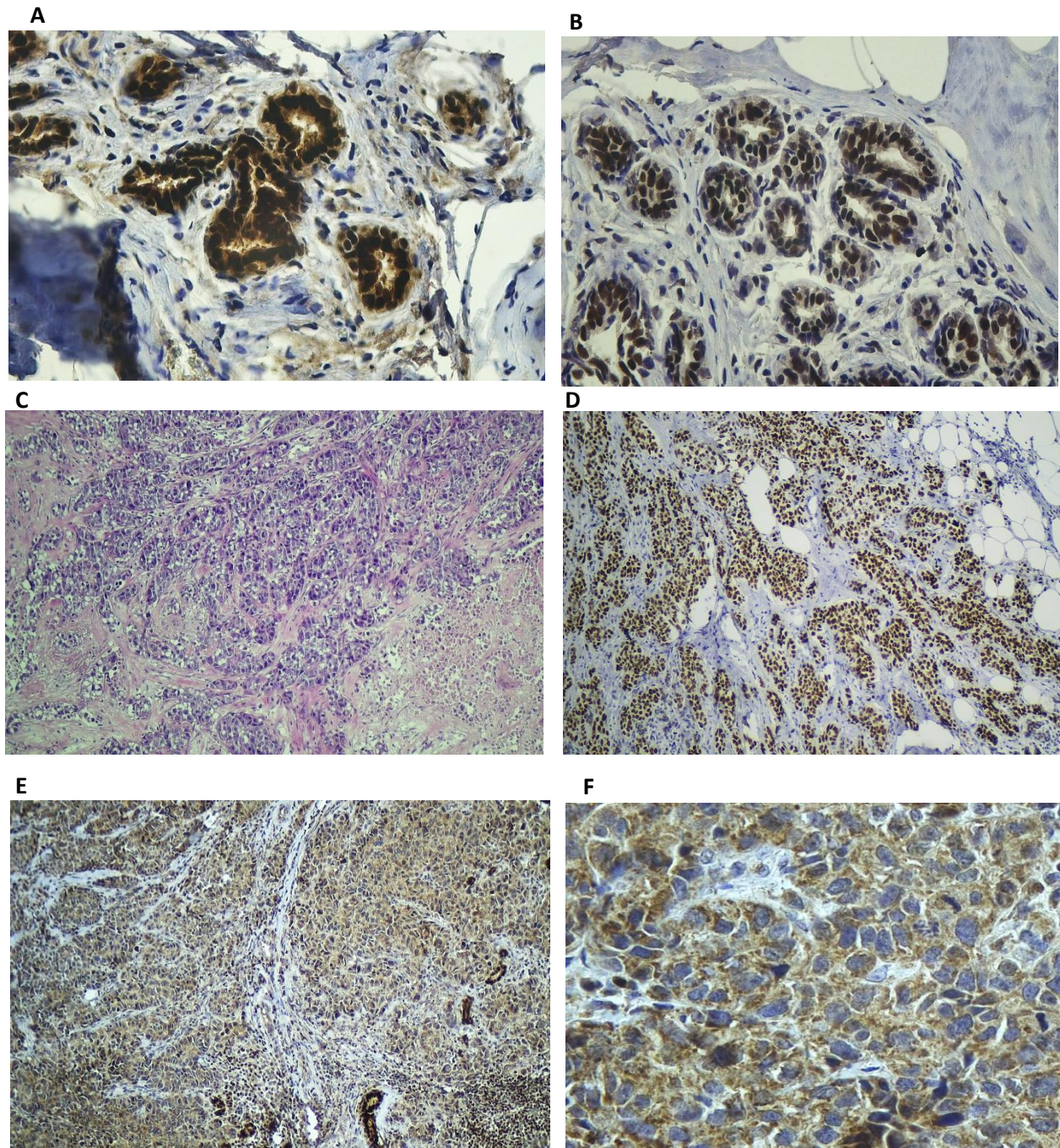


Figure 1: Positive controls and representative immunohistochemical findings in Case 1. (A) Normal breast epithelium with strong nuclear GATA3 expression as a positive control (×400). (B) Normal breast epithelium with strong nuclear TRPS1 expression as a positive control (×400). (C) H&E section of Nottingham grade 3 invasive breast carcinoma of no special type (×100). (D) Diffuse strong nuclear TRPS1 expression in tumor cells (immunoreactivity score = 9 [extent score 3 × intensity score 3]; ×100). (E) GATA3 immunohistochemistry showing minimal tumor-cell nuclear staining but nonspecific cytoplasmic and background staining (×100). (F) High power view confirming minimal nuclear GATA3 expression (immunoreactivity score = 1; negative according to prespecified threshold) (×400).

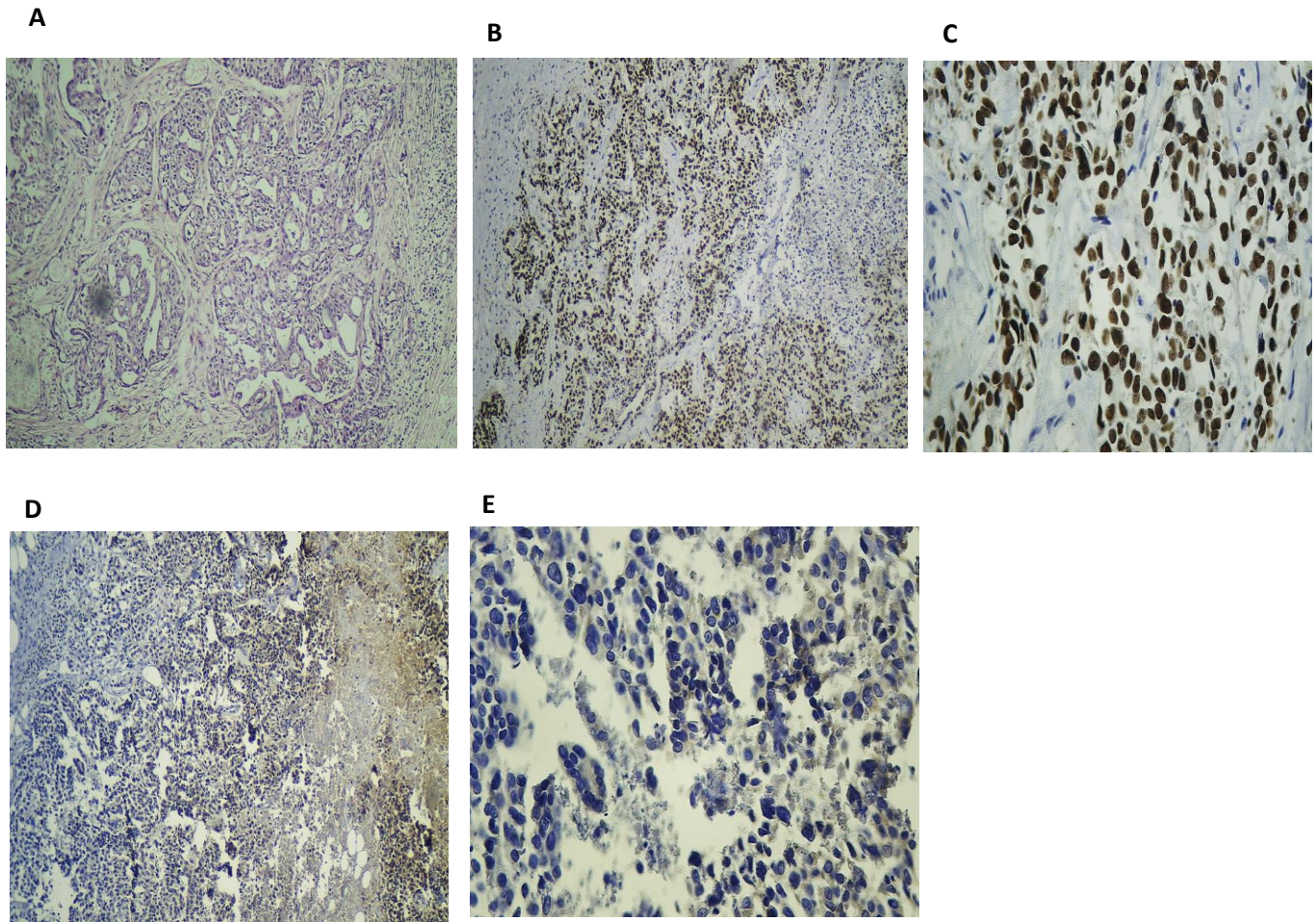


Figure 2: Representative immunohistochemical findings in Case 2. (A) H&E section showing Nottingham grade 2 invasive breast carcinoma of no special type (×100). (B) TRPS1 immunohistochemistry showed nuclear positivity in tumor cells at low power (×100). (C) High-power view shows strong nuclear TRPS1 expression (×400). Immunoreactivity score = extent score 2 × intensity score 3 = 6. (D) GATA3 immunohistochemistry showed minimal tumor-cell nuclear staining with massive nonspecific background staining at low power (×100). (E) High-power view only focal weak GATA3 nuclear staining was present; immunoreactivity score=1 therefore the tumor was GATA3 negative according to prespecified positivity threshold (×400).

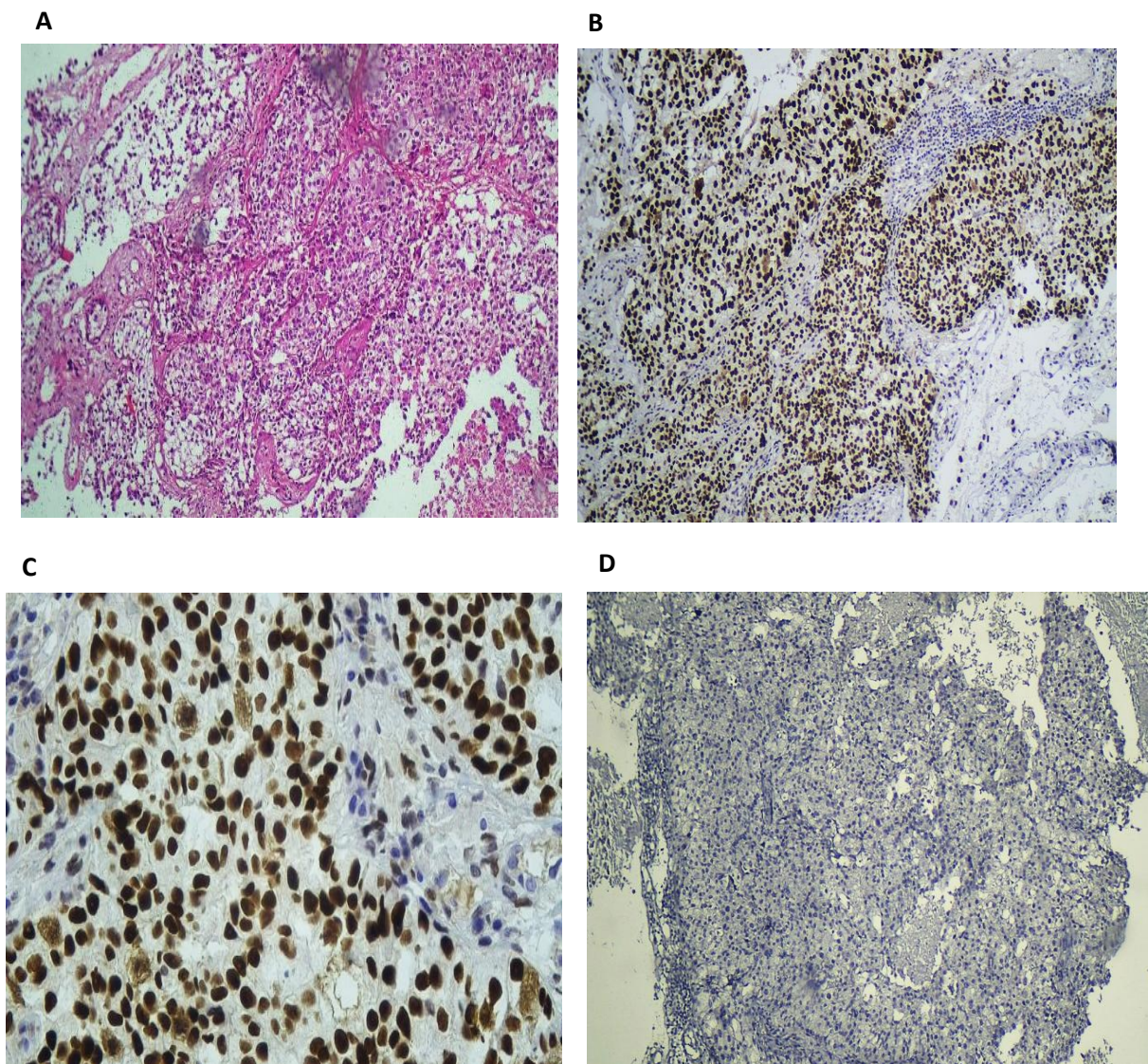


Figure 3: Representative metastatic triple-negative breast carcinoma involving the brain. (A) H&E section showing metastatic carcinoma involving brain tissue (×100). (B) Diffuse strong nuclear TRPS1 expression in metastatic tumor (score 9: extent score 3 × intensity score 3; ×100). (C) High power view confirming strong nuclear expression of TRPS1 in tumor cells (×400). (D) No nuclear GATA3 expression in metastatic tumor (score 0 immunoreactivity and classified as GATA3-negative) (×100).

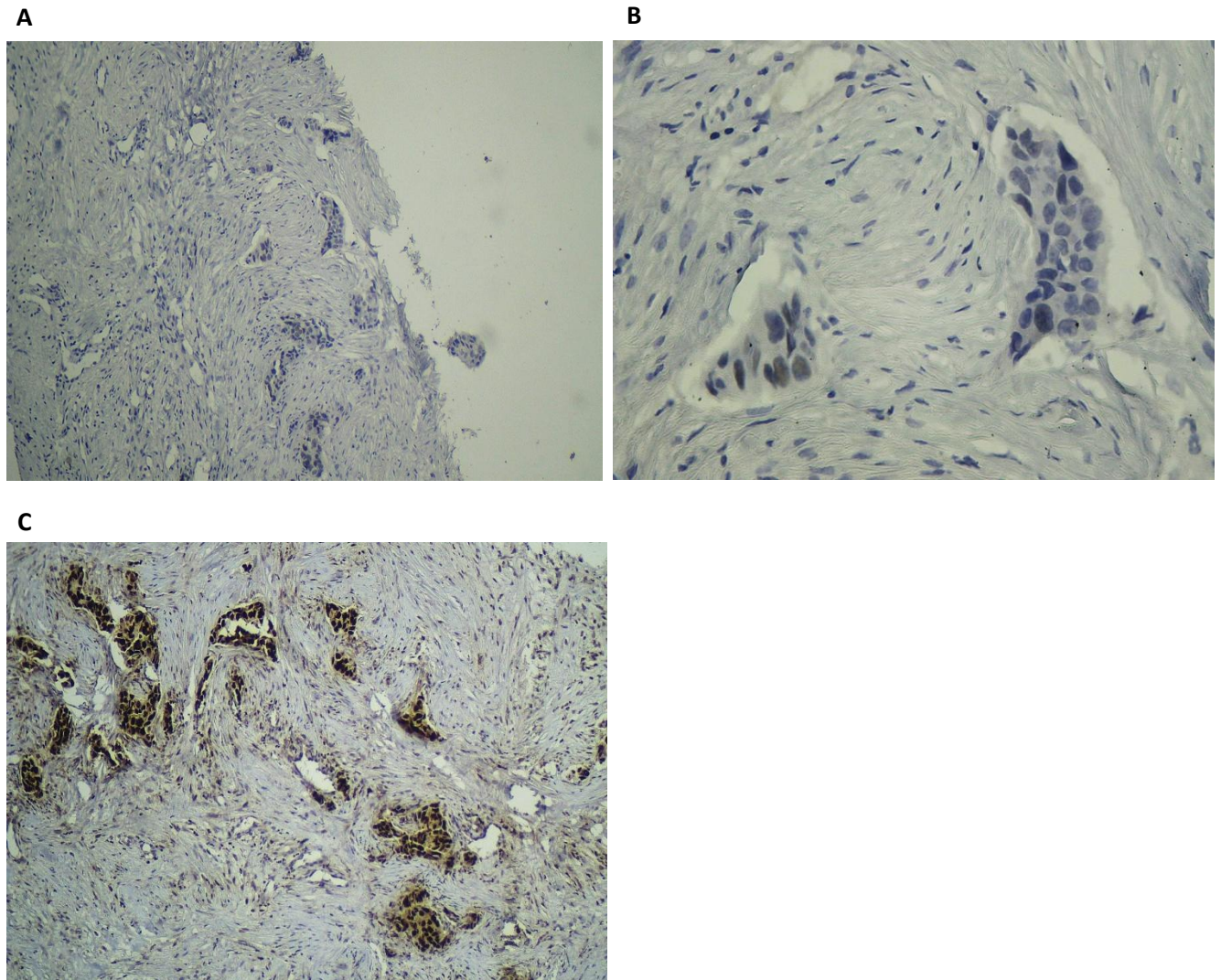


Figure 4: Representative reciprocal TRPS1 and GATA3 expression in Case 4. (A) TRPS1 immunohistochemistry showing no nuclear staining in Nottingham grade 2 invasive breast carcinoma of no special type with immunoreactivity score 0 ($\times 100$). (B) High-power view confirms absent TRPS1 nuclear staining in tumor cells ($\times 400$). (C) GATA3 immunohistochemistry showing strong nuclear expression in tumor-cell nests with immunoreactivity score 9 (extent score 3 $\times$ intensity score 3; $\times 100$).

DISCUSSION

In the current paired multi-institutional TNBC series, TRPS1 was positive in 93.3% of tumors compared with 73.3% for GATA3. However, the clinically significant aspect was that the discordance between them was in favor of TRPS1, since 7 out of 8 GATA3-negative tumors expressed TRPS1, whereas only 1 out of 2 TRPS1-negative tumors retained GATA3. The combination of these markers yielded positive results in 29 of 30 cases. Although there was a marginal difference of 20.0 percentage points and the matched-pairs odds ratio favored TRPS1, the *P*-value of the McNemar test was 0.070, and the confidence interval around the matched odds ratio was large. Consequently, the results suggest a considerable observed incremental yield, given the imprecision of the 30-case series.

The findings of TRPS1 positivity are in agreement with the existing literature. Ai et al. found broad TRPS1 expression in breast cancers irrespective of the subtype and high prevalence of TRPS1 in TNBC cases with the absence of breast markers (21, 22). Geetha et al. showed that TRPS1 stained almost all conventional and metaplastic TNBC and served as a good complement to GATA3 and SOX10 (18). The studies involving metaplastic carcinoma and lineage marker panels confirmed the superiority of TRPS1 in numerous TNBC settings (23, 24). Multi-institutional and tissue microarray research also revealed the high sensitivity of TRPS1 in detecting breast carcinoma, although the percentages vary depending on subtype, clone, cutoff, and specimen type (15, 19, 25, 26).

This methodological variation matters. The present research involved whole sections, clone EP392, citrate retrieval, and a product score combining staining extent and intensity. Some studies utilized different clones, tissue microarrays, percentage-only cutoffs, or other scoring methods. A low cutoff increases the

positivity rate but complicates the interpretation of weak staining; a high cutoff decreases the likelihood of missing tumors but makes the staining less confident. Consequently, direct comparison of quantitative positivity rates between studies is only meaningful when taking into account the same analytical platform, cutoff point, and type of tissue examined (13, 14, 15, 16, 19, 25, 26).

The seven TRPS1-positive/GATA3-negative cases are highly relevant to diagnostic practice. In the case of a poorly differentiated metastasis with a history of TNBC, nuclear expression of TRPS1 supports breast origin provided that the morphology and the rest of the immunopanel are concordant. Cytopathologic and effusion studies showed similar results, demonstrating the capacity of TRPS1 to identify tumors missed by GATA3, including small specimens with low tumor cellularity (27, 28, 29, 30). Additional yield is beneficial since high-grade TNBC loses mamoglobin and GCDFP-15. However, a positive stain in a known TNBC series is not equivalent to accuracy in an unknown primary.

TRPS1-negative/GATA3-positive tumors are equally educational. In some apocrine-differentiating tumors, TRPS1 may be present only weakly or be absent, whereas GATA3 remains positive (31). The minimal difference has been demonstrated in some effusion cohorts (14, 16, 18). These findings suggest that GATA3 cannot replace TRPS1. The reciprocal approach seems to be much more effective. TRPS1 can increase the sensitivity among GATA3-negative TNBCs, while GATA3 remains useful in selected TRPS1-negative tumors and in the differential diagnosis due to its broader profile.

The markers should be chosen in accordance with the differential diagnosis. TRPS1 has been reported to be expressed in gynecologic carcinomas, prostatic and urothelial neoplasms, salivary neoplasms, cutaneous tumors, phyllodes tumors, and several mesenchymal

tumors (13, 14, 15, 16, 17). On the other hand, GATA3 is not limited to the breast and is well known in urothelial and other neoplasms (17). Hence, neither marker alone identifies the mammary origin of tumors. In a tumor located in the liver, for example, a panel for breast lineages should be combined with hepatobiliary and gastrointestinal markers, while a tumor in the brain would require lung, melanoma, and other appropriate markers. Morphological and clinical imaging concordance are still important.

The study-specific expression scores were high in 73.3% of TRPS1 specimens and in 46.7% of GATA3 specimens. This difference is descriptive since the aggregated data did not contain a patient-level four-category matrix for both markers. It is inappropriate to use an unpaired ordinal test to compare two measurements made on the same specimens. Further research should record extent and intensity scores on the patient level to allow for a paired ordinal test and evaluate not only the difference in positivity but also the distribution of the scores in the same patients.

No significant clinicopathologic association was found. GATA3 positivity was numerically lower in patients 50 years or older and in grade III tumors, while TRPS1 negativity occurred in some T3 and N2 tumors. None of these tendencies were statistically significant after multiplicity adjustment. A higher incidence of high TRPS1 expression was seen in stage III–IV compared to stage I–II patients, although the difference was not statistically significant ($P=0.385$). Stage at sampling is not a surrogate for recurrence, response, or survival. Hence, these results do not support prognostic claims. Outcome-based research with appropriate follow-up and correction for standard prognostic factors is needed (32, 33).

This analysis possesses several strengths. Both markers were evaluated in the same specimens; the scoring rules were prespecified; whole sections were used; and the main comparison was based on exact paired statistics.

The variable-specific denominators were kept, and multiplicity correction was used for exploratory clinicopathologic tests. Representative examples from primary and metastatic tissues illustrate both types of discordance.

The principal limitations of the study include the moderate non-random sample size, the small number of discordant pairs, and single testing in each patient. There were three patients without complete stage information and one patient without T category information. The current cohort did not include non-breast mimics; thus, the specificity, predictive values, and diagnostic accuracy cannot be calculated. The original information about receptor testing was not rescored in this study; the triple-negative status was retrieved from the diagnostic records. The blinded second-reader concordance and patient-level paired ordinal scores were not available; there was no outcome-based follow-up. All these limitations delimit the inference range but do not affect the results of paired comparisons of the positivity.

The future validation study should cover a broader range of TNBC morphology and clinically relevant mimics, pre-specify the staining cutoffs, and keep the patient-level scores. Blinded double reading would provide the reproducibility evaluation, while the paired tests should remain the primary method of marker comparison in the same tumors. A control cohort chosen around realistic differential diagnoses would allow estimation of the specificity and diagnostic accuracy. Prognostic questions should be addressed in a separate study with treatment and time-to-event data.

CONCLUSION

In this study, the TRPS1 positivity rate was higher than that of GATA3 in paired TNBC, and we detected seven out of eight tumors negative for GATA3. Both markers demonstrated positivity in 96.7% of the cases, but one case was GATA3-positive in the absence of TRPS1

positivity. Paired test results did not achieve statistical significance at the 0.05 level; due to the small number of discordant cases, this limits the precision of the results. These results indicate that TRPS1 can serve as an additional marker for the breast lineage immunohistochemical panel.

DECLARATION

ETHICAL APPROVAL

The study was approved by the ethical committee from the Iraqi Board for Medical Specializations in Histopathology with approval ethical# IBMS-SUMS00125. The study adhered to the ethical principles of the Helsinki Declaration. As per the current study, since it is a retrospective study involving only the existing anonymous archived formalin fixed paraffin embedded tissue samples, the requirement for informed consent was waived by the IRB.

CONFLICT OF INTEREST

The authors declare that they have no competing interests

FUNDING

This research received no external funding.

DATA AVAILABILITY

The data set that analyzed and generated through the study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

R.M.H.; study design, data analyses, conceptualization, histopathological examination, manuscript drafting. M.F.ALQ.; slides staining, data collection. S.J.; manuscript revision, statistical analyses and supervision. All authors reviewed and approved the final version of the manuscript

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