The Relationship Between Tumor Budding and Patient's Survival in Breast Cancer

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ABSTRACT

INTRODUCTION: Breast cancer is a severe life-threatening condition in which many women are involved yearly. One factor that has recently been noticed and investigated as a diagnostic predictor of this type of cancer is the number of tumor buds and the relation of this factor with a patient's survival rate.

MATERIALS AND METHODS: This study includes 150 female patients over 18 years old with a mean age of 53.99 ± 12.56 years old with breast cancer, which was diagnosed at various medical centers, including Rouhani Hospital itself, and referred to Rouhani Hospital Medical Center, Babol, Iran. The number of intratumoral and peritumoral buds in patients' microscopic slides were archived and evaluated along with tumor microenvironment on hematoxylin and eosin (H&E) slides and compared to other clinicopathological findings. This article precisely investigated the relationship between the number of intratumoral and peritumoral buds with patients' 5-year survival rate. Also, the relationship between age, tumor stage, grade, size, the number of lymph nodes involved, and the presence of metastasis with the number of intratumoral and peritumoral buds was studied.

RESULTS AND DISCUSSION: The result showed a significant statistical association between the number of intratumoral and peritumoral buds with tumor size, tumor stage, presence of metastasis, the number of lymph nodes involved, and 5-year survival rate. On the other hand, there is not a significant statistical association between the number of intratumoral and peritumoral buds with age and tumor grade.

CONCLUSION: Our investigation revealed a significant statistical relationship between the number of tumor buds and patients' survival rate. So, this factor should be considered significant to help those patients increase their survival ratio.

KEYWORDS: Breast cancer, intratumoral buds, peritumoral buds, 5-year survival rate, tumor budding

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Introduction

According to the WHO (World Health Organization) GLOBOCAN 2020 reports, breast cancer (BC) is the most common type of cancer and the fifth cause of mortality among all genders worldwide and also in Iran. The cumulative incidence risk of breast cancer is 3.67%, and the cumulative death risk of it is 1.15% in Iran.[1-4] Therefore, finding new methods to help diagnose as a complementary method or predict breast cancer prognosis is essential.

Multiple studies show that several clinicopathological variables can effectively predict BC prognosis and patients' survival rates, such as tumor size, grade, stage, metastasis, lymph nodes involved, and the number of intratumoral and peritumoral buds. Furthermore, there are a variety of opinions on the purposeful use of the number of intratumoral or peritumoral buds, especially in breast cancer.[5-8]

Tumor budding is a histopathological phenomenon defined as a single cancer cell or small clusters of up to 4, located on the tumor's invasive side. This phenomenon can be further classified into peritumoral budding (PTB), observed at the tumor front, and intratumoral budding (ITB), identified within the tumor center. The assessment of PTB is restricted to endoscopic or surgical resection specimens, while ITB can be evaluated in both colorectal cancer biopsies and resection specimens. ITB and PTB are morphological indicators of the epithelial-mesenchymal transition (EMT) process in colorectal cancer.[9] Several studies have shown that tumor budding can be used as a prognostic biomarker in solid cancers such as rectal and colon cancers and also in breast cancer, especially in early-stage.[10,11]

On the other hand, several studies have been done recently, and their results reveal some weak and poor associations

between tumor budding and some clinicopathological variables such as tumor size, grading, stage, and lymphatic invasion.[12-16]

Therefore, in this study, we try to reveal if any relationships exist between intratumoral or peritumoral budding and the number of them with patients' 5-year-survival rate and some other clinicopathological variables such as age, tumor size, grade and stage, presence of metastasis, lymphatic invasion; and if any, find and design a method to use them to diagnose the progression and cure patients effectively and more accurate and increase their survival rate and improve the quality of life.

Materials and Methods

In this retrospective study, 150 patients over 18 years old with no underlying disease with breast cancer were referred and admitted to Rouhani Hospital Medical Center, Babol, Iran, between 2015 and 2020 and were identified. This study was conducted as an analytical cohort study after the approval of the research assistant and receiving the code of ethics (IR.MUBABOL.HRI.REC.1398.295) from the Babol University of Medical Science ethical committee. Patients' medical histories were reviewed, and 150 of them whose medical information was complete and available and met our inclusion criteria were examined in this research project.

Inclusion criteria:

- Female patients over 18 years old with breast cancer, without any underlying disease, with their microscopic tissue samples were sent to the Pathology Department of the Rouhani Hospital for diagnosis and investigation, and blocks fixed with formalin embedded with paraffin were available.

Exclusion criteria:

- Samples that could not be evaluated or patients' files that information was not entirely recorded.

Specimens were provided by lumpectomy or core needle biopsy. Tumor stroma was evaluated on H&E slides by Olympus microscope (ocular size 26.6 mm and field size 0.096 mm^2. First, microscopic tissue samples were examined with 4× and 10× microscope objective lenses to select areas with the most tumor buds. After that, those areas were investigated by a 20× microscope objective lens, and tumor buds were counted by our team's pathologists. To classify tumors according to grades, Patients were divided into 3 groups based on the number of tumor buds. Group I (grade I), tumor with 0 to 4 tumor buds, Group II (grade II), tumor with 5 to 10 tumor buds; and Group III (grade III), tumor with more than 10 tumor buds.[17]

After data gathering, they were encoded and then analyzed using the statistical package for the social sciences (SPSS edition 22) software in this study. Mean and standard deviation, frequency (percentage), chi-square test, survival analysis test, and long rank were used to describe the quantitative variables. The significance level in all tests was considered 0.05, which means P-value $< .05$ was significant.

Results

The study population consisted of females over 18 years old with a mean age of 53.99 ± 12.56, while 66 (44%) patients were under 50 years and 84 patients (56%) were over 50 years old.

In this article, various quantitative and qualitative variables were investigated, which included age, tumor size and grade, the number of lymph nodes involved, presence or absence of metastasis, 5-year-survival rate, and the number of intratumoral or/and peritumoral buds.

The highest frequency of the variable, "the number of lymph nodes involved," was related to patients without involvement of lymph nodes, which included 96 (64%) cases. The highest frequency of variable "tumor stage" was related to stage "2a," which included 57 (38%) of patients, and about variable "tumor grade" was related to grade 2, which included 94 (62.7%) cases. The files of 138 patients did not have any evidence in favor of metastasis.

Based on the chi-square test, our study showed significant relations between the number of intratumoral and peritumoral buds with tumor size and grade, the number of lymph nodes involved, the presence of metastasis, and the 5-year-survival rate, as can be seen in Tables 1 and 2. There is no significant relationship between age with the number of intratumoral and peritumoral buds.

Patients' mean five-year survival rate was 51.86 ± 1.53 months. More investigations revealed a significant relationship between the five-year survival rate and the number of lymph nodes involved, tumor size, the number of intratumoral and peritumoral buds, and the presence of metastasis. However, there is no relation between five-year survival and patients' age and also their tumor grade.

Based on log-rank test results, as can be seen in Table 3, it can be concluded that there is no significant relationship between age or tumor grade with the five-year survival rate. On the other hand, our investigations revealed that, as can be seen in Table 3 and Figures 1 and 2, patients who had less than 4 lymph nodes involved or with tumor size equal to or less than 2 cm, patients without metastasis, and patients with less than 10 intratumoral or peritumoral buds significantly had a better chance to live more than 5 years after BC diagnosis.

As seen in Figure 1, as the number of intratumoral buds increases, the patients' 5-year survival rate decreases.

As seen in Figure 2, as the number of peritumoral buds increases, the patients' 5-year survival rate decreases.

For more investigation, we used cox regression with a backward conditional way. It was done in 6 stages, and the results of the first and the last step are shown in Table 4. The results presented in this table demonstrate that the increase in the number of intratumoral buds is related to a decrease in patients' survival rate ($P < .001$), so the risk of death in patients with

Table 1. The relation between the number of intertumoral buds with patients' clinicopathological variables.

INTERTUMORAL BUDS		GRADE I	GRADE II	GRADE III	*P*-VALUE
Age	50⩾	33 (50%)	17 (25.8%)	16 (24.2%)	.57
	<50	41 (48.8%)	17 (20.2%)	26 (31%)	
Tumor stage	1a	19 (65.5%)	7 (24.1%)	3 (10.3%)	.004*
	2a	31 (54.4%)	17 (29.8%)	9 (18.5%)	
	2b	14 (50%)	5 (17.9%)	9 (32.1%)	
	3a	4 (36.4%)	2 (18.2%)	5 (45.5%)	
	3b	1 (33.3%)	0 (0%)	2 (66.7%)	
	3c	1 (10%)	2 (20%)	7 (70%)	
	4	4 (33.3%)	1 (8.3%)	7 (58.3%)	
Tumor grade	1	19 (76%)	2 (8%)	4 (16%)	.04*
	2	43 (45.7%)	22 (23.4%)	29 (30.9%)	
	3	12 (38.7%)	10 (23.3%)	9 (29%)	
The number of lymph node involved	0	54 (56.2%)	24 (25%)	18 (18.8%)	.001*
	1-3	16 (55.2%)	4 (13.8%)	9 (31%)	
	4-9	2 (16.7%)	4 (33.3%)	6 (50%)	
	⩾10	2 (15.4%)	2 (15.4%)	9 (69.2%)	
Metastasis	Yes	4 (33.3%)	1 (8.3%)	7 (58.3%)	.04*
	No	70 (50.7%)	33 (23.9%)	35 (25.4%)	

*Means there is a significant relationship between that variable and the number of intertumoral buds.

Table 2. The relation between the number of peritumoral buds with patients' clinicopathological variables.

PERITUMORAL BUDS		GRADE I	GRADE II	GRADE III	P-VALUE
Age	⩾50	28 (42.4%)	18 (27.3%)	20 (30.3)	.85
	<50	39 (46.4%)	20 (23.8%)	25 (29.8%)	
Tumor stage	1a	22 (75.9%)	2 (6.9%)	5 (17.2%)	<.001*
	2a	30 (52.6%)	22 (38.6%)	5 (8.8%)	
	2b	11 (39.3%)	8 (28.6%)	9 (32.1%)	
	3a	1 (9.1%)	1 (9.1%)	9 (81.8%)	
	3b	1 (3.3%)	1 (3.3%)	1 (3.3%)	
	3c	1 (10%)	3 (30%)	6 (60%)	
	4	1 (8.3%)	1 (8.3%)	10 (83.4%)	
Tumor grade	1	18 (72%)	3 (12%)	4 (16%)	.049*
	2	38 (40.4%)	25 (26.6%)	31 (33%)	
	3	11 (34.5%)	10 (32.3%)	10 (32.3%)	
The number of lymph node involved	0	55 (57.3%)	28 (29.2%)	13 (13.5%)	<.001*
	1-3	10 (34.5%)	6 (20.6%)	13 (44.9%)	
	4-9	1 (8.3%)	1 (8.3%)	10 (83.4%)	
	⩾10	1 (7.7%)	3 (23.1%)	9 (69.2%)	
Metastasis	Yes	1 (8.3%)	1 (8.3%)	10 (83.4%)	<.001*
	No	66 (47.8%)	37 (26.8%)	35 (25.4%)	

*Means there is a significant relationship between that variable and the number of intertumoral buds.

Table 3. The relation between 5-year-survival rate with patients' clinicopathological variables.

		FIVE-YEAR-SURVIVAL RATE (MONTHS)	*P*-VALUE
Age	⩾50	52.89	.86
	<50	51.6	
The number of lymph nodes involved	0-3	54.62	<.001*
	4-9	42	
	⩾10	34.46	
Tumor size	⩽2	55.6	.03*
	2-5	52.63	
	<5	42.27	
Tumor grade	1	54.47	.38
	2	51.87	
	3	48.93	
The number of intertumoral buds	Grade I	56.89	<.001*
	Grade II	56.82	
	Grade III	39.04	
The number of peritumoral buds	Grade I	58.12	<.001*
	Grade II	53.94	
	Grade III	40.51	
Metastasis	Yes	26.5	.001*
	No	54.07	

*Means there is a significant relationship between that variable and the 5-year-survival rate.

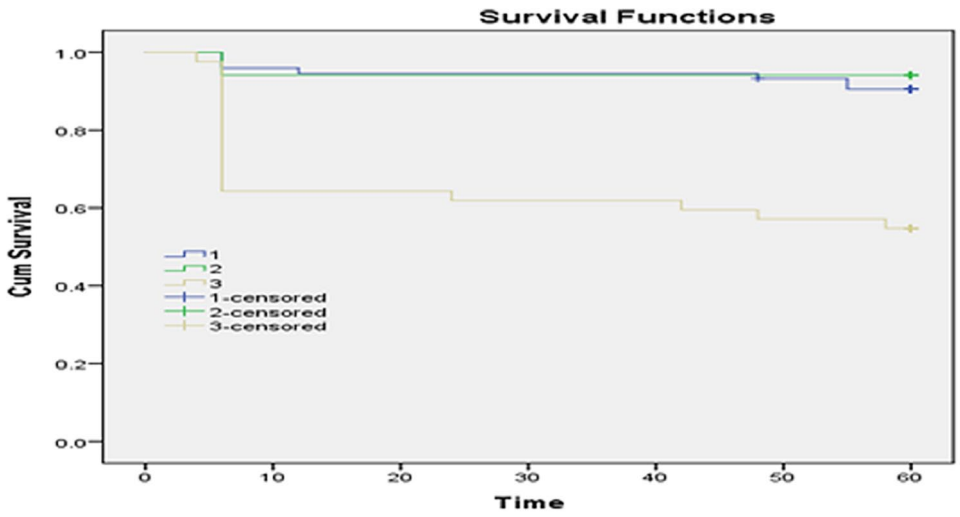

Figure 1. The number of intratumoral buds versus 5-year-survival rate.

more than 10 intratumoral buds is 2.85 times higher than that of patients who had 4-0 tumor buds (P=.03) (Figures 3–5). Further studies on the tumor stage and patients' survival rate showed that their relationship is the opposite of each other (P<.001). So that the risk of death in patients with stages 3c and 4 are 8.80 times (P=.04) and 22.33 times (P=.004), respectively, higher than in patients with stage 1a, which is significantly bold.

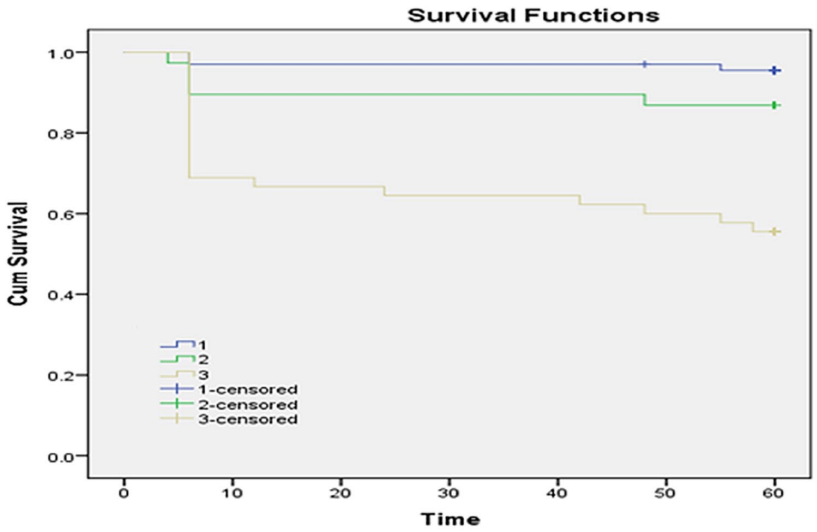

Figure 2. The number of peritumoral buds versus 5-year-survival rate.

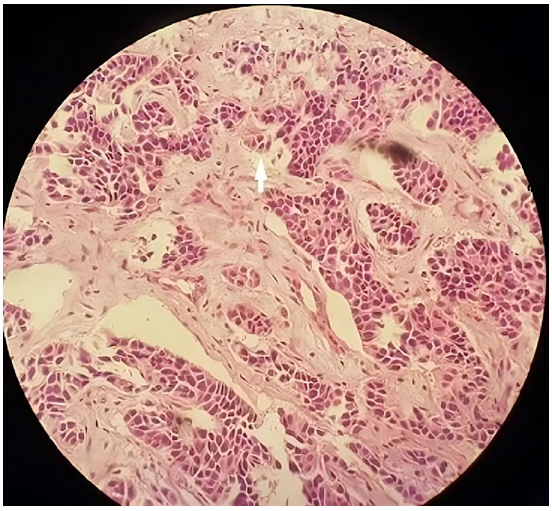

Figure 3. Examples of tumor budding in microscopic view of breast cancer tissue.

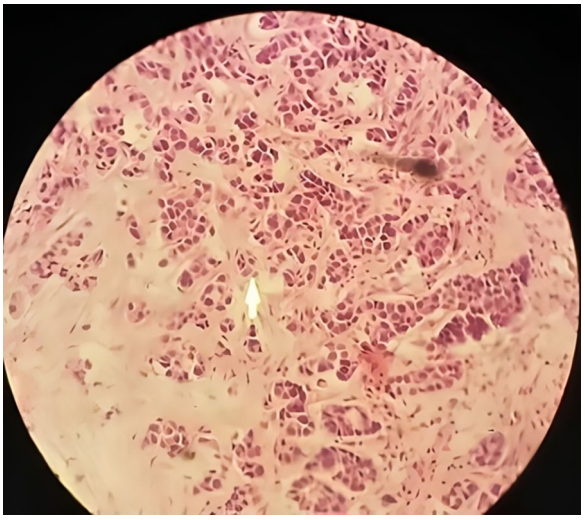

Figure 4. Examples of tumor budding in microscopic view of breast cancer tissue.

Discussion

Breast cancer, the foremost cause of female mortality globally, ranking fifth in 2020 according to WHO GLOBOCAN, underscores the urgency for advancing precise prognostic methods to enhance survival rates.[1,3,5] Multiple studies have been done up to now and approve that some clinicopathological variables can be helpful for early diagnosing or determining the probability of patients' survival rate, which can be very effective in determining treatment methods or policies and plans.[4-8]

The results of our study also confirmed that the risk of death in patients with more than 10 intratumoral buds and patients with tumor stages 3c and 4 is higher than in other patients, and these variables could be more effective than other variables on patients' survival rate.

More investigation proved that, as the other studies revealed,[13,18-22] there was a reverse relationship between tumor stage, grade, and size, the number of lymph nodes involved, and

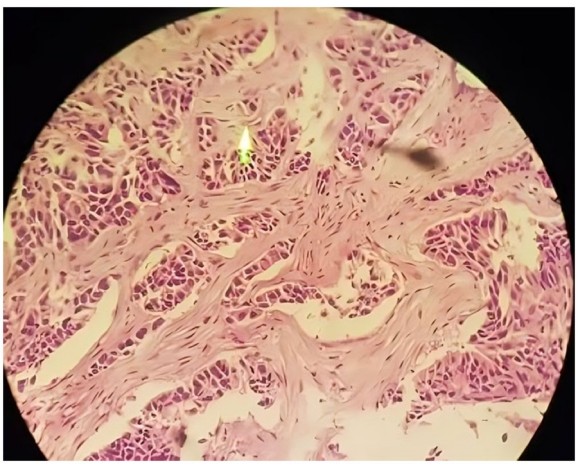

Figure 5. Examples of tumor budding in microscopic view of breast cancer tissue.

Table 4. Results based on COX regression.

STAGE	VARIABLES	OR	95.0% CI FOR OR		P-VALUE
			LOWER	UPPER	
First Stage (1)	Age > 50	1.33	0.59	3.03	0.48
	The number of lymph nodes (⩾1)	0.42	0.13	1.3	0.13
	Tumor size (⩽2 cm)	1	-	-	0.22
	Tumor size (2-5 cm)	0.57	0.15	2.06	0.39
	Tumor size (>5 cm)	1.32	0.32	5.43	0.69
	Tumor grade I	1	-	-	0.37
	Tumor grade 2	0.57	0.12	2.57	0.47
	Tumor grade 3	1.16	0.23	5.87	0.85
	The number of intertumoral buds (Grade I)	1	-	-	0.07
	The number of intertumoral buds (Grade II)	0.79	0.14	4.45	0.79
	The number of intertumoral buds (Grade III)	3.15	1.02	9.7	0.45
	The number of peritumoral buds (Grade I)	1	-	-	0.30
	The number of peritumoral buds (Grade II)	2.42	0.47	12.43	0.29
	The number of peritumoral buds (Grade III)	3.50	0.71	17.21	0.12
	Tumor stage 1a	1	-	-	0.001
	Tumor stage 2a	3.34	0.29	37.54	0.32
	Tumor stage 2b	2.08	0.13	32.8	0.60
	Tumor stage 3a	6.60	0.41	106.35	0.18
	Tumor stage 3c	14.66	0.86	249.32	0.06
	Tumor stage 4	35.20	3.08	402.38	0.004
Final Stage (6)	The number of intertumoral buds (Grade I)	1	-	-	0.03
	The number of intertumoral buds (Grade II)	0.63	0.12	3.08	0.57
	The number of intertumoral buds (Grade III)	2.85	1.1	7.38	0.03
	Tumor stage 1a	1	-	-	<0.001
	Tumor stage 2a	2.47	0.28	21.27	0.4
	Tumor stage 2b	1.52	0.13	17.11	0.73
	Tumor stage 3a	5.87	0.59	58.31	0.13
	Tumor stage 3c	8.80	0.94	81.66	0.04
	Tumor stage 4	22.33	2.72	183.05	0.004

Abbreviations: OR, odds ratio; CI, confidence interval.

the presence of metastasis, with the 5-year survival rates of the patients, so that as the stage or grade or size of the tumor or the number of lymph nodes involved increases, the five-year survival rate of the patients decreased. Our study also stated that an increase in the number of intratumoral or peritumoral buds could be associated with a decrease in the five-year survival rates of the patients.

In a study by Kumarguru et al,[18] 50 patients were studied, and the result, considering 20 tumor buds as cut-off, point revealed a significant direct relationship between the number of tumor buds and metastasis to lymph vessels and nodes.

In a study prepared by Agarwal et al,[19] 40 patients were studied for one and a half years, and the result, considering 10 tumor buds as the cut-off point, showed that the larger the

tumor in size, the higher the stage or presence of lymphovascular metastasis, the number of tumor buds is higher.

In the study conducted by Huang et al, the medical history of 102 patients was investigated, and the results, considering 10 tumor buds as the cut-off point, revealed a significant statistical association between tumoral budding with lymph node metastasis, lymphovascular emboli, and tumor grade. However, there was not a significant relationship between age and size of the tumor with the number of tumoral budding.[5]

It should be mentioned that our study not only confirms the results of other studies that have been done before,[5,18,19] it clearly shows that the number of intratumoral and peritumoral buds could be used as a proper clinicopathological parameter in diagnosing and determining the prognosis of breast cancer and determining a patient's survival rate.

In this study, the strength lies in and limitations of our approach. The strength lies in addressing the multiplicity of variables inherent in breast cancer prognostication, allowing for a comprehensive assessment of patients' survival rates. However, it is crucial to acknowledge certain limitations, notably the existing discrepancies among pathologists in this field.[23] The subjective nature of pathological interpretation and the potential oversight of important prognostic factors, such as Immunohistochemistry (Er, Pr, Her2, and Ki-67), pose challenges in the interpretation of prognostic findings, which demonstrate the need for more research on the matter incorporating these factors. Despite these limitations, our study endeavors to contribute valuable insights into the predictive potential of tumor buds, offering a nuanced understanding of their role in predicting survival outcomes for breast cancer patients.

Conclusion

According to the WHO investigation results, breast cancer is a common problem threatening women's lives. One of the controversial clinicopathological factors that could be helpful in breast cancer prognosis is "The number of intratumoral and peritumoral buds."This study clearly revealed that this factor could be used as a beneficial factor in the early-stage diagnosis of breast cancer, and by confirming the results of some other studies, this factor could be used to diagnose progression earlier and increase patients' survival rates. Resolving studied limitations and reaching more certain results is crucial to saving patients' lives.

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Author Contributions

All authors contributed equally to the manuscript and read and approved the final version of the manuscript.

Patient's Contest

The article is a retrospective study.

REFERENCES

1. World Health Organization (WHO). International Agency for Research on Cancer. https://gco.iarc.fr/today/data/factsheets/cancers/20-Breast-fact-sheet.pdf
2. World health Organization (WHO). International Agency for Research on Cancer. https://gco.iarc.fr/today/data/factsheets/populations/364-iran-islamic-republic-of-fact-sheets.pdf
3. Aghajanzadeh M, Torabi H, Najafi B, Talebi P, Shirini K. Intermammary breast cancer: a rare case of cancer with origin of breast cells in an unusual location. *Sage Open Med Case Rep*. 2023;11:1–6.
4. Torabi H, Rodd M, Shirini K. Presentation and treatment of a rare case of metaplastic breast cancer: a case report. *Sage Open Med Case Rep*. 2023;11:1–5.
5. Huang T, Bao H, Meng YH, et al. Tumour budding is a novel marker in breast cancer: the clinical application and future prospects. *Ann Med*. 2022;54: 1303-1312.
6. Voutsadakis IA. Prognostic role of tumor budding in breast cancer. *World J Exp Med*. 2018;8:12-17.
7. Okcu O, Öztürk Ç, Şen B, Arpa M, Bedir R. Tumor budding is a reliable predictor for death and metastasis in invasive ductal breast cancer and correlates with other prognostic clinicopathological parameters. *Ann Diagn Pathol*. 2021; 54:1–7.
8. Hiratsuka M, Hasebe T, Ichinose Y, et al. Tumor budding and fibrotic focus-proposed grading system for tumor budding in invasive carcinoma no special type of the breast. *Virchows Arch*. 2022;481:161-190.
9. Lugli A, Kirsch R, Ajioka Y, et al. Recommendations for reporting tumor budding in colorectal cancer based on the international tumor budding consensus conference (ITBCC) 2016. *Mod Pathol*. 2017;30:1299-1311.
10. Grigore AD, Jolly MK, Jia D, Farach-Carson MC, Levine H. Tumor budding: the name is EMT. Partial EMT. *J Clin Med*. 2016;5:51.
11. Kim S, Huh JW, Lee WY, et al. Lymphovascular invasion, perineural invasion, and tumor budding are prognostic factors for stage I colon cancer recurrence. *Int J Colorectal Dis*. 2020;35:881-885.
12. Mozarowski P, Rasaiah B, Reed M, et al. Prognostic role of tumor budding in breast cancer patients receiving neo-adjuvant therapy. *J Clin Med*. 2021;10:827.
13. Liang F, Cao W, Wang Y, et al. The prognostic value of tumor budding in invasive breast cancer. *Pathol Res Pract*. 2013;209:269-275.
14. Kocsmár É, Lotz G, Kiss A, et al. Prognostic impact of tumor budding and EMT in periampullary adenocarcinoma: a quantitative approach. *Cancer*. 2020; 11:6474-6483.
15. Jun SY, Chung JY, Yoon N, et al. Tumor budding and poorly differentiated clusters in small intestinal adenocarcinoma. *Cancers*. 2020;12:2199.
16. Park JY, Chong GO, Park JY, et al. Tumor budding in cervical cancer as a prognostic factor and its possible role as an additional intermediate-risk factor. *Gynecol Oncol*. 2020;159:157-163.
17. Singh T, Chandra K, Kumar N, et al. A retrospective study of association of tumor budding, tumor microenvironment, and clinicopathological characteristics of invasive breast carcinoma. *J Lab Physicians*. 2022;14:485-490.
18. Kumarguru BN, Ramaswamy AS, Shaik S, et al. Tumor budding in invasive breast cancer - an indispensable budding touchstone. *Indian J Pathol Microbiol*. 2020;63:S117-S122.
19. Agarwal R, Khurana N, Singh T, Agarwal PN. Tumor budding in infiltrating breast carcinoma: correlation with known clinicopathological parameters and hormone receptor status. *Indian J Pathol Microbiol*. 2019;62:222-225.
20. Salhia B, Trippel M, Pfaltz K, et al. High tumor budding stratifies breast cancer with metastatic properties. *Breast Cancer Res Treat*. 2015;150:363-371.
21. Gujam FJ, McMillan DC, Mohammed ZM, Edwards J, Going JJ. The relationship between tumour budding, the tumour microenvironment and survival in patients with invasive ductal breast cancer. *Br J Cancer*. 2015;113: 1066-1074.
22. van Wyk HC, Roseweir A, Alexander P, et al. The relationship between tumor budding, tumor microenvironment, and survival in patients with primary operable colorectal cancer. *Ann Surg Oncol*. 2019;26:4397-4404.
23. Taraz Jamshidi S, Gharib M, Etemadi M, et al. Pathology reports: discrepancy patterns of second opinions in a referral cancer center. *Cancer Investig*. 2023;41: 284-291.