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Biliary Brush Cytology: Diagnostic Yield and Predictors of Positive Cytology in A Moroccan Cohort Study

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Research Article

Keywords: Biliary brush cytology, biliary strictures, malignant biliary strictures, ERCP, cholangiocarcinoma

Posted Date: August 7th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10240977/v1

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Additional Declarations: No competing interests reported.

Biliary Brush Cytology: Diagnostic Yield and Predictors of Positive Cytology in A Moroccan Cohort Study

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Keywords: Biliary brush cytology; biliary strictures; malignant biliary strictures; ERCP; cholangiocarcinoma.

Funding declaration:

This research received no external funding.

Author contributions:

Chifaa Belkhayat wrote the manuscript and participated in data collection and analysis.

Ghizlane Kharrasse critically reviewed the manuscript.

Rachid Jabi provided surgical data and interpretation.

Hajar Koulali provided patients data.

Ouiam El Mqaddem participated in endoscopic procedures.

Amal Bennani and Chaimae Bekhakh provided valuable insight in the anatomopathological analysis

Abdelkrim Zazour performed ERCP procedures and biliary brushings

Zahi Ismaili supervised the study and critically revised the manuscript.

Declaration of interests:

The authors declare that they have no conflicts of interest.

Abstract:

Background
Biliary strictures pose significant diagnostic challenges because of the anatomical complexity of the biliary tree and their broad etiological spectrum. Brush cytology during ERCP is commonly used for tissue sampling because it is simple, accessible, and safe. However, its limited sensitivity continues to hinder diagnostic accuracy. This study aimed to **evaluate the diagnostic performance of ERCP-guided biliary brush cytology and to explore factors associated with positive cytology** in a Moroccan tertiary-care cohort.

Methods
We conducted a single-center observational study with retrospective and prospective data collection from January 2016 to January 2025. Adult patients who underwent ERCP-guided biliary brush cytology for suspected biliary strictures were included. Cytological results were classified according to the Papanicolaou Society of Cytopathology terminology. Categories V, suspicious for malignancy, and VI, positive for malignancy, were considered positive **cytology results**. Categories I to IV were considered non-positive for malignancy. Final diagnoses were established using histology, imaging findings, and/or clinical follow-up. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated. An exploratory multivariable logistic regression analysis was performed to identify factors independently associated with positive cytology.

Results
Among 2,190 ERCP procedures performed during the study period, 184 patients were identified for inclusion. Ten patients were excluded because of failed cannulation or unavailable cytology results, leaving 174 patients for final analysis. The mean age was 66.4 ± 12.6 years, and 85 patients were male (48.9%). The most frequent final diagnoses were cholangiocarcinoma in 54 patients (31.0%), pancreatic cancer in 52 patients (29.9%), and gallbladder cancer in 23 patients (13.2%). Brush cytology yielded 60 true-positive results, no false-positive results, 84 false-negative results, and 30 true-negative results. The overall sensitivity, specificity, positive predictive value, and negative predictive value were 41.7%, 100%, 100%, and 26.3%, respectively. Sensitivity was highest for cholangiocarcinoma (50.0%) and lowest for gallbladder cancer (17.4%). In exploratory multivariable analysis, male sex remained independently associated with positive cytology (adjusted OR 3.04; 95% CI 1.43–6.46; $p = 0.004$).

Conclusion
ERCP-guided biliary brush cytology remains a safe, accessible, and highly specific diagnostic tool for biliary strictures. However, its limited sensitivity confirms that a negative or non-positive result cannot exclude malignancy. These findings support the need to optimize sampling techniques and to consider complementary diagnostic modalities, particularly when clinical or radiological suspicion of malignancy remains high.

Introduction:

Biliary strictures remain a diagnostic and therapeutic challenge because of the anatomical complexity of the biliary tree and the broad range of underlying etiologies. A comprehensive approach combining clinical assessment, laboratory tests, imaging, and tissue sampling is often required to differentiate benign from malignant strictures and guide timely management, particularly when surgery is indicated. With the development of endoscopic techniques, endoscopic tissue acquisition has become central to the distinction between benign and malignant biliary strictures.

One of the most widely used strategies for obtaining tissue samples is brush cytology performed during endoscopic retrograde cholangiopancreatography (ERCP). It is a simple and safe method, with minimal additional procedural burden. It has a very high specificity, but the main limitation of this technique is its relatively low sensitivity for the diagnosis of malignant biliary strictures. This low sensitivity is mainly attributed to the high rate of false-negative results.

Biliary strictures can be caused by a wide range of etiologies, including benign inflammatory or postoperative causes as well as malignant and life-threatening diseases. The leading causes of malignant biliary strictures are cholangiocarcinoma and pancreatic cancer [1]. Although imaging has improved considerably, tissue confirmation is frequently required to guide oncological management and, in selected cases, surgical decision-making.

The aim of this study was to evaluate the diagnostic performance of ERCP-guided biliary brush cytology and to explore clinical, biological, radiological, and procedural factors associated with positive cytology in a Moroccan tertiary-care cohort.

Materials and methods:

Study design and participants

We conducted a single-center observational study with retrospective and prospective data collection from January 2016 to January 2025 at Mohammed VI University Hospital, Oujda, Morocco. Inclusion criteria comprised adult patients with suspected biliary strictures who were considered for ERCP-guided biliary brush cytology, encompassing both benign and suspected malignant etiologies. Patients were excluded if biliary cannulation had failed or if cytology results were unavailable (Figure 1).

Baseline data, including age, sex, medical history, clinical presentation, biological parameters, imaging findings, endoscopic data, cytological results, final diagnosis, and treatment strategy, were collected from the ERCP registry, medical records, hospital archives, electronic files, and, when necessary, phone calls with patients' family members.

Biliary brush cytology procedure

Cytology samples were obtained using Cook, Natick, or Boston Scientific cytology brushes with a standard 0.035-inch guidewire. The brush was inserted across the biliary stricture after cholangiographic identification of the stenosis and was moved back and forth at least 10 times. The brush segment was then cut and placed in Cytolyt solution before being transported to the cytology department for analysis.

Cytological classification and definition of a positive result

Cytology results were classified into six categories according to the Papanicolaou Society of Cytopathology terminology: non-diagnostic (I), negative for malignancy (II), atypical (III), neoplastic (IV), suspicious for malignancy (V), and positive for malignancy (VI) [2].

For the purpose of diagnostic accuracy analysis, specimens classified as category V or VI were considered index test-positive for malignancy. Categories I to IV were considered index test-negative or non-positive for malignancy. Non-diagnostic samples were reported separately and included in the non-positive group in the main analysis because they did not provide cytological confirmation of malignancy. Category IV was not considered positive for malignancy because this category does not necessarily establish invasive malignancy.

Reference standard and final diagnosis

The final diagnosis was established using surgical histology, percutaneous biopsy, EUS-guided fine-needle aspiration, cytology or histology obtained from another site, imaging evidence of locally advanced or metastatic disease, and/or clinical follow-up. In the absence of histological confirmation, the final classification was based on the available imaging findings and clinical follow-up data.

These data were used as the composite reference standard to classify cytology results as true-positive, false-positive, true-negative, or false-negative. For diagnostic-performance analysis, final diagnoses were categorized as malignant or non-malignant according to this composite reference standard.

Statistical analysis

All data were processed and analyzed using the Statistical Package for the Social Sciences, version 25.0 (SPSS 25.0). Quantitative variables were expressed as mean ± standard deviation or median with interquartile range, as appropriate. Categorical variables were expressed as numbers and percentages.

Sensitivity, specificity, positive predictive value, and negative predictive value were calculated using 2 × 2 contingency tables. Exact 95% confidence intervals were calculated for diagnostic-performance estimates.

Associations between positive brush cytology and clinical, biological, radiological, and procedural variables were first assessed using the chi-square test or Fisher’s exact test, as appropriate. An exploratory multivariable logistic regression analysis was then

performed. Positive brush cytology, defined as Papanicolaou categories V or VI, was used as the dependent variable.

The predefined model included age, male sex, elevated CA 19-9, metastatic disease, visible mass on imaging, and distal stricture location. **Elevated CA 19-9 was defined as a value above the local upper limit of normal.** Because CA 19-9 values were missing in some patients, the multivariable analysis was performed as a complete-case analysis. Adjusted odds ratios (aORs) with 95% confidence intervals (CIs) were reported. All tests were two-sided, and **p values < 0.05** were considered statistically significant.

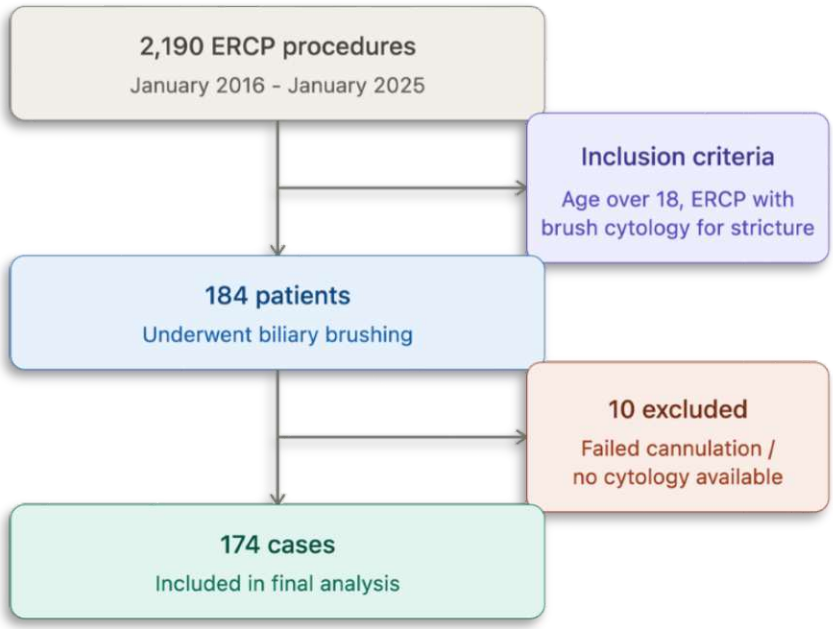


Figure 1: Patient selection Flowchart.

Results:

Study population and baseline characteristics

A total of 2,190 ERCP procedures were performed in our center during the study period, among which 184 patients were considered for ERCP-guided biliary brush cytology. Ten patients were excluded due to failed biliary cannulation or unavailable cytology results, leaving 174 cases for final analysis.

The cohort had a slight female predominance, with 89 women (51.1%) and 85 men (48.9%). The mean age was 66.4 ± 12.6 years, with a range from 26 to 95 years. The predominant presenting symptom was cholestatic jaundice, reported in 163 patients (93.7%), followed by weight loss in 151 patients (86.8%). Physical examination confirmed jaundice in 162 patients (93.1%), with abdominal tenderness in 109 patients (62.6%). Most patients presented with signs of acute cholangitis, observed in 126 cases (72.4%) (Table 1).

Biologically, liver tests were abnormal in 166 patients (95.4%). The mean hemoglobin level was 11.8 ± 1.8 g/dL, mean total bilirubin was 174.5 ± 117.7 mg/L, mean CRP was 54.9 ± 64.7 mg/L, and mean albumin was 31.9 ± 6.2 g/L. CA 19-9 was available in 146 patients and was elevated in 115 of them, corresponding to 78.8% of patients with available values and 66.1% of the overall cohort. The threshold used to define elevated CA 19-9 is reported in Table 1.

Radiological imaging, mainly CT scan and MRCP, identified a biliary stricture in 169 patients (97.1%). The stricture was distal in 96 cases (55.2%) and proximal in 73 cases (42.0%). In five patients (2.9%), the stricture location was not clearly specified. A visible mass was found in 85 patients (48.9%), and 47 patients (27.0%) had metastatic disease.

ERCP allowed biliary stent placement in 165 patients (94.8%). Plastic stents were used in the vast majority of stented patients, 161 out of 165 cases (97.6%). Endoscopic ultrasound (EUS) was performed in 41 patients (23.6%), detected a pancreatic or biliary mass in 17 patients, and allowed fine-needle aspiration in 17 patients (9.8% of the overall cohort). Only 21 patients (12.1%) underwent curative treatment, including nine pancreaticoduodenectomies, one major hepatectomy, five bile duct resections, and six isolated curative cholecystectomies.

The most frequent final diagnoses were cholangiocarcinoma in 54 patients (31.0%), pancreatic cancer in 52 patients (29.9%), and gallbladder cancer in 23 patients (13.2%). Other diagnoses included ampullary cancer in five patients (2.8%), gastric cancer in three patients (1.7%), metastatic or other cancers in seven patients (4.0%), and benign or indeterminate strictures in 30 patients (17.2%) (Table 4).

Cytological findings and diagnostic performance

Cytological analysis of the 174 biliary brushings revealed 15 non-diagnostic samples (8.6%), 59 negative samples (33.9%), 39 atypical samples (22.4%), one neoplastic sample (0.6%), 32 samples suspicious for malignancy (18.4%), and 28 samples positive for malignancy (16.1%). Overall, 60 samples (34.5%) were considered positive for malignancy when categories V and VI were grouped together.

Using categories V and VI as positive cytology for malignancy, ERCP-guided biliary brush cytology yielded 60 true-positive results, no false-positive results, 84 false-negative results, and 30 true-negative results. The overall sensitivity, specificity, positive predictive value, and negative predictive value were 41.7% (95% CI, 33.5–50.2), 100% (95% CI, 88.4–100), 100% (95% CI, 94.0–100), and 26.3% (95% CI, 18.5–35.4), respectively (Tables 2 and 3).

Sensitivity varied according to tumor type. It was highest for cholangiocarcinoma, reaching 50.0%, followed by pancreatic cancer at 44.2%, ampullary cancer at 40.0%, and gallbladder cancer at 17.4%. The remaining malignant diagnoses, including gastric and metastatic or other cancers, were included in the overall diagnostic-performance analysis and are detailed in Table 4. Because specificity, positive predictive value, and negative predictive value are less informative when calculated separately for each tumor subtype, subtype analysis was focused on sensitivity.

Factors associated with positive cytology

In univariate analysis, male sex was significantly associated with positive cytology results ($p = 0.014$). Elevated CA 19-9 showed a borderline association with positive cytology in univariate analysis ($p = 0.049$), whereas age, stricture location, visible mass on imaging, and metastatic disease were not significantly associated with cytological positivity.

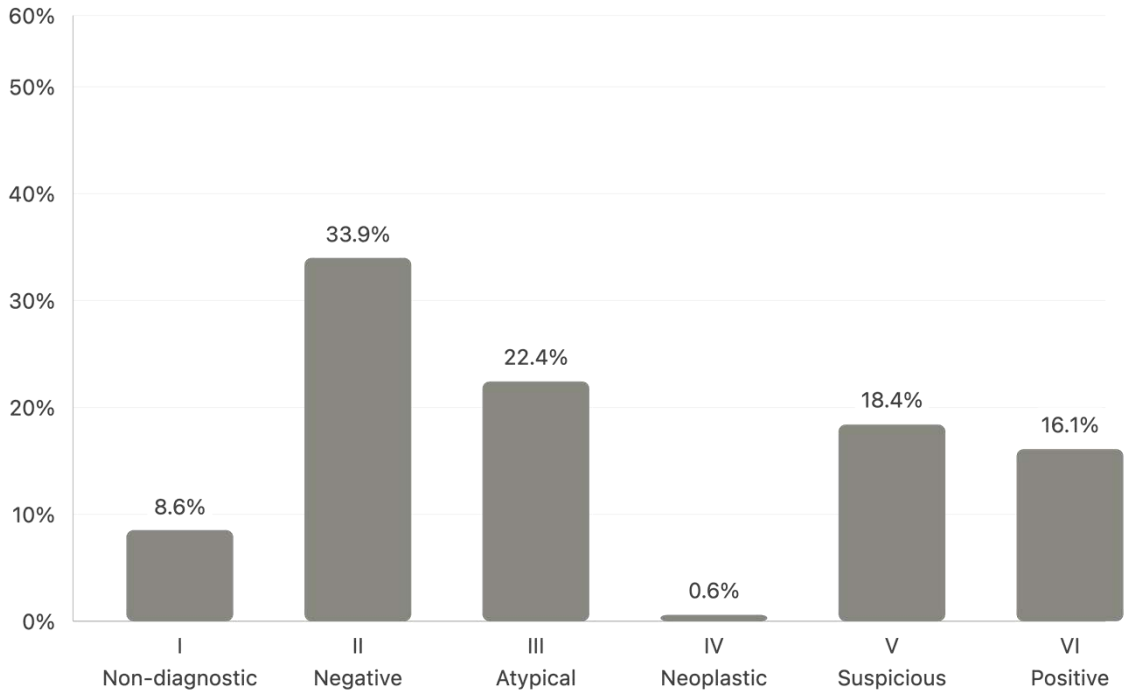
An exploratory multivariable logistic regression analysis was performed in 143 complete cases, including 50 patients with positive cytology. After adjustment for age, elevated CA 19-9, metastatic disease, visible mass on imaging, and distal stricture location, male sex remained independently associated with positive brush cytology (adjusted OR 3.04; 95% CI 1.43–6.46; $p = 0.004$). Elevated CA 19-9 showed a non-significant trend toward positive cytology (adjusted OR 2.48; 95% CI 0.90–6.87; $p = 0.080$). No significant association was found for age, metastatic disease, visible mass, or distal stricture location (Table 5).

Variable	Mean ± SD or n (%)
Demographics	
Age, years	66.4 ± 12.6
Male sex	85 (48.9%)
Female sex	89 (51.1%)
Medical history	
Hypertension	47 (27.0%)
Diabetes	46 (26.4%)
Previous cholecystectomy	25 (14.4%)
Smoking	35 (20.1%)
Clinical presentation	
Jaundice	163 (93.7%)
Acute cholangitis	126 (72.4%)
Weight loss	151 (86.8%)
Abdominal tenderness	109 (62.6%)
Laboratory findings	
Abnormal liver function tests	166 (95.4%)
Hemoglobin, g/dL	11.8 ± 1.8
Total bilirubin, mg/L	174.5 ± 117.7
CRP, mg/L	54.9 ± 64.7
Albumin, g/L	31.9 ± 6.2
CA 19-9, IU/mL	2466.0 ± 4868.9
Elevated CA 19-9*	115/146 (78.8%)
Imaging findings	
Visible biliary stricture on imaging	169 (97.1%)

*Among patients with available values (n=146)

Table 1: Baseline characteristics of the study cohort

*CA 19-9 values were available in 146 patients. The percentage of elevated CA 19-9 was calculated among patients with available values.



Histogram 1: Distribution of biliary brush cytology results according to the Papanicolaou Society of Cytopathology terminology

Categories V and VI were grouped as positive for malignancy, corresponding to 60 patients (34.5%). Categories I to IV were considered non-positive for malignancy for the purpose of diagnostic accuracy analysis.

	Final malignant diagnosis	Final benign or indeterminate	Total
Positive cytology, categories V–VI	60	0	60
Non-positive cytology, categories I–IV	84	30	114
Total	144	30	174

Table 2: Diagnostic accuracy of ERCP-guided biliary brush cytology for malignancy, Two-by-two diagnostic accuracy table

Parameter	Value
Sensitivity	41.7%
Specificity	100%
Positive predictive value	100%
Negative predictive value	26.3%

Table 3: Diagnostic performance

Final diagnosis	Total cases	Positive cytology, categories V–VI	Sensitivity
Cholangiocarcinoma	54	27	50.0%
Pancreatic cancer	52	23	44.2%
Gallbladder cancer	23	4	17.4%
Ampullary cancer	5	2	40.0%

Table 4: Sensitivity according to final malignant diagnosis

Variable	Adjusted OR	95% CI	p value
Age	1.00	0.97–1.03	0.902
Male sex	3.04	1.43–6.46	0.004
Elevated CA 19-9	2.48	0.90–6.87	0.080
Metastatic disease	1.85	0.79–4.31	0.156
Visible mass on imaging	0.62	0.27–1.40	0.250
Distal stricture location	1.27	0.57–2.85	0.560

Table 5: Exploratory multivariable logistic regression analysis of factors associated with positive brush cytology.

Discussion:

Biliary strictures remain a major diagnostic challenge because malignant and benign etiologies may have overlapping clinical, biological, and radiological presentations. In this context, tissue acquisition is essential, particularly when curative or oncological treatment depends on histological or cytological confirmation. Surgical resection remains the only potentially curative treatment for selected patients with cholangiocarcinoma and other localized pancreatobiliary malignancies [3,4]. In our cohort, 27.0% of patients already had metastatic disease at diagnosis, and only 12.1% underwent curative treatment, highlighting the advanced stage at presentation in a substantial proportion of patients.

ERCP-guided biliary brush cytology remains one of the most widely used sampling techniques for biliary strictures. It is simple, accessible, technically feasible during therapeutic ERCP, and associated with a low additional procedural burden. However, its main limitation is its low sensitivity, despite consistently high specificity. In our study, using Papanicolaou categories V and VI as positive for malignancy, brush cytology showed 41.7% sensitivity, 100% specificity, 100% positive predictive value, and 26.3% negative predictive value. These findings confirm that a positive cytology result is highly reliable for confirming malignancy, whereas a negative or non-positive result cannot exclude cancer.

Our results are consistent with published data. Burnett et al. reviewed 16 studies including more than 1500 patients and reported an overall sensitivity of approximately 41.6% for standard ERCP cytology [5]. Similarly, the systematic review and meta-analysis by Navaneethan et al. reported a pooled sensitivity of 45% and a pooled specificity of 99% for brush cytology in malignant biliary strictures [6]. These values are very close to our findings and support the external validity of our results. In contrast, diagnostic performance varies widely across studies, reflecting differences in case

selection, sampling technique, cytological processing, interpretation criteria, and local expertise [7,8].

Several mechanisms may explain the limited sensitivity of brush cytology. Malignant biliary strictures may result from extrinsic compression rather than direct epithelial infiltration, particularly in pancreatic cancer, gallbladder cancer, or metastatic disease. In such cases, brushing may fail to retrieve malignant cells. In addition, submucosal tumor spread, dense fibrosis, well-differentiated tumors, mucinous or papillary features, insufficient cellularity, inflammation, and reactive atypia may all reduce diagnostic yield or complicate cytological interpretation [9]. These factors are particularly relevant in real-life practice, where systematic adjunctive techniques are not always available.

In our cohort, sensitivity varied according to tumor type. It was highest for cholangiocarcinoma, at 50.0%, followed by pancreatic cancer at 44.2%, ampullary cancer at 40.0%, and gallbladder cancer at 17.4%. This pattern is clinically plausible. Cholangiocarcinoma more often directly involves the biliary epithelium, making malignant cells more likely to be retrieved by brushing. By contrast, gallbladder cancer and pancreatic cancer may cause biliary obstruction through extrinsic compression or secondary invasion, which may explain the lower yield of cytology in these subgroups.

Several studies have evaluated factors associated with positive biliary brush cytology. Mahmoudi et al. reported that positive cytology was associated with older age, mass size greater than 1 cm, and stricture length greater than 1 cm [10]. Mehmood et al. also evaluated factors affecting the diagnostic yield of biliary brush cytology in patients with biliary strictures [8]. Park and Jeon, in patients with atypical or suspicious cells on brush cytology, found that several clinical and biological parameters, including CA 19-9 and stricture-related features, were associated with malignant biliary strictures [11].

In our study, male sex was significantly associated with positive cytology in univariate analysis and remained independently associated with positive brush cytology after multivariable adjustment. This association should be interpreted cautiously. It may reflect differences in tumor distribution, disease stage, or other unmeasured characteristics of the study population, rather than a direct effect of sex on cytological yield. Elevated CA 19-9 showed only a non-significant trend after multivariable adjustment, and other variables, including age, metastatic disease, visible mass on imaging, and distal stricture location, were not independently associated with cytological positivity.

The low negative predictive value observed in our cohort has direct clinical implications. A non-positive cytology result should not reassure clinicians when clinical, biological, or radiological suspicion of malignancy remains high. In such cases, further diagnostic work-up should be considered rather than delaying treatment decisions. Current endoscopic guidelines support this approach. The ASGE suggests adding fluoroscopy-guided intraductal biopsy sampling to brush cytology during ERCP and recommends considering cholangioscopy-guided or EUS-guided tissue acquisition after nondiagnostic sampling, depending on stricture location and suspected disease extension [12]. The ESGE also recommends combined diagnostic strategies, including EUS-guided tissue acquisition and ERCP-based sampling in selected strictures, as

well as cholangioscopy-guided biopsies when standard ERCP-based sampling remains inconclusive [13].

This study has several strengths. It reflects real-life practice in a Moroccan tertiary-care center and includes a relatively large cohort of patients undergoing ERCP-guided biliary brush cytology over a long study period. It also provides practical data from a resource-limited setting, where access to cholangioscopy, rapid on-site cytological evaluation, molecular testing, or systematic EUS-guided tissue acquisition may be limited.

However, several limitations should be acknowledged. First, the study included retrospective data, which may have introduced selection and information biases. Second, brush cytology is operator-dependent, and variations in brushing technique, number of passes, stricture dilation, and sample handling may have influenced diagnostic yield. Third, rapid on-site cytopathological evaluation was not available. Fourth, complementary techniques such as fluoroscopy-guided forceps biopsies, cholangioscopy-guided biopsies, EUS-guided tissue acquisition, or molecular testing were not systematically performed. Fifth, final diagnosis was not based on histology in all patients and relied on imaging or clinical follow-up in some cases. Finally, the multivariable analysis was exploratory and limited by the number of positive cytology events and by missing CA 19-9 values, which reduced the number of complete cases to 143. Therefore, the identified association between male sex and positive cytology should be considered hypothesis-generating and requires confirmation in larger prospective cohorts.

In summary, our findings confirm that ERCP-guided biliary brush cytology is highly specific but has limited sensitivity for detecting malignant biliary strictures. It remains useful as a confirmatory diagnostic tool when positive, but a negative or non-positive result should not exclude malignancy. Optimizing sampling techniques and integrating complementary diagnostic modalities are essential to improve diagnostic accuracy, particularly in patients with persistent suspicion of malignancy despite negative cytology.

Conclusion:

In conclusion, our study confirms that ERCP-guided biliary brush cytology remains a safe, accessible, and highly specific diagnostic tool for evaluating biliary strictures. However, its limited sensitivity, **of** 41.7% in our cohort, restricts its ability to exclude malignancy when cytology is negative or non-positive.

While our findings are consistent with existing literature, the diagnostic yield of biliary brush cytology appears to be influenced by several factors, including tumor type, lesion characteristics, sampling technique, and local cytopathological expertise. In our study, sensitivity was highest for cholangiocarcinoma and lowest for gallbladder cancer. Male sex remained associated with positive cytology in exploratory multivariable analysis, but this finding should be interpreted cautiously and regarded as hypothesis-generating.

These results highlight the need to optimize ERCP-guided tissue acquisition and to consider complementary diagnostic modalities, such as fluoroscopy-guided forceps biopsy, EUS-guided tissue acquisition, or cholangioscopy-guided biopsy when available. In resource-limited settings, biliary brush cytology remains useful, but its limitations must be clearly recognized to avoid delayed diagnosis of pancreatobiliary malignancies.

Declarations:

Consent for publication: not applicable.

Competing interests:

The authors declare no competing interests

Ethical approval and consent to participate:

All procedures for this retrospective and prospective study were reviewed and approved by the Ethics Committee of the Digestive Diseases Research Laboratory at Mohammed VI University Hospital in Oujda (Approval No. 34/2024). Research Laboratory of Pathology and Digestive Oncology at Mohammed VI University Hospital in Oujda has an established ethics committee/Institutional Review Board (IRB) that reviewed and approved this study.

Written informed consent was obtained from all participants prior to their inclusion in the study. Data anonymity was ensured following national and international guidelines. All methods were carried out in accordance with relevant guidelines and regulations of the Declaration of Helsinki.

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