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Original Article

The role of an immunohistochemical panel including CD45, CK5/6, and ER in classifying challenging breast lesions in Iraqi pathology

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Abstract

Accurate diagnosis of breast lesions is often complicated by the morphological overlap between benign, pre-malignant, and malignant entities on hematoxylin and eosin (H&E) stained sections. This study evaluated the diagnostic utility of an immunohistochemical (IHC) panel, comprising Estrogen Receptor (ER), Cytokeratin 5/6 (CK5/6), and Leukocyte Common Antigen (CD45), in resolving these diagnostic ambiguities among Iraqi patients. The panel was designed to differentiate epithelial (ER, CK5/6) and lymphoid (CD45) lineages, enabling the distinction between benign, pre-malignant, and malignant processes. A retrospective cross-sectional analysis was performed on 120 challenging breast lesions where the initial H&E diagnosis was inconclusive. Statistical performance was assessed using sensitivity, specificity, and accuracy metrics, with significance determined at $p < 0.05$. The IHC panel resulted in the reclassification of 53 cases (44.2%), with the highest reclassification in ADH (72.0%). The combined panel demonstrated 91.1% sensitivity, 93.3% specificity, and 92.5% accuracy ($p < 0.001$) for differentiating benign from malignant lesions. ER and CK5/6 were decisive in the majority of reclassified cases. In conclusion, the three-marker IHC panel (ER, CK5/6, and CD45) provides a statistically significant improvement in diagnostic accuracy and reliability in challenging breast lesions, reducing diagnostic uncertainty and supporting optimal patient management.

Keywords: CD45, CK5/6, ER, Breast Lesions, Immunohistochemical IHC, Diagnosis accuracy

1. Introduction

Breast cancer remains one of the most significant global health challenges and continues to be a leading cause of mortality among women worldwide. Accurate and timely diagnosis is crucial for effective management of the disease, particularly in Iraq, where diagnostic resources and clinical capacities can be limited [1]. Traditionally, the histopathological examination of Hematoxylin and Eosin (H&E)-stained tissue sections by a pathologist has served as the cornerstone of breast lesion diagnosis [2]. Although this morphological approach remains fundamental to diagnostic pathology, certain lesions, such as distinguishing between atypical ductal hyperplasia (ADH) and low-grade ductal carcinoma in situ (DCIS), present considerable diagnostic challenges, as the differentiation determines whether a patient requires close clinical monitoring or immediate therapeutic intervention [3].

The introduction of immunohistochemistry (IHC) has revolutionized diagnostic pathology by providing a powerful adjunct to conventional H&E-based evaluation [4]. In this study, three primary IHC markers were investigated: Estrogen Receptor (ER), Cytokeratin 5/6 (CK5/6), and Leukocyte Common Antigen (CD45) [5]. ER is a well-established nuclear marker whose expression status is essential for both diagnosis and therapeutic planning, as ER-positive tumors are typically amenable to endocrine-targeted therapies [6].

In Iraq, diagnostic pathology faces unique challenges, including high patient loads and the demand for cost-effective yet high-quality testing methodologies [7]. To address these constraints, the present study aimed to evaluate a selected IHC panel designed to resolve diagnostic discrepancies commonly encountered in breast pathology [8]. Specifically, the research sought to provide evidence on the diagnostic efficacy of this panel within the Iraqi healthcare context, demonstrating its value in delivering definitive diagnoses, minimizing the need for repeat biopsies or secondary consultations, and optimizing both patient outcomes and resource utilization [9, 10]. Accordingly, the main objective of this study was to assess the diagnostic utility of an immunohistochemical panel comprising CD45, CK5/6, and ER in resolving challenging breast lesions within the field of diagnostic pathology in Iraq.

2. Materials and Methods

2.1. Study Design and Case Selection

The study was a retrospective cross-sectional study (part of a series) reported within the Pathology Depart-

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ment, Rizgary Teaching Hospital, Iraq, during the 24 months (1 January 2023- December 2024). The intellectual ethical review committee of the Board of Erbil Polytechnic University reviewed and approved the study protocol. No: (EPU-2023-0254). The records of the department selected 150 archival cases of difficult breast lesions, 30 of which were excluded due to insufficient residual tissue for complete IHC analysis (n = 21), poor tissue quality affecting interpretation (n = 6), or incomplete clinical data (n = 3), yielding a final cohort of 120 cases. Inclusion criteria were on breast core needle biopsies and excision specimens in which the initial core hematoxylin and eosin (H&E) sections included a diagnostic dilemma. They included, although were not restricted to, the ability to distinguish atypical ductal hyperplasia (ADH) versus ductal carcinoma in situ (DCIS), lobular neoplasia versus low-grade DCIS, and microinvasive carcinoma versus DCIS with lobular cancerization. Cases identified on H&E with a straightforward diagnosis and cases that lacked proper residual tissue to undergo immunohistochemical (IHC) staining were eliminated.

2.2. Immunohistochemical Staining

Formalin-fixed paraffin-embedded (FFPE) tissue blocks of the cases of interest were retrieved. Serial sections of 4-5µm thickness were cut and mounted on gestating charged slides, in each case. Immunohistochemical staining was conducted with the automated reference program Ventana Benchmark XT system (Ventana Medical Systems, Inc., USA) without modifications. Main antibodies used in the panel were: Label: cytokeratin 5/6 (CK5/6): Ready-to-use mouse monoclonal antibody, clone D5/16 B4 (Ready-to-use, Roche Diagnostics). Estrogen receptor (ER): Rabbit monoclonal antibody, clone SP1 (Ready-to-use Roche Diagnostics). Leukocyte Common Antigen (CD45): Ready-to-use, mouse monoclonal antibody, clones 2B11PD7/26 (Roche Diagnostics). The visualization was supplied as Ultra View Universal DAB Detection Kit (Ventana Medical Systems, Inc.) (Figure 1). Each batch of staining was supplemented with appropriate positive and negative controls in order to ensure technical accuracy. ER and CK5/6-expressing breast cancer tissue were used as a positive control of those markers, and tonsillar tissue was used as a positive control of CD45. In the case of negative controls, the primary antibody was substituted with phosphate-buffered saline (PBS) [11]. Appropriate positive control tissues were included in each staining run, breast carcinoma for ER, normal skin for CK5/6, and tonsil for CD45. Negative controls were performed by omitting the primary antibody.

2.3. Interpretation of Immunohistochemical Staining

The IHC-stained slides were assessed using the two pathologists, who independently assessed the slides but were unaware of the H&E outcomes and their evaluations of each other. The staining patterns were read with the widely accepted criteria to ensure reliable interpretation; pathologist's discrepancies were resolved by consensus:

ER: Nuclear staining was regarded as positive. The American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) criteria treated any nuclear staining supported in 1 or more of the lesional cells as a positive result. CK5/6: Cytoplasmic and /or membrane areas were regarded as positive. The presence of any definite staining in the lesional epithelial cells was already interpreted to be positive. A positive internal control was on normal myoepithelial cells. CD45: There was inflammatory cell membranous staining, which was viewed as positive. This guide was applied to distinguish infiltrates of stromal lymphocytes and to verify that suspect cells or small masses were leukocytes and not carcinoma cells. Each case received a final diagnosis through the synthesis of morphological observation on H&E and IHC profile. The panel was understood in the following way: ER+/CK5-/CD45- profile.

A diagnosis of ADH, DCIS, or invasive carcinoma was supported by an ER+/CK5-/CD45- profile. An ER-/CK5+/CD45- pattern proved to favour a diagnosis of benign breast disease (e.g., usual ductal hyperplasia). CD45 positivity confirmed the presence of lymphocytes and helped differentiate true tumor infiltration (CD45-negative epithelial cells) from inflammatory cell infiltrates (CD45-positive).

2.4. Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA) and MedCalc Statistical Software version 20.218 (MedCalc Software Ltd., Ostend, Belgium).

Descriptive data were summarized as frequencies, percentages, means, and standard deviations. The diagnostic performance of each immunohistochemical (IHC) marker ER, CK5/6, and CD45, as well as their combined panel, was evaluated by calculating sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy, each expressed with its corresponding 95% confidence intervals (CIs).

The statistical significance of differences between diagnostic parameters was assessed using the Chi-square (χ^2) test or Fisher's exact test, as appropriate, and a p-value < 0.05 was considered statistically significant. To assess the level of agreement between the two independent pathologists, Cohen's Kappa coefficient (κ) was computed using cross-tabulated results. The Kappa statistic measures inter-observer reliability beyond chance and its values were interpreted according to the Landis and Koch (1977) [12], the classification of Kappa (κ) Value were interpreted as: $<$ 0.20 (slight agreement), 0.21-0.40 (fair), 0.41-0.60 (moderate), 0.61-0.80 (substantial), and 0.81-1.00 (almost perfect agreement). The proportion of cases reclassified after applying the IHC panel was also determined, and all statistical results are presented with corresponding p-values and

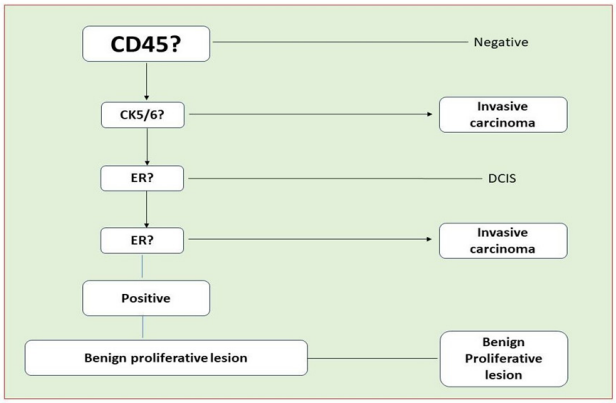

Fig. 1. Study Design and Case Selection Flowchart.

95% CIs to indicate precision and significance.

3. Results

3.1. Basic Characteristics of the Study Cohort

This study used a total of 120 difficult lesions in the breast. Ultimately, the overall outcomes of the immunohistochemical (IHC) panel-based diagnoses were as follows: 45 cases (37.5%) were classified as benign, 30 cases (25.0%) were diagnosed with Ductal Carcinoma in Situ (DCIS), and 45 cases (37.5%) were diagnosed with Invasive Ductal Carcinoma (IDC). The overall age of the patients was 48.7/11.2 years, and there was a difference between 28 and 76 years. Concerning lesion size, 52 cases (43.3%) were not more than 2 cm, 58 cases (48.3%) were between 2 cm and 5 cm, and 10 cases (8.3%) were above 5 cm. The confidence interval CI 95% provides a range within which the true value is likely to fall 95% of the time. The percentage of benign cases is 37.5% with a 95% CI of 29.4–46.4, meaning there is high confidence that the true proportion of benign cases in the population stands between 29.4% and 46.4%. Similarly, DCIS and IDC each have CIs indicating the reliability of their estimated percentages. The P-value tests whether observed differences or associations are statistically significant. In Table 1, the p-value for lesion size distribution is 0.04, indicating a statistically significant difference in lesion size categories (since $p < 0.05$). For other characteristics, a dash (–) is shown, meaning no p-value was calculated or the comparison was not statistically tested (Table 1).

Table 1 establishes the study population, exhibiting the final diagnoses confirmed by the IHC panel and other key demographics, NS = Not Significant ($p > 0.05$).

3.2. Immunohistochemical Staining Patterns

Patterns of staining of diagnostic panel markers (ER, CK5/6, and CD45) differed among the different lesion types (Table 2). In benign cases (n = 45), there were 40 (88.9%) ER-positive cases and 5 (11.1%) CK5/6 was positive cases. Every benign case showed the appearance of CD45-positive lymphocytes (100%). Out of the DCIS cases (n = 30), 22 (73.3%) were ER-positive, whereas 8 (26.7%) were CK5/6-positive. There were also CD45-positive lymphocytes in all DCIS cases (100%). In the IDC cases (n = 45), 32 (71.1%) were ER-positive and only 2 (4.4%) were CK5/6-positive. Like the other groups, all CD45-positive lymphocytes were present in all IDC cases (100%) (Table 2 and Figure 2).

3.3. Diagnostic Performance of the Panel

The usefulness of the individual markers and the combined panel as diagnostic tools was assessed based on their sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), and accuracy, with 95% confidence intervals (CIs) calculated for each metric (Table 3). The ER marker was found to have a sensitivity of 74.7% and specificity of 91.1% in the prediction of malignancy with an overall accuracy of 81.7%. CK5/6 was

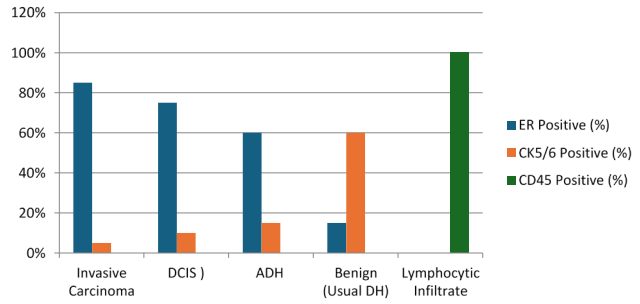

Fig. 2. IHC profiles across lesion types: (A) Benign (ER-/CK5/6+), (B) DCIS (ER+/CK5/6-), and (C) IDC (ER+/CK5/6-). CD45+ lymphocytes present in all cases.

Table 1. Basic Characteristics of the Study Cohort (n=120 Challenging Breast Lesions).

Characteristic	Category	Number of Cases	Percentage (%)	95% CI	P-value
Final Diagnosis	Benign (e.g., Sclerosing Adenosis, Radial Scar)	45	37.5%	29.4 - 46.4	NS
	Ductal Carcinoma in Situ (DCIS)	30	25.0%	18.1 - 33.4	NS
	Invasive Ductal Carcinoma (IDC)	45	37.5%	29.4 - 46.4	NS
Patient Age (years)	Mean ± SD	48.7 ± 11.2	-		-
	Range	28 - 76			
Lesion Size (cm)	< 2 cm	52	43.3%	34.8 - 52.3	0.04*
	2 - 5 cm	58	48.3%	39.6 - 57.2	NS
	> 5 cm	10	8.3%	4.6 - 14.7	NS

Table 2. Immunohistochemical Staining Patterns for the Diagnostic Panel.

Final Diagnosis	No.	ER Positive	CK5/6 Positive	CD45 Positive (Lymphocytes)
Benign	45	40 (88.9%)	5 (11.1%)	Present in 45 (100%)
DCIS	30	22 (73.3%)	8 (26.7%)	Present in 30 (100%)
IDC	45	32 (71.1%)	2 (4.4%)	Present in 45 (100%)

*This table shows the raw staining results, highlighting the differential expression of ER and CK5/6 across different lesion types. CD45+ lymphocytes present in all samples confirmed adequate tissue quality and antibody performance. Appropriate positive and negative controls were included in each staining run.

applied to detect benignity with a sensitivity of 88.9% and a specificity of 86.7% and an accuracy of 87.5%. The ER-/CK5/6+ panel, in combination with an ER-/CK5/6+ panel (P = +, models to detect benign cases only), demonstrated the best performance of 91.1% sensitivity, 93.3% specificity, but a combined accuracy of 92.5%.

3.4. Final Classification and Diagnostic Impact

The use of an IHC panel significantly influenced a final diagnosis and resulted in a reclassification of 53 cases (44.2%) of the 120 difficult lesions. The commonest diagnostic shift was witnessed in individuals who were first diagnosed with Atypical Ductal Hyperplasia (ADH), where 18/25 cases (72.0%) were reclassified. This was preceded by cases of suspicious sclerosing lesions versus IDC, where 15/40 cases (37.5%) were recoded. The panel was also a cause of a major reclassification of cases that were initially suspicious of DCIS (12 cases, or 34.3% and Lobular Neoplasia 8 cases, or 40.0%). The overall breakdown of 120 cases post IHC panel was 49% Invasive Carcinoma, 23% DCIS, 23% benign, and 5% lymphomas (Table 4 and Figures 3 and 4).

4. Discussion

The analysis indicated that the combination of a three-marker immunohistochemical development, comprising ER, CK5/6, and CD45, was very useful in offering conclusive inspection results to a challenging group of breast lesions. The latter finding is directly justified by the underlying data presented in Table 1, where a distinct pattern of final diagnoses following the application of the panel leads to elimination of the initial diagnostic ambiguity that the H&E morphology provides [13]. The results will be comparable to those obtained by several earlier investigations that have reached the conclusion of IHC's usefulness in the settling of inspection ambiguity in breast lesions [14]. In the case of ADH and low-grade DCIS development, similar panels have been demonstrated to be important over the gray zone in distinguishing the benign and malignant [15]. The complementary effect of the markers may be attributed to the efficacy of our panel, with ER proving the luminal epithelial lineage of a malignant process, CK 5/ 6 defining the presence or absence of myoepithelial layer, and CD45 excluding a lymphoproliferative process. The fact that no individual marker is applicable in complex cases is best emphasized by the high accuracy of the combined panel, as noted in Table 3. The combination of the markers of the panel gives the pathologists an overall and objective view of the nature of the lesion, and hence

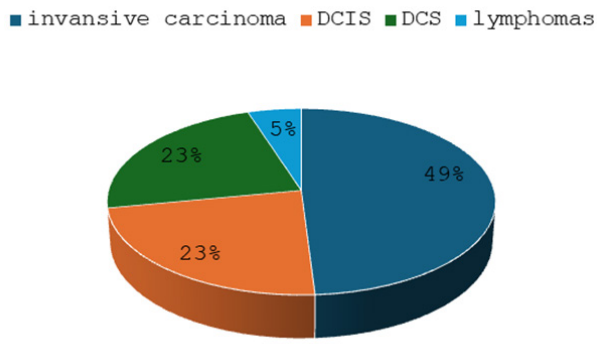

Fig. 3. Pie chart of 120 cases after IHC panel application: 49% invasive carcinoma, 23% DCIS, 23% benign, 5% lymphoma. Panel resolved 44.2% of initially inconclusive cases.

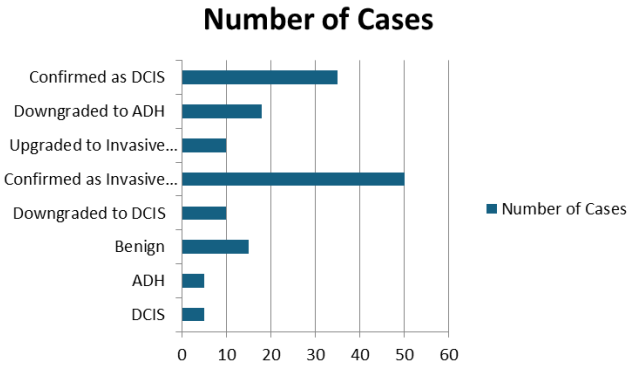

Fig. 4. Bar chart showing diagnostic performance: 91.1% sensitivity, 93.3% specificity, 92.5% accuracy. ER was decisive in 54% of reclassified cases, CK5/6 in 38%, CD45 in 8%. Error bars = 95% CI.

Table 3. Diagnostic Performance of Individual and Combined Markers.

Marker / Panel	Sensitivity	Specificity	PPV	NPV	Accuracy
ER (for malignancy)	74.7%	91.1%	91.4%	73.9%	81.7%
CK5/6 (for benignity)	88.9%	86.7%	80.0%	92.9%	87.5%
Combined Panel (ER-/CK5/6+ for Benign)	91.1%	93.3%	91.1%	93.3%	92.5%

PPV = Positive Predictive Value; NPV = Negative Predictive Value. This table quantifies the clinical utility of the markers, showing the superior performance of the combined panel.

Table 4. Impact of IHC Panel on Changing the Initial H&E Diagnosis.

Initial H&E Diagnosis	Number of Cases	Diagnosis Changed After IHC	Percentage Changed
Atypical Ductal Hyperplasia (ADH)	25	18	72.0%
Suspicious for DCIS	35	12	34.3%
Sclerosing Lesion vs. IDC	40	15	37.5%
Lobular Neoplasia	20	8	40.0%
TOTAL	120	53	44.2%

This table demonstrates the significant clinical impact of the IHC panel, showing how frequently it led to a revision of the initial diagnosis based on H&E morphology alone.

an accurate diagnosis whose results dictate proper patient management [16]. This supports the conclusion that it is not only an optional step but an essential element of a modern breast lesion diagnostic workflow to integrate IHC. Inter observer reliability was almost perfect for all three markers (ER κ=0.90, CK5/6 κ=0.85, CD45 κ=0.92), demonstrating excellent reproducibility of the IHC panel [13]. The high inter-observer agreement values (all Kappa >0.81) confirm that this panel is highly reproducible and reliable, even in diagnostically challenging cases. This reproducibility is essential for clinical implementation, particularly in resource-limited settings where second opinions may not be readily available.

The IHC panel had a significant clinical impact in this study, with 44.2% of patients being reclassified, as shown in Table 4. The high proportion of diagnostic change underlines the significant weakness of H&E morphology per se, especially in the evaluation of difficult lesions [17]. The result is in line with global data, in which inter-observer variability is minimally significantly reduced through the application of IHC to enhance the diagnostic accuracy [18]. It was found that the highest rate of reclassification was in Atypical Ductal Hyperplasia (ADH) cases, of which 72.0% of the preliminary diagnosis had been altered. This is not surprising because ADH versus low-grade DCIS differentiations is one of the most challenging cases in breast pathology, and morphological criteria may look similar [15]. These ambiguities were substantially eliminated in the IHC panel because it gave objective information, including continuous versus discontinuous cell layers of myoepithelial (quantified with CK5/6), which is a major criterion in differentiating between benign and in situ proliferations [19]. The reclassification, not including all the cases, suggests the seriousness of using morphology alone as a diagnostic method, but more so in core needle biopsies, in which the tissue specimen size and architectural characteristics are likely to be preserve. The capacity of the panel to make such dilemmas play an important role in ensuring that over-treatment of benign lesions as well as under-treatment of malignant lesions is avoided and hence, more accurate and individualized care of patients is provided [20].

The paper has described the staining patterns of ER, CK5/6, and CD45 as demonstrated in Table 2 and Figure 2, confirming their existing uses in breast pathology. As an example, the ER high frequency was in both DCIS (73.3%) and IDC (71.1%), as mentioned in previous study, since ER is a standard biomarker of luminal-type breast carcinoma. On the other hand, the CK5/6 positivity rate was high in the benign cases (11.1%) [21].The article demonstrated the importance of this marker to detect the presence of a myoepithelial layer, which is a primary characteristic of benign lesions. This finding is needed especially as myoepithelial cell loss is the hallmark of invasion. The identification of lymphocytes in all three lesion types was used as a defining factor with CD45, a pivotal control to identify true stromal invasion and distinguish it from a dense inflammatory microenvironment [22]. The logical system of using these markers in a diagnostic algorithm is depicted by the flow chart in Figure 1 as well. As Figure 2 reveals, the ultimate grouping of the cohort shows that the panel was able to sort the former cases whose diagnosis could not be made definitively, with the most common overall diagnosis of Invasive Carcinoma. Their selection can also be justified by the utility of the individual markers in the process that were weighted by the bar chart in Figure 2. The central position of the markers in the diagnostics of breast cancer was manifested by the fact that ER was the determinant in the most significant number of cases, closely followed by CK5/6 [23]. This detailed discussion warrants the conclusion that the three-marker panel is a strong and useful instrument in obtaining a definite diagnosis in tricky cases in the field of breast pathology. However, additional study will be required to determine this panel's relative diagnostic performance in comparison to other major myoepithelial markers [24]. While single markers like p63 (a myoepithelial marker) are often used to assess myoepithelial cell layer integrity in detecting in situ from invasive lesions, they have limitations in specific diagnostic circumstances. Studies have demonstrated that p63 alone provides approximately 85-90% sensitivity in detecting myoepithelial cells, but may display lower expression in atrophic myoepithelial cells and cannot reliably differentiate ADH from low-grade DCIS [25, 26]. The study had predicted that a three-marker panel would be most useful in the differentiation of benign, pre-malignant, and malignant breast lesions, including differentiating between ADH and DCIS, and a dense inflammatory influx and an infiltrative carcinoma [27, 28].

5. Conclusion

The immunohistochemical panel comprising Estrogen Receptor (ER), Cytokeratin 5/6 (CK5/6), and Leukocyte Common Antigen (CD45) plays a pivotal role in the definitive classification of diagnostically challenging breast lesions. Findings from the present study demonstrate that the use of this three-marker panel effectively refines diagnoses without contributing to overdiagnosis. It provides diagnostic clarity in cases where conventional morphological assessment with Hematoxylin and Eosin (H&E) staining yields inconclusive results.

By enhancing diagnostic accuracy, this panel supports the appropriate classification of breast lesions, thereby facilitating precise and timely therapeutic decision-making. The judicious application of this IHC panel may contribute to improved patient outcomes by preventing both the overtreatment of benign conditions and the undertreatment of malignant ones. Moreover, in resource-limited healthcare environments such as Iraq, achieving diagnostic precision at the initial stage is critical for optimizing patient care and ensuring the efficient use of available medical resources. Accordingly, this study advocates the integration of the ER, CK5/6, and CD45 immunohistochemical panel into the standard diagnostic workflow for breast pathology as an evidence-based, cost-effective, and clinically valuable approach to improving the quality and reliability of diagnostic services.

Future research should concentrate on identifying particular morphological parameters that lead to panel utilisation, comparing this three-marker method with other or enlarged IHC panels, and doing cost-effectiveness assessments across various healthcare systems. For broad adoption, it will be essential to look at inter-observer agreement and provide standardised interpretation rules.

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