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EVALUATION OF PANCYTOKERATIN IMMUNOHISTOCHEMISTRY FOR DETECTING OCCULT LYMPH NODE METASTASIS IN EPITHELIAL MALIGNANCIES

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Abstract

Background: Lymph node metastasis is a critical prognostic factor in epithelial malignancies. Routine hematoxylin and eosin (H&E) staining may miss occult deposits such as micrometastases and isolated tumor cells, leading to understaging. Immunohistochemistry using pancytokeratin (PANCK) can improve detection.
Aim: To evaluate the utility of PANCK immunohistochemistry in detecting occult lymph node metastasis in histologically negative nodes and its impact on TNM staging.
Materials and Methods: A cross-sectional study was conducted from June 2024 to March 2026 on 530 lymph nodes from resected epithelial malignancies. All nodes were examined by H&E and PANCK (AE1/AE3) immunohistochemistry. Clinicopathological parameters and TNM staging were assessed.
Results: PANCK identified occult metastases in 5 of 496 H&E-negative nodes (1%), increasing sensitivity from 87.2% to nearly 100% ($p < 0.0001$). Additional positivity was highest in uterine (9.5%) and colorectal (2.4%) cancers, with minimal gain in breast carcinoma (0.5%). PANCK was more useful in low- and intermediate-grade tumors. TNM staging was upgraded in 3 cases (8.6%).
Conclusion: PANCK immunohistochemistry significantly improves detection of occult nodal metastases, enhances diagnostic sensitivity, refines pathological staging, and may guide adjuvant therapy in selected epithelial malignancies.

Keywords: Lymph node metastasis, Micrometastasis, Pancytokeratin, AE1/AE3, Immunohistochemistry, Epithelial malignancy.

INTRODUCTION

Lymph nodes are organized lymphoid structures situated along lymphatic channels and play a critical role in immune surveillance. In epithelial malignancies, they represent the most common initial site of metastatic spread, making nodal involvement an important determinant of staging, prognosis, and treatment planning[1,2,3].

Lymphatic metastasis is a multistep biological process involving tumor cell detachment, extracellular matrix invasion, intravasation into lymphatic vessels, survival during transit, and colonization within lymph nodes [1]. Thin-walled lymphatic capillaries with discontinuous basement membranes facilitate tumor cell entry, while molecular mechanisms such as matrix metalloproteinase activation, epithelial–mesenchymal transition, and VEGF-C/VEGF-D–mediated lymphangiogenesis promote metastatic dissemination [1,4,5].

Within lymph nodes, metastatic tumor cells initially localize to the subcapsular sinus before infiltrating deeper cortical and medullary compartments. Metastatic tumor deposits within lymph nodes are categorized based on their size into isolated tumor cells (ITCs), micrometastases, and macrometastases, as these categories have important staging and prognostic implications [3]. According to TNM and WHO criteria, ITCs are single tumor cells or small clusters measuring less than 0.2 mm in greatest dimension, usually containing fewer than 200 tumor cells in a single histological section [3,4]. These deposits are often identified only by immunohistochemistry or molecular methods, as they may be difficult to recognize on routine H&E staining. Although ITCs may represent the earliest stage of nodal dissemination, their prognostic significance varies among different malignancies [3].

Micrometastases are defined as metastatic deposits measuring between 0.2 mm and 2 mm [3,4]. They represent an intermediate stage between isolated tumor cells and overt nodal metastasis. Micrometastases are clinically important because they indicate established metastatic potential and are associated with an increased risk of recurrence and disease progression compared with node-negative cases [2]. In many cancers, including breast and colorectal carcinoma, the presence of micrometastases may alter pathological staging and influence decisions regarding adjuvant chemotherapy or radiotherapy [3].

Macrometastases are metastatic deposits larger than 2 mm and usually indicate a significant nodal tumor burden [3]. These lesions are generally identifiable on routine gross and microscopic examination and are associated with advanced disease stage, greater likelihood of extranodal extension, distant metastasis, and poorer overall survival [5,6].Progressive enlargement of metastatic deposits may eventually replace the normal nodal architecture and extend beyond the lymph node capsule into surrounding soft tissue, a phenomenon termed extranodal extension, which is considered an adverse prognostic factor in several malignancies [5].

Because ITCs and micrometastases can be subtle and easily overlooked on conventional histopathology, immunohistochemistry using epithelial markers such as pancytokeratin (AE1/AE3) plays an important role in their detection [7]. PANCK immunostaining highlights occult epithelial tumor cells within lymphoid tissue, thereby improving the sensitivity of lymph node evaluation and enabling more accurate TNM staging [2,3].

The extent of nodal involvement, including the number of positive nodes and the size of metastatic deposits, correlates strongly with disease progression and survival outcomes [3,4,5].Detection of micrometastases and isolated tumor cells therefore has considerable clinical significance.

Routine H&E staining remains the standard method for lymph node evaluation; however, small metastatic deposits may escape detection. Cytokeratins are intermediate filament proteins expressed in epithelial cells and are preserved in most carcinomas, making them reliable immunohistochemical markers for identifying metastatic epithelial cells within lymphoid tissue [7]. Pancytokeratin (AE1/AE3) immunostaining has been shown to improve the detection of occult metastasis missed on conventional microscopy.

The present study was undertaken to evaluate the utility of PANCK immunohistochemistry in detecting occult lymph node metastasis in epithelial malignancies and to assess its impact on pathological staging.

METHODOLOGY

Study Design and Setting

This was a cross-sectional, descriptive study conducted in the Central Laboratory, Department of Pathology, Sree Balaji Medical College and Hospital, Chennai. The study was carried out over a period of 22 months, from June 2024 to March 2026. The purpose of the study was to evaluate the diagnostic utility of pancytokeratin (PANCK) immunohistochemistry in detecting occult lymph node metastases in epithelial malignancies and to assess its impact on pathological TNM staging. The study protocol was approved by the Institutional Human Ethics Committee prior to initiation, and all procedures followed the ethical standards of the institution.

Study Population and Sampling

A total of 530 lymph nodes were obtained from patients who underwent radical surgical resection for various histologically confirmed epithelial malignancies with concurrent lymph node dissection. Consecutive sampling was employed, and all lymph nodes submitted for routine histopathological examination during the study period were included. The sample size was determined based on the volume of surgical resections for

epithelial malignancies performed at the institution during the study timeframe, with the aim of capturing a diverse range of tumor types and nodal statuses.

INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria: All lymph nodes retrieved from radically resected specimens of histologically confirmed epithelial malignancies (including breast, colorectal, gastric, pancreatic, uterine, and oral cancers) were included in the study. Only nodes that were adequately processed and yielded interpretable sections were considered.
Exclusion criteria: Cases of in situ malignancies without invasive component, non-epithelial tumors (sarcomas, lymphomas, melanomas), and autolyzed or poorly preserved specimens that precluded accurate histopathological assessment were excluded from the study.

Data Collection and Clinical Parameters

Clinical and histopathological data were recorded for each patient using a pre-structured case report form. The following variables were documented: age, sex, type of surgical procedure, primary tumor site, histological type, tumor grade (differentiated as Grade 1, 2, or 3 based on standard criteria), lymph node status on H&E and PANCK, cytokeratin expression patterns, and final TNM stage according to the AJCC 8th edition criteria. All data were entered into a secure database for subsequent statistical analysis.

Histopathological Processing and H&E Staining

All resected lymph nodes were carefully dissected from the surgical specimens, counted, and measured. Each lymph node was bisected along its long axis and processed routinely for formalin fixation and paraffin embedding. Tissue sections of 4 µm thickness were cut using a rotary microtome and mounted on clean glass slides. Routine hematoxylin and eosin (H&E) staining was performed on all sections following standard laboratory protocols. The H&E-stained slides were examined independently by two pathologists blinded to the immunohistochemistry results. Lymph nodes were classified as positive for metastatic tumor deposits (macrometastases >2 mm) or negative (no visible tumor cells). Micrometastases (0.2–2 mm) and isolated tumor cells (<0.2 mm) were considered negative on H&E for the purpose of this study, as they are often overlooked on routine microscopy.

Immunohistochemistry Protocol for PANCK (AE1/AE3)

For immunohistochemistry, 3 µm thick sections were cut from the same paraffin blocks onto positively charged slides to ensure tissue adhesion. The sections were dried overnight at 37°C. Antigen retrieval was performed using Tris-EDTA buffer at pH 9.0, with heating in a pressure cooker for 3 minutes followed by cooling at room temperature for 20 minutes. Endogenous peroxidase activity was blocked by incubating the sections in 3% hydrogen peroxide for 10 minutes. Non-specific binding was minimized using a protein block. The primary antibody used was a mouse monoclonal anti-pancytokeratin antibody (clone AE1/AE3, ready-to-use or diluted as per manufacturer's instructions). Sections were incubated with the primary antibody for 60 minutes at room temperature. After washing with Tris-buffered saline containing Tween-20, a polymer-based detection system (HRP-conjugated secondary antibody) was applied for 30 minutes. Diaminobenzidine (DAB) was used as the chromogen, yielding a brown precipitate at the site of antibody binding. The sections were then counterstained with Mayer's hematoxylin for 30 seconds, dehydrated through graded alcohols, cleared in xylene, and mounted with a coverslip using DPX mountant. Positive controls (known carcinoma tissue) and negative controls (omission of primary antibody) were included in each run.

Microscopic Evaluation and Interpretation

All PANCK-stained slides were examined independently by two pathologists who were unaware of the H&E results. Any brown cytoplasmic staining in epithelial cells within the lymph node parenchyma, regardless of cell number or cluster size, was considered a positive finding for metastatic carcinoma. The size of the metastatic deposit was measured using an ocular micrometer to classify as isolated tumor cells (<0.2 mm), micrometastasis (0.2–2 mm), or macrometastasis (>2 mm). Discrepancies between the two observers were resolved by consensus discussion using a multi-headed microscope.

Statistical Analysis

Statistical analysis was performed using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA). Continuous variables were summarized as mean ± standard deviation, while categorical variables were expressed as frequencies and percentages. Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of H&E staining and PANCK immunohistochemistry were calculated using standard formulas. Comparison of detection rates between H&E and PANCK was performed using Fisher's exact test (for 2×2 contingency tables). A p-value less than 0.0001 was considered statistically significant, reflecting the stringent threshold required due to the multiple comparisons and the high specificity of the diagnostic tests.

RESULTS

A total of 530 lymph nodes from radically resected epithelial malignancies were evaluated. On routine H&E staining, 34 nodes (6.4%) showed macrometastatic deposits, while 496 nodes (93.6%) were negative. Among the H&E-negative nodes, PANCK immunostaining identified occult micrometastases in 5 additional nodes (1%) [Table 1].

H&E staining demonstrated a sensitivity of 87.2%, specificity of 100%, and overall diagnostic accuracy of 99.1%. The addition of PANCK improved sensitivity and accuracy to nearly 100%, while maintaining 100% specificity ($p < 0.0001$) [Table 2].

Organ-wise analysis showed the highest additional detection rates in uterine malignancies (9.5%) (Figure 1,2) and colorectal carcinomas (2.4%) (Figure 3,4), whereas minimal additional positivity was observed in breast carcinoma (0.5%) (Figure 5,6). No added benefit was noted in pancreatic, gastric, tongue, or other malignancies [Table 3].

Grade-wise analysis revealed that PANCK was most useful in low- and intermediate-grade tumors, increasing positivity from 3.8% to 5.2% in Grade 1 tumors and from 5.3% to 5.8% in Grade 2 tumors. No additional metastatic deposits were identified in Grade 3 tumors [Table 4].

TNM staging was revised in 3 cases (8.6%) following PANCK immunostaining, including two colorectal carcinomas and one uterine malignancy [Table 5]. Fisher's exact test demonstrated a significant improvement in metastatic detection with PANCK compared to H&E alone ($p < 0.0001$) [Table 6].

DISCUSSION

Epithelial malignancies are among the most prevalent cancers worldwide, and cancer-related mortality is primarily due to metastatic disease rather than the primary tumor itself [9].Occult metastases, particularly within lymph nodes, remain a major diagnostic challenge because micrometastases and isolated tumor cells may not be detected on routine histopathological examination [10]. Accurate lymph node evaluation is therefore critical for TNM staging, prognostic assessment, and decisions regarding adjuvant therapy [2,3]. Conventional H&E staining, although highly specific, has limited sensitivity in identifying minimal tumor burden, resulting in possible understaging and suboptimal treatment [11]. Cote et al. and Turner et al. reported that small metastatic deposits are frequently overlooked on routine sections, emphasizing the importance of detecting occult nodal disease [10,12].

Immunohistochemistry using epithelial markers such as pancytokeratin has emerged as a valuable adjunct to routine histopathology because cytokeratins are consistently expressed in epithelial malignancies and enable identification of even isolated tumor cells within lymph nodes[13]. In the present study, most patients belonged to older age groups, particularly 51–60 years, consistent with observations by Carter et al. and Jemal et al., who attributed increasing cancer incidence with age to cumulative genetic damage and prolonged carcinogen exposure [14,15]. Female predominance in the cohort was largely due to the higher frequency of breast carcinoma, in agreement with global cancer statistics reported by Parkin et al [16].Breast carcinoma was the most common malignancy, followed by colorectal carcinoma, paralleling GLOBOCAN 2020 data [17]. Adequate lymph node retrieval is essential for reliable staging, and the analysis of 530 lymph nodes in this study strengthens the validity of the findings, as emphasized by Goldstein et al [18].

A major finding of the study was the improved detection of occult lymph node metastasis with PANCK immunohistochemistry. While H&E staining demonstrated high specificity (100%), its sensitivity was lower (87.2%), reflecting its limitation in detecting micrometastases and isolated tumor cells, similar to findings by Weaver DL et al. and Greenson JK et al [19,20].Addition of PANCK increased detection of occult metastasis by 1%, with near 100% sensitivity and accuracy, supporting previous reports by Cote et al. and Braun et al.,

who demonstrated that immunohistochemistry improves detection of micrometastatic disease missed on routine staining [10,21].

Tumor-specific analysis showed higher incremental detection in colorectal and uterine malignancies compared with breast carcinoma. Colorectal tumors demonstrated an additional detection rate of 2.4%, while uterine malignancies showed a marked increase of 9.5%. Similar findings were reported by Noura et al., Kahn et al., and Van Schaik PM et al., who noted that adenocarcinomas of the gastrointestinal and gynecological tract frequently exhibit subtle micrometastatic spread that may not be readily apparent on H&E sections [22,23,24]. In contrast, breast carcinoma showed only minimal additional positivity (0.5%), likely because metastatic deposits are often larger and more cohesive. However, in lobular carcinomas, where tumor cells are discohesive and morphologically subtle, immunohistochemistry significantly improves detection, as reported by Weaver DL et al. and Wells CA et al [19,25].

The study also demonstrated that PANCK was more effective in detecting micrometastases in low- and intermediate-grade tumors than in high-grade tumors. Pantel and Brakenhoff suggested that micrometastases may occur early in tumor progression and are not always associated with high-grade morphology [26]. Zhuang Y et al. further proposed that micrometastases in low-grade tumors may indicate a worse prognosis than previously assumed [27]. In the present study, micrometastases were detected more frequently in Grade 1 and Grade 2 tumors than in Grade 3 tumors, highlighting the role of immunohistochemistry in identifying subtle metastatic deposits that may otherwise remain undetected.

Importantly, identification of additional metastatic lymph nodes resulted in TNM upstaging in 3 of 35 cases, particularly in colorectal and uterine malignancies. Even limited nodal involvement can significantly influence prognosis and eligibility for adjuvant chemotherapy or radiotherapy. Similar observations were made by Turner RR et al. and Weaver et al., who demonstrated that detection of micrometastases may alter staging and treatment strategies [12,19]. The strong statistical significance observed in this study ($p < 0.0001$) further supports the utility of PANCK immunohistochemistry in improving detection of occult nodal metastasis. Comparable conclusions were drawn by Cote et al., who emphasized the superior sensitivity of immunohistochemistry over conventional staining methods [10].

Despite these findings, the study has certain limitations, including relatively small sample size, especially for non-breast malignancies, its single-center design, and the absence of long-term follow-up data to evaluate survival and recurrence outcomes. Nevertheless, the results highlight the diagnostic and clinical value of combining H&E with PANCK immunohistochemistry for more accurate lymph node assessment in epithelial malignancies.

CONCLUSION

PANCK immunohistochemistry is a valuable adjunct to routine H&E staining for detecting occult lymph node metastasis in epithelial malignancies. Its application significantly improves diagnostic sensitivity and staging accuracy, particularly in low- and intermediate-grade tumors and in selected malignancies such as colorectal and uterine carcinomas. Although the incremental detection rate is modest, its impact on TNM upstaging and therapeutic decision-making is clinically important, supporting the combined use of H&E and PANCK in lymph node evaluation.

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TABLES

Table 1: Descriptive analysis of lymph node evaluation performed in the study

S.No	Parameter	n	%
1	Total Nodes Assessed	530	100
2	By H & E method- nodes classifies as (n=530)		
	Positive for tumor deposits	34	6.4
	Negative for tumor deposits	496	93.6
3	By PANCK method- nodes classified as (n=496)		
	Positive for tumor deposits	5	1
	Negative for tumor deposits	491	99

Out of 530 lymph nodes, 6.4% were positive for carcinoma on H&E, while 93.6% were negative.Among H&E-negative nodes, PANCK detected an additional 1% positivity, indicating minimal but clinically significant occult metastasis detection.

Table 2: Descriptive statistics of H& E and PANCK method observed in this study

S.No	**Method**	**Metastatic deposits in Lymph node**		**Sensitivity**	**Specificity**	**Accuracy**
		Positive	**Negative**			

1	H& E Method	Positive	34	0	87.2	100	99.1
		Negative	5	491			
2	HE + PANCK Method	Positive	39	0	100	100	100
		Negative	0	491			

H&E showed 87.2% sensitivity with 100% specificity and 99.1% accuracy.With PANCK, sensitivity, specificity, and accuracy reached 100%, detecting occult metastases missed on H&E and improving overall diagnostic performance.

Table 3: Comparative analysis of tumor nodal involvement between H&E staining and PANCK in correspondence to tumor site

S.No	Tumor site	Method	Tumor Positive in lymph node	Tumor Negative in lymph node	Additional Detection by achieved by PANCK
1	Breast (N=207)	H & E	17 (8.2)	190(91.7)	**0.5%**
		H & E + PANCK	18 (8.7)	189(91.3)	
2	Colon (N=84)	H & E	1 (1.2)	83 (98.8)	**2.4 %**
		H & E + PANCK	3 (3.6)	81 (96.4)	
3	Pancreas (N=51)	H & E	2 (3.9)	49 (96.1)	**0**
		H & E + PANCK	2 (3.9)	49 (96.1)	
		H & E	12 (34.2)	23 (65.8)	

4	Stomach (N= 35)	H & E + PANCK	12 (34.2)	23 (65.8)	**0**
5	Tongue (N=75)	H & E	0	75 (100)	**0**
		H & E + PANCK	0	75 (100)	
6	Uterus (N=21)	H & E	0	21 (100)	**9.5%**
		H & E + PANCK	2 (9.5)	19 (90.5)	
7	Others (N=57)	H & E	2(3.5)	55(96.5)	**0**
		H & E + PANCK	2(3.5)	55(96.5)	

PANCK added detection mainly in uterus (9.5%), colon (2.4%), and minimally in breast (0.5%), with no added positivity in pancreas, stomach, tongue, or other organs. Overall, it helped identify occult metastasis in selected organs, especially uterus and colon, with limited benefit elsewhere.

Table 4: Comparison of tumor nodal involvement and grading of primary tumor using H&E staining versus the PANCK method.

S.No	Grading of primary tumor in resection specimen	Method	Tumor Positive Lymph Node	Tumor Negative Lymph Node	Additional Detection by achieved by PANCK
1	Grade 1 (n=289)	H & E	11(3.8)	278 (96.2)	1.4%
		H & E + PANCK	15(5.2)	274 (94.8)	
2	Grade 2 (n=206)	H & E	11 (5.3)	195 (94.6)	0.5%

		H & E + PANCK	12 (5.8)	194 (94.2)	
3	Grade 3 (n=25)	H & E	12 (48)	13 (52)	0%
		H & E + PANCK	12 (48)	13 (52)	
4	Grading not done (n=10)	H & E	0	10	0%
		H & E + PANCK	0	10	

Among 530 nodes, PANCK increased detection in Grade 1 tumors from 3.8% to 5.2% and in Grade 2 from 5.3% to 5.8%, with no added benefit in Grade 3 or ungraded cases.Thus, PANCK mainly aids detection of occult micrometastasis in low–intermediate grade tumors, with minimal value in high-grade lesions.

Table 5: Distribution of tumor staging modifications based on PANCK findings observed in this study

S.No	Organ	Tumor Stage Modified due to PANCK finding		
		Observation	n	%
1	Breast(n=14)	No	14	100
		Yes	0	0
2	Colon (n=5)	No	3	60
		Yes	2	40
3	Pancreas (n=4)	No	4	100
		Yes	0	0

4	Stomach (n=3)	No	3	100
		Yes	0	0
5	Tongue (n=4)	No	4	100
		Yes	0	0
6	Uterus (n=2)	No	1	50
		Yes	1	50
7	Others (n=3)	No	3	100
		Yes	0	0

Out of 35 cases analysed, TNM staging was revised in 3 cases, among which Colon malignancy showed stage modification in 40% (2/5 cases), and Uterine malignancy in 50% (1/2 cases). No changes were observed in breast, pancreas, stomach, tongue, and other organ cases (100%).

Table 6: Distribution of changes in nodal involvement based on PANCK findings observed in this study

	H& E Positive	H& E Negative	Fisher's Exact Test, P Value
PANCK Positive	34	5	457.4, <0.0001
PANCK Negative	0	491	

All H&E-positive cases were also PANCK-positive, with 5 additional cases detected only by PANCK, showing strong concordance.Fisher's Exact Test ($p < 0.0001$) indicates PANCK significantly enhances detection over H&E alone.

Figures

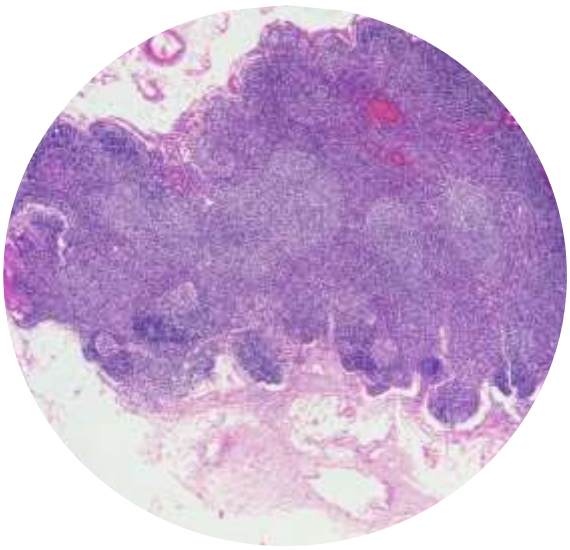

Figure 1- Endometrioid Carcinoma showing Metastatic Foci Missed On H&E

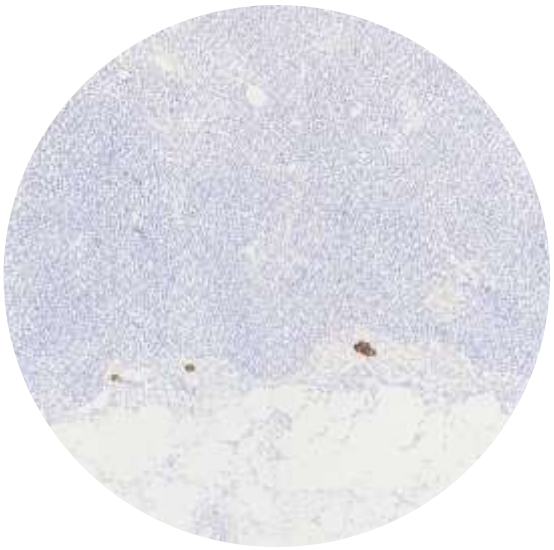

Figure 2-Micrometastatic Foci of Endometrioid Carcinoma Detected On IHC

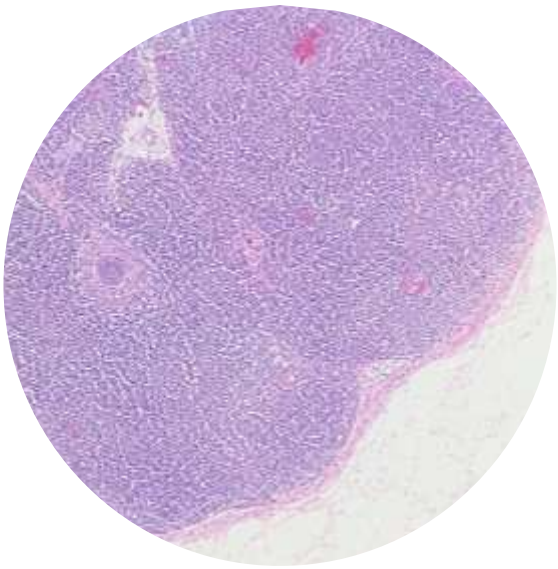

Figure 3- Moderately Differentiated Adenocarcinoma Of Colon showing Metastatic Foci Missed On H&E

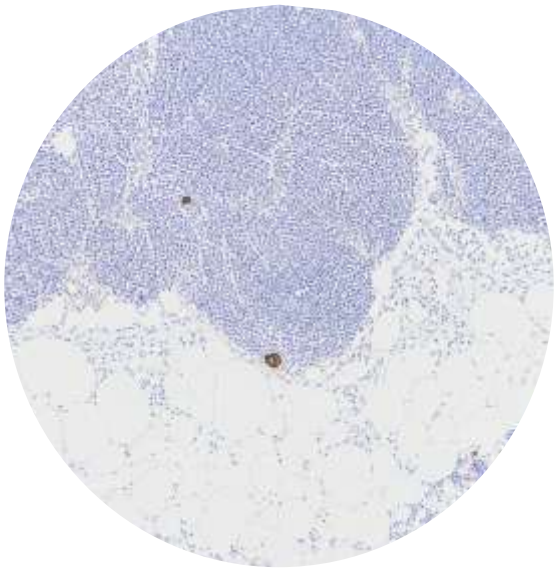

Figure 4-Micrometastatic Foci of Moderately Differentiated Adenocarcinoma Of Colon Detected On IHC

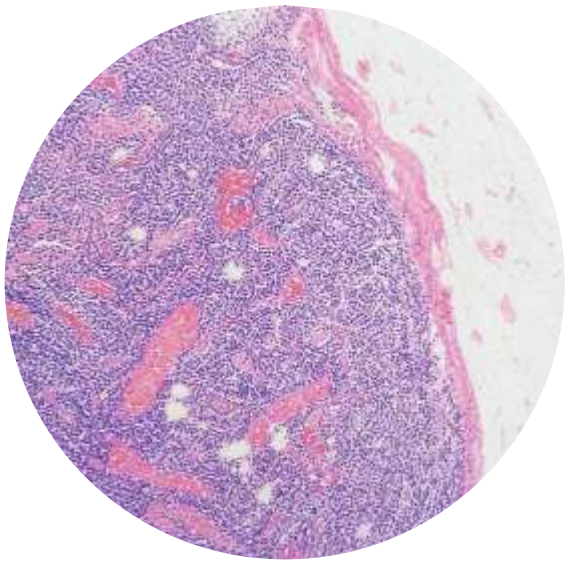

Figure 5-Invasive Breast Carcinoma With Mixed Ductal And Lobular Features showing Metastatic Foci Missed On H&E

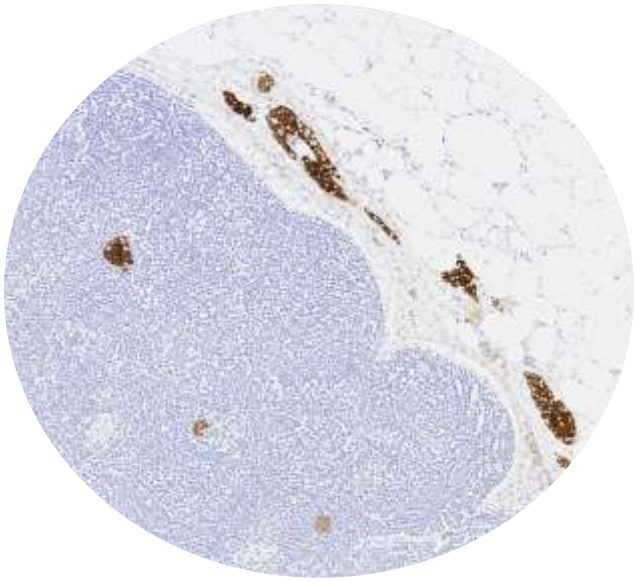

Figure 6- Micrometastatic Foci of Invasive Carcinoma of Breast With Mixed Ductal And Lobular Features Detected On IHC