Molecular Testing with BiliSeq Improves the Detection of Malignancy Among Indeterminate Biliary Strictures: A Prospective Analysis of 720 Patients

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INTRODUCTION

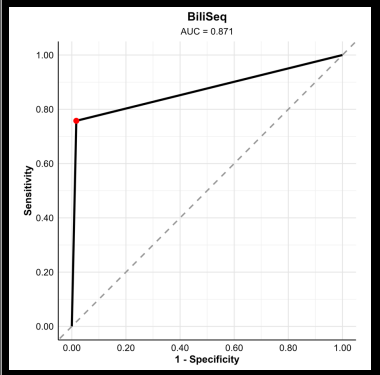
- Indeterminate biliary strictures, defined by inconclusive imaging and pathology, represent a clinical dilemma that often leads to repeat procedures and delays in diagnosis
- The aim of this study was to examine the diagnostic performance of BiliSeq, a targeted next generation sequencing (NGS) panel designed for ERCP-associated specimens, in the evaluation of indeterminate biliary strictures.

METHODS

- Between 2019 and 2025 (6-year time frame), 2,116 patients from 28 medical institutions across the United States were prospectively evaluated using BiliSeq, totaling 2908 ERCP-associated specimens.
- A sub analysis of our multicenter prospective cohort study enrolled 720 patients with indeterminate biliary strictures.
- Indeterminate strictures were defined as those with inconclusive imaging and pathology, and a diagnosis was established through surgical pathology, tissue sampling, or follow-up of >12 months.
- Diagnostic performance of BiliSeq, CA19-9, and their combination were evaluated across the study cohort and PSC patients.

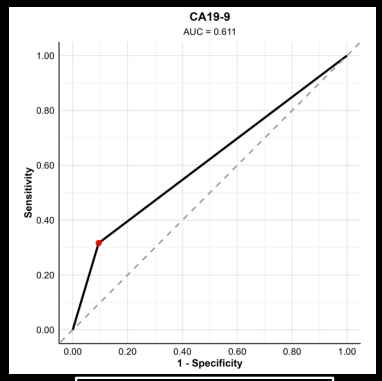
Patient or Tumor Characteristics	Total, n = 720	Positive, n = 232 (32%)	Negative n = 488 (68%)	p
Intrahepatic CCA	99 (14%)	82 (35%)	17 (3%)	< 0.001
Perihilar CCA	6 (1%)	5 (2%)	1 (1%)	
Extrahepatic CCA	70 (10%)	58 (25%)	12 (2%)	
Pancreatic adenocarcinoma	78 (11%)	51 (22%)	27 (6%)	
Ampullary adenocarcinoma	11 (2%)	9 (4%)	2 (1%)	
Gallbladder carcinoma	12 (2%)	8 (3%)	4 (1%)	
B-cell lymphoma	6 (1%)	5 (2%)	1 (1%)	
Epithelial dysplasia	7 (1%)	5 (1%)	2 (1%)	
Metastatic carcinoma	9 (1%)	3 (1%)	6 (1%)	
Benign cholangiopathy	385 (53%)	6 (3%)	379 (78%)	
IgG4 disease	37 (5%)	0 (0%)	37 (8%)	

BiliSeq



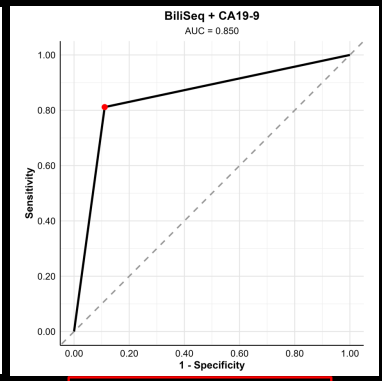
75.8% Sensitivity
98.3% Specificity

Serum CA19-9



31.6% Sensitivity
90.5% Specificity

BiliSeq + CA19-9



81.1% Sensitivity
88.9% Specificity

RESULTS

- Overall, among 720 patients with indeterminate biliary strictures, 232 (32%) had malignant strictures, and 488 (68%) had benign strictures. Within this total cohort, 156 patients (22%) had primary sclerosing cholangitis.
- BiliSeq demonstrated 75.8% sensitivity and 98.3% specificity for malignant strictures compared to CA 19-9 elevation, which demonstrated 31.6% sensitivity and 90.5% specificity. When combined, BiliSeq and CA 19-9 performed with 81.1% sensitivity and 88.9% specificity.
- In the PSC subgroup, BiliSeq achieved a sensitivity of 78.4% and specificity of 99.2%, while CA 19-9 achieved a sensitivity of 29.7% and specificity of 91.6%.
- BiliSeq performance was consistent across specimen types, with similar performance characteristics with intraductal brushing and biopsy specimens.

CONCLUSION

- For truly indeterminate biliary strictures, BiliSeq testing exhibited high sensitivity and specificity for malignancy and demonstrated high diagnostic performance across specimen types and in PSC patients.
- The combination of CA 19-9 and BiliSeq testing provided optimal sensitivity while maintaining adequate specificity.