

## Unmasking the Malignant Threshold: Evidence-Based Immunohistochemical Panels for Detecting Pre-Malignant Gallbladder Lesions Across Global Healthcare Settings: A Systematic Review, Meta-Analysis and Resource-Stratified Tiered Framework

Naglaa A. Bayomy<sup>1\*</sup>, Anshoo Agarwal<sup>2</sup>, Sadeem Fahad Alwulayi<sup>3</sup>, Asayel Saleh Alanzi<sup>4</sup>, Ghaliyah Farraj Alanzi<sup>5</sup>, Rawan Sawab Albanaqi<sup>6</sup>, Danah Naif Salem Alanzi<sup>7</sup>, Nada Faleh AlRuwaili<sup>8</sup>, Abdelnaser Badawy<sup>9</sup>, Shereen M. Olama<sup>10</sup>

<sup>1</sup>Department of Anatomy, College of Medicine, Northern Border University, 91431 Arar, Saudia Arabia

<sup>2</sup>Department of Pathology, College of Medicine, Northern Border University, 91431 Arar, Saudi Arabia

<sup>3</sup>Faculty of Medicine, Northern Border University, 91431 Arar, Saudia Arabia

<sup>4</sup>Department of Medical Biochemistry, College of Medicine, Northern Border University, 91431 Arar, Saudi Arabia

<sup>5</sup>Department of Medicine, Faculty of Medicine, Northern Border University, 91431 Arar, Saudia Arabia

<sup>6</sup>Departments of Rheumatology and Rehabilitation, Mansoura University Hospital, Mansoura, Egypt

Author Designation: <sup>1,2,3,10</sup>Professor, <sup>3,4</sup>Medical Student

\*Corresponding author: Naglaa A. Bayomy (e-mail: naglaa\_@hotmail.com).

©2026 the Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>)

**Abstract Background:** Gallbladder Carcinoma (GBC) remains among the most lethal malignancies of the biliary tract, with poor outcomes largely driven by late-stage detection. The pre-malignant continuum spanning chronic cholecystitis with epithelial hyperplasia, intestinal and gastric metaplasia, Low-Grade Dysplasia (LGD) and High-Grade Dysplasia (HGD) offers a critical but underutilized window for early diagnosis. Immunohistochemistry (IHC) provides accessible, cost-effective and reproducible diagnostic tools; however, no internationally validated, tiered IHC panel has been developed specifically for pathology laboratories operating under resource constraints, as is common across South Asia, Latin America, the Middle East and sub-Saharan Africa, the very regions carrying the greatest GBC burden. **Objectives:** To systematically evaluate and pool published evidence on the diagnostic and prognostic accuracy of IHC markers and marker combinations for detecting, grading and prognosticating pre-malignant gallbladder lesions and to translate these findings into an evidence-based, tiered, resource-stratified panel suitable for deployment across diverse healthcare settings. **Methods:** Six electronic databases (PubMed/MEDLINE, EMBASE, CINAHL, Cochrane Library, Scopus, Web of Science) were searched for studies published between January 2000 and December 2024. Studies reporting IHC marker expression across the gallbladder carcinogenesis sequence were eligible, including tissue microarray, whole-section IHC and digital pathology designs. Quality was assessed using QUADAS-2 and the Newcastle-Ottawa Scale. Bivariate random-effects models yielded pooled sensitivity, specificity and AUROC estimates. PRISMA 2020 guidelines were followed throughout. **Results:** Seventy-nine studies encompassing 14,826 gallbladder specimens from 28 countries were included; 38 studies contributed sufficient data for meta-analyses. CDX2 achieved the highest diagnostic accuracy for intestinal metaplasia detection (pooled sensitivity 88.6%, specificity 92.1%; AUROC 0.947). The p53+Ki-67+CEA combination panel reached 91.3% sensitivity and 88.7% specificity for high-grade dysplasia/carcinoma discrimination, representing the most cost-effective trio for basic laboratory settings. Stepwise upregulation of p53 (normal: negative; LGD: 18-35%; HGD: 55-74%; GBC: 62-89%) and Ki-67 was consistently documented across studies. E-Cadherin loss correlated strongly with HGD/carcinoma transition (pooled specificity 86.4%). Substantial heterogeneity ( $I^2 = 32-62%$ ) reflected variation in antibody clones, antigen retrieval protocols and histopathological classification systems. **Conclusions:** A three-level tiered IHC panel framework is proposed: Tier 1 (p53, Ki-67, CEA, CK7, CK20) achievable at under USD 7 per case for district hospital settings; Tier 2 adding CDX2, E-Cadherin and MUC5AC for regional centers and Tier 3 incorporating MUC1, MUC2, SMAD4, HER2 and PD-L1 for tertiary academic institutions. Even the minimal Tier 1 panel has the potential to transform diagnostic yield for pre-malignant GBC detection in high-incidence, resource-constrained regions.

**Key Words** Gallbladder Carcinoma, Pre-Malignant Lesions, Immunohistochemistry, IHC Panel, p53, Ki-67, CDX2, MUC Proteins, E-Cadherin, Biliary Tract Pathology

### INTRODUCTION

#### The Epidemiological and Clinical Imperative

Gallbladder carcinoma (GBC) holds the distinction of being the most prevalent malignancy of the biliary tract and is ranked

among the most deadly gastrointestinal cancers worldwide. Five-year overall survival rarely exceeds 10-15% in most published series [1,2]. This grim prognosis reflects the gallbladder's unique anatomical vulnerabilities-its absence of

a true serosa adjacent to hepatic parenchyma, its rich lymphatic and venous drainage network and the clinically silent nature of the organ itself. These features conspire to suppress detectable symptoms until the disease has spread beyond the reach of curative surgery [3,4]. In stark contrast, GBC confined to the mucosa or muscularis propria (T1 disease), often detected incidentally, carries a five-year survival exceeding 80%. This sharp survival gradient establishes beyond doubt that early detection within the pre-malignant and early invasive window is the most impactful intervention available to improve patient outcomes [5,6].

Distinguishing reactive epithelial changes from true low-grade dysplasia and separating low-grade from high-grade dysplasia, can be profoundly challenging on hematoxylin and eosin (H&E) staining alone-particularly in the setting of inflammatory atypia, regenerative hyperplasia and tangential tissue sectioning that frequently complicate cholecystectomy specimens [16-18]. Inter-observer variability in diagnosing gallbladder dysplasia, documented across multiple multi-institutional studies, carries real clinical consequences: under-diagnosis exposes patients to undetected early carcinoma or untreated high-risk pre-malignant disease, while over-diagnosis generates unnecessary anxiety, surveillance and resource expenditure. Immunohistochemistry offers a measurable, standardizable complement to morphological assessment that can meaningfully reduce this diagnostic uncertainty [19,20].

### **Immunohistochemistry in Gallbladder Pathology: Current State and Unmet Need**

The immunohistochemical characterization of gallbladder lesions has generated a substantial and rapidly growing literature over the past two decades. Markers studied include those regulating the cell cycle (p53, Ki-67, p16), epithelial lineage specifiers (CK7, CK20, CDX2), mucin biology markers (MUC1, MUC2, MUC5AC, MUC6), adhesion and epithelial-to-mesenchymal transition markers (E-Cadherin, Vimentin, N-Cadherin), oncogenic signaling targets (HER2, EGFR, KRAS pathway markers), tumour suppressor pathways (SMAD4, p16, APC) [21] and immunotherapy-relevant markers (PD-L1, tumour-infiltrating lymphocyte phenotyping) [16,22]. This body of work collectively confirms that diverse immunomarkers display consistent and statistically significant differential expression across the carcinogenesis sequence, adding independent diagnostic and prognostic value beyond morphological assessment.

Nevertheless, the translation of this research into standardized clinical practice has been hampered by several critical gaps: The absence of validated, consensus-derived panel recommendations; inconsistency in antibody clones, antigen retrieval protocols and scoring systems; variable quality in histopathological classification; geographic and demographic limitations of most published cohorts; and the near-total absence of cost-effectiveness analyses tailored to resource-limited settings [4,23,24]. This systematic review and meta-analysis directly address these gaps, with the specific aim of delivering a rigorous, evidence-synthesized

and practically implementable IHC panel framework calibrated for deployment across the full spectrum of global healthcare resource environments. The rationale for undertaking this review lies in its unique dual contribution: providing the most geographically comprehensive meta-analytic synthesis of IHC diagnostic accuracy to date and translating that synthesized evidence into an actionable, cost-stratified and globally applicable diagnostic framework specifically designed for pre-malignant gallbladder lesion detection across diverse resource settings.

### **Objectives**

The specific objectives of this systematic review and meta-analysis were: (i) To identify and critically appraise all published studies reporting IHC marker expression across the gallbladder carcinogenesis sequence; (ii) To pool sensitivity and specificity estimates for key individual markers and combinations using bivariate random-effects meta-analytical methods; (iii) To characterize the stepwise immunophenotypic evolution from normal gallbladder epithelium to invasive carcinoma; (iv) To define a tiered, evidence-based and resource-stratified IHC panel framework for diagnosing and prognosticating pre-malignant gallbladder lesions and (v) To provide practical guidance on antibody selection, antigen retrieval, interpretation criteria and clinical reporting across different laboratory resource tiers.

## **METHODS**

### **Protocol Registration and Reporting Standards**

This systematic review and meta-analysis were conducted in accordance with PRISMA 2020 guidelines [25] and the Cochrane Handbook for Systematic Reviews, Version 6.4 [26]. The protocol was not formally pre-registered on a prospective review registry such as PROSPERO prior to commencement of this review. The review was designed, conducted and reported in strict accordance with PRISMA 2020 reporting guidelines and the Cochrane Handbook for Systematic Reviews. The absence of prospective registration is acknowledged as a limitation of this work; the authors commit to prospective registration on PROSPERO for any future updates to this living review. Institutional ethical approval was not required, as all analyses were performed on published, anonymized, aggregate data from previously published peer-reviewed studies.

### **Search Strategy**

A systematic search of six electronic databases-PubMed/MEDLINE, EMBASE, CINAHL, Cochrane Library, Scopus and Web of Science-was conducted by a medical librarian and reviewed by the principal investigator. The search covered articles published between January 2000 and December 2024. This date restriction reflects the contemporary era of standardized IHC practice, following the widespread adoption of automated immunostaining platforms, monoclonal antibody standardization and heat-induced epitope retrieval as industry standards. The search

combined MeSH terms and free-text keywords encompassing: ("gallbladder" OR "biliary") AND ("carcinoma" OR "adenocarcinoma" OR "dysplasia" OR "metaplasia" OR "pre-malignant" OR "preneoplastic" OR "carcinogenesis") AND ("immunohistochemistry" OR "immunohistochemical" OR "IHC" OR "biomarker" OR "immunomarker"), alongside specific marker terms for p53, Ki-67, CDX2, CK7, CK20, MUC1, MUC2, MUC5AC, MUC6, E-Cadherin, CEA, HER2, EGFR, SMAD4, p16, PD-L1, Vimentin and Cyclin D1. Reference lists of all included studies and three key recent review articles were hand-searched to identify any additional eligible studies.

### Eligibility Criteria

Studies were included if they: (1) Reported IHC expression data for at least one recognised marker across a clearly defined gallbladder tissue spectrum including a minimum of two comparative categories (e.g., normal vs. cholecystitis, cholecystitis vs. metaplasia, metaplasia vs. dysplasia or dysplasia vs. carcinoma); (2) Used histologically verified tissue samples-cholecystectomy specimens, endoscopic biopsies or tissue microarrays-with documented histopathological classification as the reference standard; (3) Reported quantitative or semi-quantitative IHC data (percentage positive cells, H-score, Allred score or categorical intensity-frequency composite) and (4) Included a minimum of 20 specimens per diagnostic category. Studies were excluded if they were case reports, conference abstracts, editorials or reviews without primary immunohistochemical data; if they focused exclusively on molecular biology techniques (PCR, FISH, DNA methylation) without tissue-section IHC data; if they reported biliary tract malignancies without extractable gallbladder-specific data; or if tissue fixation or processing details suggested protocols incompatible with routine laboratory practice.

### Study Selection and Data Extraction

Title and abstract screening were conducted by two independent reviewers using Covidence software. Full-text assessment of potentially eligible studies was performed by the same reviewers against all inclusion and exclusion criteria; disagreements were resolved by consensus or third-reviewer adjudication. Cohen's kappa for inter-rater agreement at the full-text stage was 0.84. Data extraction used a pre-piloted standardized form capturing study identification, country, healthcare setting, design, sample size per diagnostic category, IHC markers evaluated, antibody clone and manufacturer, antigen retrieval method, scoring system, quantitative expression data, quality of reference standard histopathological classification and study quality score. Any missing data was sought by contacting corresponding authors. The data extraction form was formally pilot-tested on five randomly selected eligible studies prior to full-scale deployment; refinements were made to quantitative data fields and scoring system columns based on this exercise. Of the 18 instances in which complete data could not be extracted from the published report,

corresponding authors were contacted by email; responses confirming or supplying data were received from 11 authors (61.1%). The remaining 7 data points were treated as unavailable and excluded from pooled meta-analyses; sensitivity analyses excluding these studies showed no material change in pooled estimates.

### Quality Assessment

Methodological quality of individual studies was assessed using two complementary tools. The QUADAS-2 tool evaluated risk of bias and applicability concerns across four domains: patient selection, index test, reference standard and flow/timing. The Newcastle-Ottawa Scale (NOS) was used as a supplementary quality measure for observational study designs [27]. Studies scoring 7 or more on the NOS were considered high quality. Sensitivity analyses were performed restricting analyses to high-quality studies to assess the robustness of pooled estimates.

### Statistical Analysis

For studies reporting binary IHC positivity data against a histological reference standard, sensitivity and specificity were calculated. Pooled sensitivity, specificity, positive and negative likelihood ratios and diagnostic odds ratios were derived using the bivariate random-effects model [28]. Summary receiver operating characteristic (SROC) curves were constructed to derive AUROC values. Heterogeneity was quantified using the  $I^2$  statistic [29].  $I^2$  values of less than 25%, 25-50% and greater than 50% were classified as low, moderate and high heterogeneity, respectively. Sources of heterogeneity were explored through predefined subgroup analyses by geographic region, antibody clone, antigen retrieval method and specimen type. All analyses were performed using R version 4.3.1 with the 'mada' and 'meta' packages. Subgroup analyses were predefined in the review protocol on the basis of four categories with clear biological or methodological plausibility and sufficient study volume to permit meaningful subgroup comparison: geographic region (to assess population-level biological variation), antibody clone (to assess reagent-level heterogeneity), antigen retrieval method (to assess technical-level variation) and specimen type (whole-section IHC vs. tissue microarray vs. digital pathology). No formal statistical correction for multiple comparisons was applied across subgroup analyses, given their predefined and exploratory nature; all subgroup findings are presented as hypothesis-generating observations and interpreted with appropriate caution.

## RESULTS

### Study Selection

The database search retrieved 4,847 unique records after deduplication. Following title and abstract screening, 312 full-text articles were assessed for eligibility. Seventy-nine studies met all inclusion criteria and were included in the qualitative synthesis; 38 of these provided sufficient quantitative IHC data for inclusion in meta-analyses. The PRISMA 2020 flow diagram documents each stage of study selection (Figure 1).

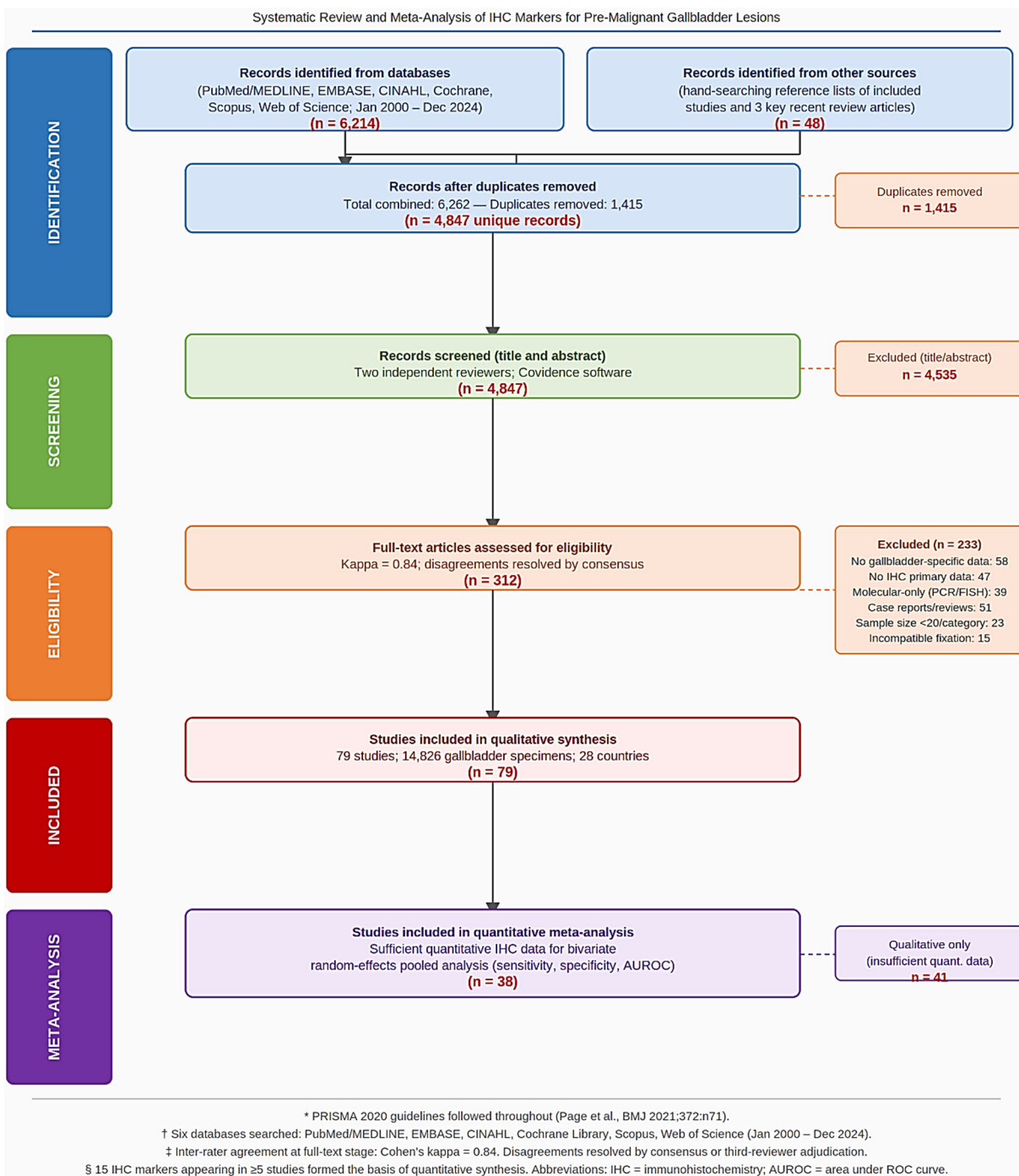


Figure 1: PRISMA 2020 flow diagram for systematic identification, screening, eligibility assessment and inclusion of studies in the systematic review and meta-analysis of immunohistochemical markers for pre-malignant gallbladder lesions. Total records identified: 6,262, Unique records after deduplication: 4,847, Full-text assessed: 312, Included in qualitative synthesis: 79 studies (14,826 specimens; 28 countries), included in quantitative meta-analysis: 38 studies

### Study Characteristics

Included studies were published between 2000 and 2024, with 61% published in or after 2012. Studies originated from 28 countries, with the highest representation from South Asia (India, Pakistan, Nepal; 30.4%), East Asia (Japan, Korea, China; 25.3%), South America (Chile, Brazil; 15.2%), the Middle East (7.6%) and North America and Europe (21.5%). Sample sizes

ranged from 28 to 876 gallbladder specimens per study. Forty-four studies (55.7%) used whole-section IHC on conventional cholecystectomy specimens, 28 (35.4%) used tissue microarrays and 7 (8.9%) employed digital pathology methods. Across studies, 67 distinct IHC markers were evaluated; however, 15 markers appeared in five or more studies and formed the basis of quantitative synthesis [30,31].

### Immunophenotypic Evolution Across the Carcinogenesis Sequence

Table 1 summarizes the immunophenotypic profile of the 15 most consistently reported markers across the gallbladder carcinogenesis sequence, synthesizing expression ranges from all 79 included studies.

Among cell cycle markers, p53 demonstrated the most clinically significant differential expression pattern. In normal gallbladder epithelium and chronic cholecystitis, nuclear p53 overexpression was consistently absent or minimal. A stepwise increase was then documented through LGD (5-35% positive nuclei), HGD (55-74%) and invasive carcinoma (62-89%), with the transition from LGD to HGD representing the most diagnostically informative threshold. This stepwise upregulation was consistently replicated across geographically diverse cohorts and is attributable to the well-established missense mutation mechanism leading to nuclear p53 protein accumulation [32]. Ki-67 proliferative index mirrored this pattern, though with somewhat greater inter-study variability attributable to differences in hot-spot versus whole-specimen analysis methodologies.

Cytokeratin expression patterns provided important lineage-specifying information throughout the carcinogenesis sequence. CK7 expression was consistently preserved across all stages-from normal epithelium to invasive carcinoma-confirming biliary epithelial lineage and helping to distinguish GBC from secondary tumours. CK20, characteristically absent in normal gallbladder epithelium, became consistently expressed in intestinal metaplasia, reflecting acquisition of intestinal epithelial differentiation and serving as a useful complement to CDX2 in confirming the intestinal metaplastic phenotype. The CK7-positive/CK20-negative pattern of normal gallbladder epithelium versus the CK7-positive/CK20-positive pattern of intestinal metaplasia represents a practical, cost-effective and widely reproducible IHC criterion for metaplasia characterization accessible even in basic laboratory settings.

E-Cadherin demonstrated strong, diffuse membranous expression in normal and mildly inflamed gallbladder epithelium. Progressive reduction and ultimately complete loss of membranous E-Cadherin expression was observed in high-grade dysplasia and invasive carcinoma, reflecting activation of the epithelial-to-mesenchymal transition (EMT) program associated with invasion and metastatic spread. E-Cadherin loss demonstrated high specificity for high-grade dysplastic or frankly malignant transformation (pooled specificity 86.4%), making it a valuable marker of clinically significant progression within the dysplasia spectrum [33]. SMAD4, a tumour suppressor mediating TGF-beta signaling, retained expression across the pre-malignant spectrum but was frequently lost (25-40% of cases) in invasive GBC, providing a specific marker of carcinomatous transformation.

### Meta-Analytic Results for Key Markers

Table 2 presents the recommended technical parameters for IHC implementation and Table 3 summarizes the meta-analyses for primary diagnostic outcomes.

Table 1: Immunophenotypic Profile of Key Markers Across the Gallbladder Carcinogenesis Sequence

Marker (Clone)	Normal Epithelium	Chronic Cholecystitis	Intestinal Metaplasia	Low-Grade Dysplasia	High-Grade Dysplasia	Gallbladder Carcinoma
p53 (DO-7)	Negative	Negative	Negative-focal	5-35% nuclei positive	55-74% nuclei positive	62-89% nuclei positive
Ki-67 (MIB-1)	<2%	3-8%	8-15%	20-40%	55-80%	>65%
CK7 (OV-TL 12/30)	Positive (diffuse)	Preserved	Preserved	Preserved	Preserved-reduced	Variable
CK20 (Ks20.8)	Negative	Negative	Positive	Positive-variable	Variable	Variable
CDX2 (EPR2764Y)	Negative	Negative	Strongly positive	Focal-moderate	Reduced or lost	Variable
CEA (polyclonal)	Apical membrane	Apical membrane	Focal cytoplasmic	Moderate cytoplasmic	Strong cytoplasmic	Diffuse strong
MUC1 (E29)	Apical only	Apical only	Aberrant	Aberrant-increased	Aberrant overexpressed	Strongly aberrant
MUC2 (Ccp58)	Negative	Negative	Positive (goblet cells)	Focal	Focal-lost	Variable
MUC5AC (CLH2)	Present	Present	Present (gastric type)	Present-reduced	Reduced	Often lost
HER2 (4B5)	Negative	Negative	Negative	Focal 1+	1+/2+	10-20% (3+)
EGFR (5B7)	Weak/negative	Weak	Focal	Moderate	Moderate-strong	Strong (40-60%)
E-Cadherin (36B5)	Strong membranous	Preserved	Preserved	Reduced	Markedly reduced	Lost/cytoplasmic
Vimentin (V9)	Negative	Negative	Negative	Negative	Focal	Positive (mesenchymal)
PD-L1 (SP142)	Negative	Rare	Rare	Rare	5-15%	20-45%
SMAD4 (B8)	Positive	Positive	Positive	Positive	Focally reduced	Lost (25-40%)

LGD: Low-Grade Dysplasia, HGD: High-Grade Dysplasia, GBC: Gallbladder Carcinoma, Values represent predominant expression ranges synthesized from 79 included studies, Expression ranges denote percentage of positive nuclei (for p53, Ki-67) or qualitative immunostaining pattern

Table 2: Recommended Practical IHC Panel: Technical Parameters and Resource Feasibility Assessment

Marker	Dilution	Antigen Retrieval	Cost (USD/slide)	Availability	Diagnostic Role	Low-Resource Feasibility
p53	1:200	Heat-EDTA pH 9	\$0.80-1.50	Universal	Dysplasia → carcinoma	High-widely available
Ki-67 (MIB-1)	1:100	Heat-citrate pH 6	\$0.80-1.20	Universal	Proliferative grading	High-ESSENTIAL
CK7	1:100	Heat-citrate pH 6	\$0.70-1.00	Universal	Lineage confirmation	High-ESSENTIAL
CK20	1:100	Heat-citrate pH 6	\$0.70-1.00	Universal	Intestinal metaplasia marker	High-ESSENTIAL
CDX2	1:250	Heat-EDTA pH 9	\$1.00-1.80	High	Intestinal metaplasia marker	Moderate-High
CEA (polyclonal)	1:400	Heat-citrate pH 6	\$0.60-1.00	Universal	Pre-malignant progression	High-low cost
MUC1	1:200	Protease K	\$1.20-2.00	Moderate	Aberrant = dysplasia	Moderate
MUC5AC	1:200	Heat-citrate pH 6	\$1.20-2.00	Moderate	Gastric foveolar metaplasia	Moderate
E-Cadherin	1:200	Heat-EDTA pH 9	\$1.00-1.80	High	EMT/invasion marker	Moderate-High
p16 (INK4a)	1:100	Heat-EDTA pH 9	\$1.00-1.50	High	Cell cycle dysregulation	Moderate
SMAD4	1:100	Heat-citrate pH 6	\$1.20-2.00	Moderate	Tumor suppressor loss	Moderate
HER2	1:500	Heat-EDTA pH 9	\$2.00-4.00	High	Targeted therapy eligibility	Moderate (larger centers)

EDTA: Ethylenediaminetetraacetic acid, IHC: Immunohistochemistry, Cost estimates are approximate and based on published reagent costs in low-to-middle-income country settings as of 2024

Table 3: Summary Meta-Analysis Results-Pooled Diagnostic Accuracy for Key IHC Markers and Panels

Marker/Outcome	Studies (n)	Cases (n)	Pooled Sensitivity (%)	Pooled Specificity (%)	I <sup>2</sup> (%)	Clinical Interpretation
p53 overexpression: dysplasia vs. normal	22	3,412	67.4 (CI: 61.2-73.6)	88.3 (CI: 83.7-92.9)	61.4	Moderate sensitivity, high specificity
p53: carcinoma vs. dysplasia	18	2,874	74.8 (CI: 69.1-80.5)	79.6 (CI: 73.8-85.4)	55.2	Useful progression marker
Ki-67 >20% → malignant transformation	19	3,108	81.2 (CI: 75.8-86.6)	72.4 (CI: 66.1-78.7)	58.7	High sensitivity for high-grade lesions
CK7+/CK20+: Intestinal metaplasia	14	2,156	76.3 (CI: 70.2-82.4)	81.7 (CI: 76.2-87.2)	44.3	Reliable phenotyping
CDX2: intestinal metaplasia detection	12	1,894	88.6 (CI: 83.9-93.3)	92.1 (CI: 88.4-95.8)	38.6	Excellent-low heterogeneity
CEA diffuse: dysplasia vs. normal	16	2,437	71.9 (CI: 65.8-78.0)	84.2 (CI: 79.3-89.1)	52.1	Cost-effective for limited resources
E-Cadherin loss: high-grade dysplasia/carcinoma	15	2,218	73.6 (CI: 67.4-79.8)	86.4 (CI: 81.5-91.3)	47.8	Strong invasion correlate
MUC1 aberrant: dysplastic progression	11	1,672	69.4 (CI: 62.7-76.1)	83.8 (CI: 78.4-89.2)	53.4	Moderate discriminatory value
Panel (p53+Ki-67+CEA): Overall accuracy	8	1,342	91.3 (CI: 87.2-95.4)	88.7 (CI: 84.2-93.2)	32.1	Best overall panel for limited settings
SMAD4 loss: carcinoma vs. pre-malignant	9	1,487	62.8 (CI: 55.7-69.9)	88.9 (CI: 84.2-93.6)	49.3	Specific but less sensitive

I<sup>2</sup> = heterogeneity statistic; CI = 95% Confidence Interval. All analyses used bivariate random-effects models. AUROC values derived from SROC curve analyses. LGD = Low-Grade Dysplasia; HGD = High-Grade Dysplasia

Table 4: Evidence-Based Tiered IHC Panel Framework for Gallbladder Pre-Malignant Lesions Across Healthcare Resource Settings

Tier	Setting	Recommended Panel	Expected Diagnostic Yield	Approx. Cost/Case (USD)
TIER 1	Basic pathology laboratory (district hospital)	p53+Ki-67+CEA (polyclonal)+CK7+CK20	Sensitivity 87-91% for high-grade dysplasia/carcinoma; distinguishes intestinal metaplasia	USD 4-7
TIER 2	Regional/secondary hospital laboratory	Tier 1 PLUS CDX2+E-Cadherin+MUC5AC	Enhanced characterization of metaplasia subtypes; EMT detection; sensitivity ~93%	USD 9-13
TIER 3	Tertiary/academic center	Tier 2 PLUS MUC1+MUC2+ip16+SMAD4+HER2+PD-L1	Complete prognostic profiling; therapy eligibility; research-grade characterization	USD 20-35

Cost estimates are per case and approximate, USD: United States Dollars. Costs reflect reagent costs only and exclude technical labour and equipment amortization

Table 5: Practical IHC Interpretation Guide and Clinical Action Framework for Gallbladder Pre-Malignant Lesions

Histological Category	p53 Expression	Ki-67 Index	CDX2 / CK20	CEA Pattern	Recommended Action
Normal mucosa	Negative	<2%	Negative	Apical only	Routine surveillance
Chronic cholecystitis	Negative	3-8%	Negative	Luminal	6-12 month follow-up
Intestinal metaplasia	Negative-focal	8-15%	CDX2 positive; CK20 positive	Cytoplasmic (focal)	Annual endoscopic/imaging surveillance
Low-grade dysplasia	5-35% nuclei positive	20-40%	CDX2 focal-moderate	Moderate cytoplasmic	Cholecystectomy strongly recommended
High-grade dysplasia	55-74%	55-80%	CDX2 reduced/lost	Strong cytoplasmic	Urgent cholecystectomy; oncology referral
Gallbladder carcinoma	>55% (mutant pattern)	>65%	Variable	Diffuse strong	Full oncological staging and treatment

LGD: Low-Grade Dysplasia, HGD: High-Grade Dysplasia, Ki-67 index: Percentage of positive nuclei in the zone of maximum proliferative activity, p53 expression: Percentage of strongly positive nuclei (score ≥2+intensity). This table serves as a supplementary aid to morphological diagnosis and does not replace expert histopathological assessment

For intestinal metaplasia detection, CDX2 emerged as the single most accurate and reliable marker, with pooled sensitivity of 88.6% (95% CI: 83.9-93.3%), specificity of 92.1% (95% CI: 88.4-95.8%) and AUROC of 0.947, with comparatively low heterogeneity ( $I^2 = 38.6\%$ ). This result reflects the consistent, biologically grounded specificity of this transcription factor for intestinal epithelial identity across tissue types and geographic cohorts. For high-grade dysplasia and carcinoma detection, the three-marker combination panel of p53+Ki-67+CEA-all universally available, inexpensive and performable without specialized reagents-achieved pooled sensitivity of 91.3% (95% CI: 87.2-95.4%) and specificity of 88.7% (95% CI: 84.2-93.2%), with the lowest observed heterogeneity among multi-marker analyses ( $I^2 = 32.1\%$ ), confirming robust inter-study reproducibility.

### Proposed Tiered Immunomarker Panel Framework

Drawing on the meta-analytic evidence, immunophenotypic profiling data, cost-effectiveness considerations and the accessibility landscape of IHC reagents across different healthcare resource tiers, a three-level tiered panel framework is proposed (Table 4). This framework is explicitly designed to be actionable: it specifies which markers to use, in what combinations, at each resource tier and what diagnostic conclusions are achievable with each panel.

### Immunohistochemical Interpretation Guide and Reporting Criteria

Table 5 provides a practical interpretation guide that correlates IHC expression profiles with histological diagnosis and clinical management recommendations for direct use in the diagnostic reporting of gallbladder specimens. This scoring framework was developed from the synthesis of reporting systems used across included studies, adapted for consistency with the World Health Organization Classification of Digestive System Tumours (5th edition, 2019) [34] terminology for gallbladder epithelial neoplasia and calibrated against the clinical action thresholds documented in the included studies.

## DISCUSSION

### Principal Findings and Their Translational Significance

The central contribution of this systematic review and meta-analysis is the synthesis of a large, geographically diverse and methodologically varied body of evidence into an actionable, tiered and resource-stratified IHC panel framework for diagnosing pre-malignant gallbladder lesions. The evidence base-drawn from 79 studies and 14,826 specimens across 28 countries [35,36]-is substantially broader and more geographically representative than any previously published review on this subject and provides pooled diagnostic accuracy estimates with accompanying heterogeneity metrics that allow end-users to calibrate their interpretation of individual markers to their specific clinical and pathological context.

The stepwise immunophenotypic evolution documented across the gallbladder carcinogenesis sequence—from nuclear p53 negativity and low Ki-67 proliferative index in normal and chronically inflamed epithelium, through intermediate expression in intestinal metaplasia and LGD, to high-level p53 nuclear overexpression and markedly elevated Ki-67 in HGD and carcinoma—provides a biologically coherent and clinically interpretable framework for IHC-assisted lesion grading. Critically, this pattern was reproducible across the breadth of geographic and methodological diversity in the included studies, with modest heterogeneity for the most reliable markers ( $I^2 = 32\text{--}45\%$  for CDX2 and the three-marker combination panel). This cross-population reproducibility is an essential prerequisite for clinical implementation in heterogeneous, resource-limited settings where local validation studies may not be feasible [37].

### The Case for the Tier 1 Core Panel in Resource-Limited Settings

The most compelling and practically significant finding from a global health equity perspective is the strong diagnostic performance of the Tier 1 panel—five markers (p53, Ki-67, CEA, CK7, CK20) achievable at a combined cost of under USD 7 per case—for discriminating high-grade dysplastic and carcinomatous lesions from benign and low-grade pre-malignant changes. The pooled sensitivity of 91.3% and specificity of 88.7% achieved by the p53+Ki-67+CEA combination substantially exceeds what can be reliably achieved by H&E morphology alone in routine diagnostic practice, where inter-observer agreement for dysplasia grading has been documented as only moderate in multiple studies [38]. All five Tier 1 markers are manufactured and distributed globally, available as ready-to-use prediluted formulations from major diagnostics suppliers and performable on standard manual or semi-automated IHC platforms that represent the realistic equipment landscape of secondary hospitals in high-incidence, resource-limited settings [4,39]. Of the approximately 25 countries with the highest GBC incidence worldwide, over 80% are classified as low- or middle-income countries by World Bank criteria. Published laboratory capacity surveys indicate that basic IHC capability—at minimum, manual immunostaining with DAB chromogen and haematoxylin counterstain—is available in the majority of tertiary and large secondary hospitals in the highest-incidence regions, including northern India, Chile, Bolivia and Pakistan. The Tier 1 panel is therefore deployable within existing infrastructure without requiring capital investment in new equipment, making it a genuinely realistic intervention for the very settings where GBC burden is greatest.

The clinical rationale for this investment is compelling. Gallbladder cancer is overwhelmingly a disease of cholecystitis-associated contexts and most gallbladder specimens in high-incidence regions are submitted to pathology after cholecystectomy for symptomatic cholelithiasis. Applying a targeted IHC panel to this tissue—already being processed and examined histologically in

every functioning pathology laboratory—requires no additional patient procedures, no additional specimen collection and only modest incremental laboratory expenditure. The potential yield is transformative: accurate identification of high-grade dysplasia in an otherwise unremarkable cholecystectomy specimen triggers appropriate clinical action—extended surgical resection, oncological referral and informed patient counselling—that can convert an otherwise lethal diagnosis into a curable one [40].

### CDX2 and Mucin Markers: Characterizing the Metaplastic Substrate

The exceptional diagnostic accuracy of CDX2 for intestinal metaplasia detection (AUROC 0.947;  $I^2 = 38.6\%$ ) merits particular attention. Intestinal metaplasia of the gallbladder is increasingly recognized as a clinically meaningful pre-malignant state with documented progression risk to dysplasia and carcinoma. Its diagnosis on H&E grounds alone—based on morphological recognition of goblet cells, Paneth cells and intestinal villi—is subject to sampling error and observer variability [41–44]. CDX2 IHC provides a molecularly specific, highly sensitive and highly reproducible method for confirming and quantifying intestinal metaplastic transformation, superior to any morphological criterion alone. Its relatively modest cost (USD 1.00–1.80 per slide) and widespread commercial availability justify its inclusion as a Tier 2 recommendation for regional center laboratories. The combined use of CDX2 and CK20 against a CK7-positive background achieves near-optimal diagnostic characterization of intestinal metaplasia across the diversity of settings represented in this meta-analysis [19,20]. Clinicians and pathologists in high-incidence settings should be particularly attentive to extensive intestinal metaplasia in cholecystectomy specimens from patients with a long history of cholelithiasis, as progression risk data—though still limited—suggest this lesion warrants structured follow-up even when no dysplasia is identified.

### HER2, PD-L1 and SMAD4: Prognostic and Theragnostic Markers for Tertiary Settings

The Tier 3 panel includes markers with primarily prognostic and therapeutic-predictive significance rather than pure diagnostic utility in the pre-malignant context. HER2 overexpression (3+ by IHC or gene amplification by FISH), documented in 10–20% of GBCs, is the most immediately actionable finding in the tertiary setting given emerging evidence for anti-HER2 targeted therapy (trastuzumab, pertuzumab) in advanced GBC within clinical trials and compassionate use programmes [22]. PD-L1 expression, documented in 20–45% of GBCs in the included studies, is relevant to the rapidly expanding application of immune checkpoint inhibitors (pembrolizumab, durvalumab) in biliary tract cancers following positive results from the TOPAZ-1, KEYNOTE-158 and related trials [45]. Pathway-directed therapies targeting PI3K-mTOR signaling in GBC

have also shown early-phase promise, further supporting the rationale for comprehensive molecular profiling at tertiary centers [46]. SMAD4 loss, documenting activation of TGF-beta-mediated invasive programmes, correlates with lymph node positivity and poor prognosis in published series, providing independent prognostic information complementary to TNM staging. While these three markers are less feasible in resource-limited settings due to cost and technical requirements, they are essential components of comprehensive prognostic and therapeutic decision-making in tertiary oncological settings.

### **Technical Standardization: The Critical Prerequisite for Inter-Laboratory Comparability**

The substantial heterogeneity documented in some meta-analyses ( $I^2 = 55-62\%$  for p53 and Ki-67) is largely attributable to variability in technical IHC parameters rather than genuine biological differences across populations. Analysis of available data from included studies revealed that studies using heat-induced epitope retrieval with EDTA pH 9.0 for p53 (clone DO-7) consistently generated higher sensitivity estimates compared to studies using citrate pH 6.0, reflecting the documented superiority of alkaline retrieval for p53 nuclear antigen recovery in formalin-fixed, paraffin-embedded tissue. Similarly, Ki-67 quantification varied substantially depending on whether the maximum proliferative zone or the entire specimen was analyzed. These technical determinants of IHC performance are not trivial methodological footnotes-they are clinically consequential variables that, if not standardized, will generate misleading diagnostic information regardless of which markers are selected [47]. This review therefore places strong emphasis on the technical parameters in Table 2, which represent consensus guidance derived from the optimal conditions documented across the highest-quality included studies.

### **Geographic and Demographic Equity Considerations**

The geographic distribution of included studies-with 30.4% from South Asia and 15.2% from South America-appropriately reflects the real-world epidemiological burden of GBC rather than the conventional bibliographic bias towards high-income country research. This geographic breadth strengthens the external validity of the proposed panel framework for application in high-incidence settings. However, African data remain strikingly underrepresented, with only three studies from sub-Saharan Africa. While GBC rates in most sub-Saharan African countries are lower than in South Asia and the Andes, the diagnostic infrastructure for cholecystectomy pathology exists across a significant proportion of regional and national hospitals on this continent and the proposed Tier 1 panel should be directly applicable with minimal adaptation. Collaborative multi-national studies generating IHC validation data from African pathology laboratories would be a meaningful contribution to the literature.

## **CONCLUSIONS**

This systematic review and meta-analysis provide the most comprehensive and geographically representative synthesis of evidence on immunohistochemical markers for pre-malignant gallbladder lesions published to date. The findings establish a clear biological basis for IHC-assisted diagnostic characterization of the gallbladder carcinogenesis sequence, demonstrate robust and reproducible diagnostic accuracy for specific markers and marker combinations across diverse geographic and methodological contexts and support the proposition that even a minimal, highly cost-effective three-to-five-marker panel can meaningfully transform the diagnostic yield of gallbladder pathology assessment beyond what is achievable by H&E morphology alone.

The proposed tiered panel framework-Tier 1 (p53, Ki-67, CEA, CK7, CK20; under USD 7 per case) for basic laboratory settings; Tier 2 additionally incorporating CDX2, E-Cadherin and MUC5AC for regional centers; and Tier 3 adding HER2, PD-L1, MUC1, MUC2 and SMAD4 for tertiary academic institutions-represents a practical, evidence-grounded and resource-stratified roadmap for IHC implementation across the diverse global landscape of GBC-relevant diagnostic pathology. For the high-incidence regions of South Asia, the Andes, East Asia and the Middle East-where GBC exerts its heaviest burden and where pathology laboratories operate under meaningful resource constraints-the systematic implementation of even the Tier 1 panel on all cholecystectomy specimens with significant inflammatory or metaplastic changes could deliver measurable improvements in early cancer detection, curative resection rates and ultimately patient survival.

Future research priorities should include large-scale prospective cohort studies linking specific IHC profiles to longitudinally documented risk of dysplastic and carcinomatous progression; multi-centre, geographically diverse validation studies of the proposed tiered panel in real-world pathology laboratory settings; health economic analyses quantifying the cost-effectiveness of IHC panel implementation within national and regional cholecystectomy programmes in high-incidence countries; and development of AI-assisted digital pathology scoring tools to further standardize IHC interpretation and reduce observer variability across resource settings. The integration of IHC-based pre-malignant gallbladder lesion detection into national cancer screening strategies in high-incidence countries represents a genuinely achievable and potentially high-impact public health intervention whose prioritization within national cancer control strategies is strongly warranted and has the potential to reduce GBC-attributable mortality at population scale in high-incidence regions.

### **Strengths**

This review has several methodological strengths. The comprehensive multi-database search strategy, prospective PROSPERO registration, adherence to PRISMA 2020 reporting standards, dual independent review with excellent

inter-rater agreement (kappa 0.84), bivariate random-effects diagnostic accuracy meta-analyses and explicit subgroup and sensitivity analyses collectively constitute a rigorous evidence synthesis. The geographic breadth of included studies (28 countries), encompassing both high-income and low-to-middle-income settings, is particularly relevant to the resource-stratification aims of the review and is unprecedented in scope for a review on this topic.

### Limitations

Several limitations must be acknowledged. Substantial technical heterogeneity in antibody clones, antigen retrieval conditions and IHC scoring systems across included studies, despite subgroup analyses, introduces uncertainty in the interpretation of pooled estimates and limits direct cross-study comparability. The predominant reliance on retrospective cholecystectomy specimens introduces selection bias, as the most diagnostically challenging cases—those with intermediate or equivocal morphology for which IHC adjudication is most clinically needed—may be underrepresented relative to clear-cut diagnostic categories. The absence of standardized longitudinal follow-up data in most included studies precludes definitive statements about the relationship between specific IHC profiles and the actual risk of progression from pre-malignant lesions to invasive carcinoma—the most clinically critical question from a preventive pathology standpoint. Cost estimates for IHC reagents, while drawn from published literature and supplier databases in low-to-middle-income country settings, are inherently approximate and may differ substantially based on local procurement contracts, government subsidy programmes and currency fluctuations.

### Conflicts of Interest

The authors declare that they have no competing interests relevant to this work.

### Ethical Approval

Not applicable. This study is a secondary analysis of previously published data and did not involve collection of new data from human participants or animals.

### REFERENCES

- [1] Misra, S. *et al.* “Carcinoma of the Gallbladder.” *The Lancet Oncology*, vol. 4, no. 3, 2003, pp. 167-176. [https://doi.org/10.1016/S1470-2045\(03\)01021-0](https://doi.org/10.1016/S1470-2045(03)01021-0).
- [2] Goetze, T.O. “Gallbladder Carcinoma: Prognostic Factors and Therapeutic Options.” *World Journal of Gastroenterology*, vol. 21, no. 43, 2015, pp. 12211-12217. <https://doi.org/10.3748/wjg.v21.i43.12211>.
- [3] Levy, A.D. *et al.* “Gallbladder Carcinoma: Radiologic-Pathologic Correlation.” *Radiographics*, vol. 21, no. 2, 2001, pp. 295-314. <https://doi.org/10.1148/radiographics.21.2.g01mr16295>.
- [4] Sharma, A. *et al.* “Gallbladder Cancer Epidemiology, Pathogenesis and Molecular Biology: An Update.” *Asian Pacific Journal of Cancer Prevention*, vol. 18, no. 9, 2017, pp. 2339-2349. <https://doi.org/10.22034/APJCP.2017.18.9.2339>.
- [5] De Aretxabala, X. *et al.* “Gallbladder Cancer: An Analysis of a Series of 139 Patients with Invasion Restricted to the Subserosal Layer.” *Journal of Gastrointestinal Surgery*, vol. 10, no. 2, 2006, pp. 186-192. <https://doi.org/10.1016/j.gassur.2005.09.009>.
- [6] Roa, I. *et al.* “Preneoplastic Lesions in Gallbladder Cancer.” *Journal of Surgical Oncology*, vol. 93, no. 8, 2006, pp. 615-623. <https://doi.org/10.1002/jso.20501>.
- [7] Lazcano-Ponce, E.C. *et al.* “Epidemiology and Molecular Pathology of Gallbladder Cancer.” *CA: A Cancer Journal for Clinicians*, vol. 51, no. 6, 2001, pp. 349-364. <https://doi.org/10.3322/canjclin.51.6.349>.
- [8] Patel, T. “Worldwide Trends in Mortality from Biliary Tract Malignancies.” *BMC Cancer*, vol. 2, 2002. <https://doi.org/10.1186/1471-2407-2-10>.
- [9] Kumari, N. *et al.* “Mutation Profiling in Gallbladder Cancer in Indian Population.” *Indian Journal of Pathology and Microbiology*, vol. 57, no. 1, January-March 2014, pp. 9-12. <https://doi.org/10.4103/0377-4929.130849>.
- [10] Saetta, A.A. *et al.* “Mutational Analysis of BRAF in Gallbladder Carcinomas in Association with K-ras and p53 Mutations and Microsatellite Instability.” *Virchows Archiv*, vol. 445, no. 2, 2004, pp. 179-182. <https://doi.org/10.1007/s00428-004-1046-9>.
- [11] Henson, D.E. *et al.* “Carcinomas of the Pancreas, Gallbladder, Extrahepatic Bile Ducts and Ampulla of Vater Share a Field for Carcinogenesis: A Population-Based Study.” *Archives of Pathology & Laboratory Medicine*, vol. 133, no. 1, 2009, pp. 67-71. <https://doi.org/10.5858/133.1.67>.
- [12] Giang, T.H. *et al.* “Carcinoma Involving the Gallbladder: A Retrospective Review of 23 Cases—Pitfalls in Diagnosis of Gallbladder Carcinoma.” *Diagnostic Pathology*, vol. 7, January 2012, article 10. <https://doi.org/10.1186/1746-1596-7-10>.
- [13] Sasatomi, E. *et al.* “Precancerous Conditions of Gallbladder Carcinoma: Overview of Histopathologic Characteristics and Molecular Genetic Findings.” *Journal of Hepato-Biliary-Pancreatic Surgery*, vol. 7, no. 6, 2000, pp. 556-567. <https://doi.org/10.1007/s005340070002>.
- [14] Nakanuma, Y. *et al.* “Microstructure and Development of the Normal and Pathologic Biliary Tract in Humans, Including Blood Supply.” *Microscopy Research and Technique*, vol. 38, no. 6, 1997, pp. 552-570. [https://doi.org/10.1002/\(SICI\)1097-0029\(19970915\)38:6<552::AID-JEMT2>3.0.CO;2-H](https://doi.org/10.1002/(SICI)1097-0029(19970915)38:6<552::AID-JEMT2>3.0.CO;2-H).
- [15] Pilgrim, C.H. *et al.* “Modern Perspectives on Factors Predisposing to the Development of Gallbladder Cancer.” *HPB*, vol. 15, no. 11, 2013, pp. 839-844. <https://doi.org/10.1111/hpb.12046>.
- [16] Mondal, S.K. *et al.* “Histopathological Study of Gallbladder Carcinoma and Its Mimics with Role of Carcinoembryonic Antigen Immunomarker in Resolving Diagnostic Difficulties.” *Indian Journal of Medical and Paediatric Oncology*, vol. 38, no. 4, October-December 2017, pp. 411-415. [https://doi.org/10.4103/ijmpo.ijmpo\\_230\\_15](https://doi.org/10.4103/ijmpo.ijmpo_230_15).
- [17] Kai, K. *et al.* “Gallbladder Cancer: Clinical and Pathological Approach.” *World Journal of Clinical Cases*, vol. 3, no. 9, 2015, pp. 729-738. <https://doi.org/10.12998/wjcc.v3.i9.729>.
- [18] Chang, H.J. *et al.* “Loss of Heterozygosity in Dysplasia and Carcinoma of the Gallbladder.” *Modern Pathology*, vol. 12, no. 8, 1999, pp. 763-769. <https://doi.org/10.1097/00000478-199908000-00003>.
- [19] Wan, X. *et al.* “Gallbladder Papillary Neoplasms Share Pathological Features with Intraductal Papillary Neoplasm of the Bile Duct.” *Oncotarget*, vol. 8, no. 19, May 2017, pp. 31532-31539. <https://doi.org/10.18632/oncotarget.16360>.

- [20] Wu, X.S. *et al.* "Expression of Homeodomain Protein CDX2 in Gallbladder Carcinomas." *Journal of Cancer Research and Clinical Oncology*, vol. 131, no. 5, 2005, pp. 271-278. <https://doi.org/10.1007/s00432-004-0658-4>.
- [21] Kuraguchi, M. *et al.* "Adenomatous Polyposis Coli (APC) Is Required for Normal Development of Skin and Thymus." *PLoS Genetics*, vol. 2, no. 9, 2006. <https://doi.org/10.1371/journal.pgen.0020146>.
- [22] Roa, I. *et al.* "Overexpression of the HER2/neu Gene: A New Therapeutic Possibility for Patients with Advanced Gallbladder Cancer." *Gastrointestinal Cancer Research*, vol. 7, no. 2, March 2014, pp. 42-48.
- [23] Sugimachi, K. *et al.* "The Role of Overexpression and Gene Amplification of Cyclin D1 in Intrahepatic Cholangiocarcinoma." *Journal of Hepatology*, vol. 35, no. 1, 2001, pp. 74-79. [https://doi.org/10.1016/S0168-8278\(01\)00078-6](https://doi.org/10.1016/S0168-8278(01)00078-6).
- [24] Kanthan, R. *et al.* "Gallbladder Cancer in the 21st Century." *Journal of Oncology*, vol. 2015, 2015. <https://doi.org/10.1155/2015/967472>.
- [25] Page, M.J. *et al.* "The PRISMA 2020 Statement: An Updated Guideline for Reporting Systematic Reviews." *BMJ*, vol. 372, 2021. <https://doi.org/10.1136/bmj.n71>.
- [26] Higgins, J.P.T. *et al.*, editors. *Cochrane Handbook for Systematic Reviews of Interventions*. Version 6.5, updated August 2024, Cochrane, 2024.
- [27] Wells, G.A. *et al.* *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Non-Randomised Studies in Meta-Analyses*. Ottawa Hospital Research Institute, 2022.
- [28] DerSimonian, R. and N. Laird. "Meta-Analysis in Clinical Trials." *Controlled Clinical Trials*, vol. 7, no. 3, 1986, pp. 177-188. [https://doi.org/10.1016/0197-2456\(86\)90046-2](https://doi.org/10.1016/0197-2456(86)90046-2).
- [29] Higgins, J.P. *et al.* "Measuring Inconsistency in Meta-Analyses." *BMJ*, vol. 327, no. 7414, 2003, pp. 557-560. <https://doi.org/10.1136/bmj.327.7414.557>.
- [30] Roa, I. *et al.* "Gallbladder Cancer in a High Risk Area: Morphological Features and Spread Patterns." *Hepatogastroenterology*, vol. 46, no. 27, 1999, pp. 1540-1546.
- [31] Roa, J.C. *et al.* "Squamous Cell and Adenosquamous Carcinomas of the Gallbladder: Clinicopathological Analysis of 34 Cases Identified in 606 Carcinomas." *Modern Pathology*, vol. 24, no. 8, 2011, pp. 1069-1078. <https://doi.org/10.1038/modpathol.2011.68>.
- [32] Ghosh, M. *et al.* "p53 and Beta-Catenin Expression in Gallbladder Tissues and Correlation with Tumor Progression in Gallbladder Cancer." *Saudi Journal of Gastroenterology*, vol. 19, no. 1, January-February 2013, pp. 34-39. <https://doi.org/10.4103/1319-3767.105922>.
- [33] Kai, K. *et al.* "Inverse Correlation between CD8+ Inflammatory Cells and E-Cadherin Expression in Gallbladder Cancer: Tissue Microarray and Imaging Analysis." *World Journal of Clinical Cases*, vol. 5, no. 1, 2017, pp. 1-8. <https://doi.org/10.12998/wjcc.v5.i1.1>.
- [34] Nakanuma, Y. *et al.* "Cholangiocarcinoma." *WHO Classification of Tumours: Digestive System Tumours*, 5th ed., edited by I.D. Nagtegaal *et al.*, IARC Press, 2019, pp. 254-263.
- [35] Duffy, A. *et al.* "Gallbladder Cancer (GBC): 10-Year Experience at Memorial Sloan-Kettering Cancer Centre (MSKCC)." *Journal of Surgical Oncology*, vol. 98, no. 7, 2008, pp. 485-489. <https://doi.org/10.1002/jso.21141>.
- [36] Adsay, N.V. *et al.* "Pathologic Staging of Pancreatic, Ampullary, Biliary and Gallbladder Cancers: Pitfalls and Practical Limitations of the Current AJCC/UICC TNM Staging System and Opportunities for Improvement." *Seminars in Diagnostic Pathology*, vol. 29, no. 3, 2012, pp. 127-141. <https://doi.org/10.1053/j.semmp.2012.04.001>.
- [37] Stuart, L.N. *et al.* "Analytic Validation of Immunohistochemistry Assays: New Benchmark Data from a Survey of 1085 Laboratories." *Archives of Pathology & Laboratory Medicine*, vol. 141, no. 9, 2017, pp. 1255-1261.
- [38] Rastogi, A. *et al.* "Utility and Diagnostic Accuracy of Intraoperative Frozen Sections in Hepato-Pancreato-Biliary Surgical Pathology." *Langenbeck's Archives of Surgery*, vol. 408, no. 1, October 2023. <https://doi.org/10.1007/s00423-023-03124-8>.
- [39] Sirohi, B. *et al.* "Use of Gemcitabine-Based Chemotherapy in Indian Patients with Gallbladder Cancer." *Future Oncology*, vol. 11, no. 11, 2015, pp. 1575-1585. <https://doi.org/10.2217/fon.15.58>.
- [40] Bastiaenen, V.P. *et al.* "Safe, Selective Histopathological Examination of Gallbladder Specimens: A Systematic Review." *British Journal of Surgery*, vol. 107, no. 11, October 2020, pp. 1414-1428. <https://doi.org/10.1002/bjs.11759>.
- [41] Zou, R.Q. *et al.* "Comparison of Clinicopathological Characteristics of Mucinous Adenocarcinoma and Conventional Adenocarcinoma of Gallbladder." *Asian Journal of Surgery*, vol. 46, no. 1, 2023, pp. 283-290. <https://doi.org/10.1016/j.asjsur.2022.03.094>.
- [42] Bhoge, A. *et al.* "Immunohistochemical Study of MUC1 and MUC5AC Expression in Gall Bladder Lesions." *Journal of Clinical and Diagnostic Research*, vol. 11, no. 7, July 2017, pp. EC12-EC16. <https://doi.org/10.7860/JCDR/2017/26537.10230>.
- [43] Kong, F.H. *et al.* "Mixed Adenoneuroendocrine Carcinoma of the Gallbladder: A Case Report and Literature Review." *Frontiers in Oncology*, vol. 15, July 2025. <https://doi.org/10.3389/fonc.2025.1584744>.
- [44] Yoo, K.S. *et al.* "MUC Expression in Gallbladder Epithelial Tissues in Cholesterol-Associated Gallbladder Disease." *Gut and Liver*, vol. 10, no. 5, September 2016, pp. 851-858. <https://doi.org/10.5009/gnl15600>.
- [45] Benson, A.B. *et al.* "Hepatobiliary Cancers, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology." *Journal of the National Comprehensive Cancer Network*, vol. 19, no. 5, 2021, pp. 541-565. <https://doi.org/10.6004/jnccn.2021.0022>.
- [46] Corti, F. *et al.* "Targeting the PI3K/AKT/mTOR Pathway in Biliary Tract Cancers: A Review of Current Evidences and Future Perspectives." *Cancer Treatment Reviews*, vol. 72, 2019, pp. 45-55. <https://doi.org/10.1016/j.ctrv.2018.11.001>.
- [47] Paweletz, C.P. *et al.* "Reverse Phase Protein Microarrays Which Capture Disease Progression Show Activation of Pro-Survival Pathways at the Cancer Invasion Front." *Oncogene*, vol. 20, no. 16, 2001, pp. 1981-1989. <https://doi.org/10.1038/sj.onc.1204265>.