Evaluating the Significance of Pancreatobiliary Fluorescence In Situ Hybridization Polysomy on Prognosis in De Novo Cholangiocarcinoma

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INTRODUCTION: We recently developed a fluorescence in situ hybridization probe set for evaluating suspicious biliary and pancreatic duct strictures (PB-FISH). We aimed to determine whether PB-FISH results in biliary brush cytology specimens are associated with outcomes of patients with cholangiocarcinoma (CCA).

METHODS: We performed a retrospective study of patients with CCA tested by PB-FISH from January 2015 to August 2018. CCA was stratified by primary sclerosing cholangitis (PSC) into those with (PSC CCA) or without PSC (de novo CCA). PB-FISH results were categorized as polysomy (gain of multiple loci), nonpolysomy (single locus gain, single locus gain with 9p21 loss, homozygous 9p21 loss, tetrasomy), and disomy (no abnormalities). Overall survival (OS) was estimated using Kaplan-Meier methods and compared between the PB-FISH results using log-rank tests. Cox models were adjusted for age, sex, CA 19-9, cytology results, source of brushing sample, and treatments.

RESULTS: Characteristics of 264 eligible patients (median age 60.4; range 18–92) were comparable for patients with PB-FISH polysomy vs nonpolysomy vs disomy. The median OS was similar between disomy, nonpolysomy, and polysomy in the overall population (22.7 vs 22.7 vs 20.3 months, respectively). For de novo CCA, both polysomy and nonpolysomy were associated with worse OS compared with disomy (polysomy: hazard ratio [HR] 2.09, 95% confidence interval [CI] 1.14–3.83; nonpolysomy: HR 2.4, 95% CI 0.54–2.46; P = 0.027). For PSC CCA, neither polysomy nor nonpolysomy were significantly associated with worse OS (polysomy: 0.90, 95% CI 0.47–1.75; nonpolysomy: HR 1.78, CI 0.71–4.49; P = 0.27).

DISCUSSION: PB-FISH alterations are associated with worse survival in de novo CCA, though statistical significance was lost when adjusting for confounding variables.

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INTRODUCTION

Cholangiocarcinomas (CCAs) are malignancies that arise from the biliary tract epithelium and are the second most common hepatic malignancy after hepatocellular carcinomas (1,2). They represent approximately 2% of all cancer-related deaths per year worldwide, with a 5-year survival rate of less than 10% (3). CCAs occur sporadically (de novo) and in patients with primary sclerosing cholangitis (PSC), a rare condition characterized by chronic inflammation of the bile ducts. Patients with PSC have a higher propensity to develop CCAs than those without PSC, so patients with PSC typically undergo regular surveillance with noninvasive imaging. Potential treatment options for patients with CCA include liver transplantation, surgical resection, radiotherapy, and chemotherapy. Unfortunately, most patients present with advanced stage disease and have limited treatment options (1,4). Outcomes after each therapy are determined by tumor characteristics such as location (intrahepatic, perihilar, or extrahepatic), clinical stage, lymph node metastasis, and histopathologic grade (4). Recent studies have shown that certain biomarkers in tumor tissues after resection can predict the overall prognosis.

Fluorescence in situ hybridization (FISH) assays were developed to complement routine cytology to aid in an earlier diagnosis of CCA. The UroVysion FISH (UV-FISH) assay was initially developed for bladder cancer detection and repurposed for use in CCA detection. Studies investigating high-risk patients with PSC have shown that UV-FISH has twice the sensitivity for
CCA when compared with routine cytology alone (34%–58% vs 15%–40%) (5,6). One study of 49 consecutive patients diagnosed with CCA evaluated the utility of UV-FISH assays from trans-papillary forceps biopsy specimens in determining the prognosis of patients with CCA. They found that increased chromosome 7 copy number was associated with worse outcomes (hazard ratio [HR] 2.26; 95% confidence interval [CI] 1.11–4.63; \( P = 0.025 \)) (7).

More recently, the pancreatobiliary FISH (PB-FISH) assay was developed by the selection of probes directed toward chromosomal alterations that are common in pancreatobiliary tumors (8). The final probe set is directed toward loci on 1q21 (MCL1), 7p12 (EGFR), 8q24 (MYC), and 9p21 (CDKN2A). When compared with the UV-FISH assay, polysomy by PB-FISH was found to have a significantly higher sensitivity for pancreatobiliary cancer detection than polysomy by UV-FISH or routine cytology (65% vs 46%; \( P < 0.001 \)) (8). There have been no studies determining the utility of PB-FISH assays for determining patient outcomes.

The goal of this study was to evaluate whether PB-FISH polysomy or specific PB-FISH aberrations in brush cytology specimens are associated with differences in outcomes of patients with CCA.

**METHODS**

**Study subjects**

We retrospectively identified all patients who underwent endoscopic retrograde cholangiopancreatography (ERCP) with the collection of cytologic brushings from either the biliary or pancreatic tract for routine cytology and PB-FISH analysis at Mayo Clinic between January 2015 and September 2018. The protocol was reviewed by the Mayo Clinic Institutional Review Board, and written consent was not required for this study. Electronic medical records were reviewed following the Mayo Clinic Institutional Review Board protocol to obtain patient demographics, PB-FISH and cytology results from biliary brushings, laboratory results, imaging, and outcome data. All patients older than 17 years with a diagnosis of CCA were included in the study. The diagnosis of CCA was based on positive histopathology, positive intraluminal brush cytology, polysomy demonstrated by PB-FISH with either a malignant-appearing biliary stricture on imaging or a perihilar mass with malignant appearing biliary stricture on imaging or a perihilar mass with malignancy of the pancreatic-tract for routine cytology and PB-FISH analysis at Mayo Clinic between January 2015 and September 2018. The protocol was reviewed by the Mayo Clinic Institutional Review Board, and written consent was not required for this study. Electronic medical records were reviewed following the Mayo Clinic Institutional Review Board protocol to obtain patient demographics, PB-FISH and cytology results from biliary brushings, laboratory results, imaging, and outcome data. All patients older than 17 years with a diagnosis of CCA were included in the study. The diagnosis of CCA was based on positive histopathology, positive intraluminal brush cytology, polysomy demonstrated by PB-FISH with either a malignant-appearing biliary stricture on imaging or a perihilar mass with malignant appearing biliary stricture on imaging. If multiple ERCPs were performed for the patient within the studied time frame, we used the first ERCP with available PB-FISH for the analysis. This was based on previous studies using the FISH assay because it was found to be the most consistent and least biased approach in a referral-based tertiary center (6,8). Subsequent laboratory and imaging data were obtained based on the date of the first ERCP. Patients were classified as experiencing CCA with a history of PSC (PSC CCA) or CCA without a history of PSC (de novo CCA) based on the clinical history. Staging was based on the American Joint Committee on Cancer Tumor-Node-Metastasis (TNM) classification system only in patients with CCA who had a surgical resection. Patients were followed up until their last communication, appointment, or death date.

**PB-FISH assay**

PB-FISH was performed on cytologic brushings collected in PreservCyt or CytoLyt solution. The biliary cells were obtained from the sample, fixed, and placed on a slide. The fluorescently labeled DNA probes (1q21, 7p12, 8q24, and 9p21) were hybridized to the cells on the slide. One hundred consecutive epithelial cells were examined to evaluate the probe signals through fluorescence microscopy. PB-FISH signal patterns were categorized as described in previous literature by predetermined cutoff values: polysomy (\( \geq 3 \) copies of at least 2 probes), tetrasomy (exactly 4 copies of each probe), single locus gain with concurrent 9p21 loss (\( \geq 3 \) copies of 1q21, 7p12, or 8q24 and 0–1 copy of 9p21 in the same cell), single locus gain (\( \geq 3 \) copies of a single probe with 2 copies of the other 3 probes), or homozygous 9p21 loss (0 copies of 9p21 probe and 2 copies of the other 3 probes) (8). If multiple PB-FISH abnormality cutoffs were reached for a sample, then all respective abnormalities were reported for that sample.

**Statistical analysis**

For samples with multiple PB-FISH abnormalities reported during a single ERCP, we selected the most severe abnormality for statistics according to the following hierarchy: polysomy > single locus gain with concurrent 9p21 loss > homozygous 9p21 loss > tetrasomy > single locus gain. This hierarchy was based on their association with malignancy in phase 2 of the brushing cutoff study (8). For all samples, we further categorized the PB-FISH results into 3 types: disomy (no abnormalities), nonpolysomy (tetrasomy, single locus gain with concurrent 9p21 loss, single locus gain or homozygous 9p21 loss), and polysomy. We compared the clinical characteristics and survival between the 3 PB-FISH types in patients with diagnosed CCA. We summarized categorical data as frequency counts and percentages and continuous measures as mean values, SDs, median values, and ranges. Categorical variables were compared using the \( \chi^2 \) test or Fisher exact test. Continuous variables were compared using the 1-way ANOVA test or Kruskal-Wallis test. Overall survival (OS) was defined as time from CCA diagnosis to death from any cause. The distribution of time-to-event outcome was estimated using the Kaplan-Meier methods and compared between the 3 PB-FISH types using the log-rank test. HRs and 95% CIs were estimated using both univariate Cox proportional hazards model and multivariate Cox model, adjusting for age, sex, CA 19-9, cytology results, source of ERCP sample, and whether patients received surgery, or radiation therapy. Because transplantation is considered an important alternative therapy that substantially alters patient outcome, we performed sensitivity analyses by either censoring data when patients received transplant or removing patients who received transplant. Subgroup analysis was also conducted to compare the 3 PB-FISH types by PSC status. All analyses were conducted using 2-sided tests with a significance level of 0.05. Statistical analysis was performed using SAS version 9.4M6 (SAS Institution, Cary, NC).

The study was reviewed by Mayo Clinic Institutional Review Board (ID 08-005151) under the board/committee for Expedited Review B in accordance with 45 Code of Federal Regulations (CFR) 46.110, item 5. This study constituted a minimal risk analysis of materials that had been collected, and there was a waiver of specific informed consent in accordance with 45 CFR 46.116. Medical records and samples were not used in participants who declined authorization for use of their medical records in research.

**RESULTS**

**Patient characteristics**

A total of 2,538 PB-FISH analyses were performed between January 2015 and September 2018 on brushings taken from patients at our institution. Among those, there were 1,050 unique
|                          | Disomy (N = 75) | Nonpolysomy (N = 41) | Polysomy (N = 148) | Total (N = 264) | P-value |
|--------------------------|-----------------|----------------------|--------------------|-----------------|---------|
| Age                      |                 |                      |                    |                 | 0.9634a |
| Mean (SD)                | 60.5 (14.95)    | 60.9 (15.22)         | 60.2 (15.61)       | 60.4 (15.31)    |         |
| Median                   | 61.2            | 61.7                 | 62.7               | 62.2            |         |
| Range                    | 26.9–88.0       | 28.5–84.4            | 18.6–92.6          | 18.6–92.6       |         |
| Sex                      |                 |                      |                    |                 | 0.6397b |
| Male                     | 51 (68.0%)      | 26 (63.4%)           | 105 (70.9%)        | 182 (68.9%)     |         |
| Female                   | 24 (32.0%)      | 15 (36.6%)           | 43 (29.1%)         | 82 (31.1%)      |         |
| PSC                      |                 |                      |                    |                 | 0.0826b |
| No                       | 43 (57.3%)      | 28 (68.3%)           | 73 (49.3%)         | 144 (54.5%)     |         |
| Yes                      | 32 (42.7%)      | 13 (31.7%)           | 75 (50.7%)         | 120 (45.5%)     |         |
| Cirrhosis                |                 |                      |                    |                 | 0.8632b |
| No                       | 56 (74.7%)      | 29 (70.7%)           | 106 (71.6%)        | 191 (72.3%)     |         |
| Yes                      | 19 (25.3%)      | 12 (29.3%)           | 42 (28.4%)         | 73 (27.7%)      |         |
| Ulcerative colitis       |                 |                      |                    |                 | 0.3265b |
| No                       | 59 (78.7%)      | 31 (75.6%)           | 103 (69.6%)        | 193 (73.1%)     |         |
| Yes                      | 16 (21.3%)      | 10 (24.4%)           | 45 (30.4%)         | 71 (26.9%)      |         |
| CA 19-9 (U/mL)           |                 |                      |                    |                 | 0.1913b |
| <129                     | 33 (44.0%)      | 16 (39.0%)           | 77 (53.1%)         | 126 (48.3%)     |         |
| ≥129                     | 42 (56.0%)      | 25 (61.0%)           | 68 (46.9%)         | 135 (51.7%)     |         |
| Missing                  | 0               | 0                    | 3                  | 3               |         |
| IgG4 (mg/dL)             |                 |                      |                    |                 | 0.4677a |
| Mean (SD)                | 41.1 (43.68)    | 41.7 (40.09)         | 36.8 (39.92)       | 38.9 (40.97)    |         |
| Cancer location          |                 |                      |                    |                 | 0.1050b |
| Intrahepatic             | 17 (24.3%)      | 5 (12.5%)            | 21 (15.8%)         | 43 (17.7%)      |         |
| Hilar                    | 45 (64.3%)      | 32 (80.0%)           | 86 (64.7%)         | 163 (67.1%)     |         |
| Extrahepatic             | 8 (11.4%)       | 3 (7.5%)             | 26 (19.5%)         | 37 (15.2%)      |         |
| Missing                  | 5               | 1                    | 15                 | 21              |         |
| Stage at diagnosis       |                 |                      |                    |                 | 0.3336b |
| 1                        | 9 (39.1%)       | 3 (21.4%)            | 17 (32.7%)         | 29 (32.6%)      |         |
| 2                        | 4 (17.4%)       | 5 (35.7%)            | 15 (28.8%)         | 24 (27.0%)      |         |
| 3                        | 3 (13.0%)       | 5 (35.7%)            | 11 (21.2%)         | 19 (21.3%)      |         |
| 4                        | 4 (30.4%)       | 1 (7.1%)             | 9 (17.3%)          | 17 (19.1%)      |         |
| Missing                  | 52              | 27                   | 96                 | 175             |         |
| Surgery                  |                 |                      |                    |                 | 0.7990a |
| No                       | 52 (69.3%)      | 27 (65.9%)           | 96 (64.9%)         | 175 (66.3%)     |         |
| Yes                      | 23 (30.7%)      | 14 (34.1%)           | 52 (35.1%)         | 89 (33.7%)      |         |
| Transplant               |                 |                      |                    |                 | 0.4702a |
| No                       | 66 (88.0%)      | 35 (85.4%)           | 121 (81.8%)        | 222 (84.1%)     |         |
| Yes                      | 9 (12.0%)       | 6 (14.6%)            | 27 (18.2%)         | 42 (15.9%)      |         |
| Radiation                |                 |                      |                    |                 | 0.3321a |
| No                       | 48 (64.0%)      | 22 (53.7%)           | 80 (54.1%)         | 150 (56.8%)     |         |
| Yes                      | 27 (36.0%)      | 19 (46.3%)           | 68 (45.9%)         | 114 (43.2%)     |         |
| Follow-up (yr)           |                 |                      |                    |                 |         |
| Mean (95% CI)            | 1.93 (1.65–2.38) | 1.96 (1.36–NE)       | 1.73 (1.44–2.21)   | 1.84 (1.64–2.17) |         |

CA, 19-9, cancer antigen 19-9; IgG4, immunoglobulin G4; mg/dL, milligram per deciliter; PSC, primary sclerosing cholangitis. *Kruskal-Wallis P-value. \( \chi^2 \) P-value.
patients. Two hundred sixty-four patients had a diagnosis of CCA and met inclusion criteria for the study (182 males and 82 females; median age of 60.4 years). The clinical characteristics of the included patients are summarized in Table 1. One hundred twenty patients had a diagnosis of PSC, whereas 144 patients did not. One hundred sixty-three (61.7%) patients had hilar CCA, 43

| Table 2. Characteristics of biliary brushing |
|---------------------------------------------|
| Disomy (N = 75)   | Nonpolysomy (N = 41) | Polysomy (N = 148) | Total (N = 264) | P value |
|-------------------|----------------------|--------------------|-----------------|---------|
| Primary lesion     |                      |                    |                 |         |
| No mass           | 24 (32.4%)           | 7 (17.5%)          | 28 (19.3%)      | 59 (22.8%) |
| Mass              | 34 (45.9%)           | 25 (62.5%)         | 74 (51.0%)      | 133 (51.4%) |
| Stricture         | 16 (21.6%)           | 8 (20.0%)          | 43 (29.7%)      | 67 (25.98%) |
| Missing           | 0                    | 0                  | 0               | 0       |
| Brushing location |                      |                    |                 | 0.5495a |
| Below cystic duct | 10 (13.1%)           | 8 (19.5%)          | 19 (12.9%)      | 37 (14.1%) |
| Above cystic duct | 65 (86.7%)           | 33 (80.5%)         | 128 (87.1%)     | 226 (85.9%) |
| Missing           | 0                    | 0                  | 1               | 1       |
| Cytology result   |                      |                    |                 | 0.0001a |
| Negative          | 39 (53.4%)           | 7 (17.1%)          | 13 (8.8%)       | 59 (22.6%) |
| Atypical          | 19 (26.0%)           | 13 (31.7%)         | 37 (25.2%)      | 69 (26.4%) |
| Suspicious        | 11 (15.1%)           | 21 (51.2%)         | 71 (48.3%)      | 103 (39.5%) |
| Positive          | 4 (5.5%)             | 0 (0.0%)           | 26 (17.7%)      | 30 (11.5%) |
| Missing           | 2                    | 0                  | 1               | 3       |

aχ² P value.

| Table 3. Univariate and multivariate associations between FISH results (disomy vs nonpolysomy vs polysomy) with overall survival |
|--------------------------------------------------------------------------------------------------------------------------|
| Median (95% CI) | Univariate association | Multivariate association |
|-----------------|------------------------|-------------------------|
| HR (95% CI)     | Pair-wise P value      | P value                 |
|                 |                        | HRadj (95% CI)          | Pair-wise P value (adjusted) | P value (adjusted) |
|-----------------|------------------------|-------------------------|
| Survival by polysomy status (all patients) | 0.2858d | 0.5364e |
| Disomy | 22.7 (18.8–NE) | Reference | — | Reference | — |
| Nonpolysomy | 21.7 (14.1–NE) | 1.26 (0.70–2.25) | 0.4447a | 1.02 (0.55–1.91) | 0.9425 |
| Polysomy | 20.3 (13.1–42.8) | 1.43 (0.92–2.23) | 0.1159a | 1.29 (0.78–2.13) | 0.3249 |
| Survival by polysomy status (PSC) | 0.2706d | 0.0537e |
| Disomy | 20.6 (15.9–NE) | Reference | — | Reference | — |
| Nonpolysomy | 11.4 (10.6–NE) | 1.78 (0.71–4.49) | 0.2214a | 3.16 (1.16–8.58) | 0.0240 |
| Polysomy | 12.7 (10.6–NE) | 0.90 (0.47–1.75) | 0.7625a | 1.11 (0.51–2.43) | 0.7893 |
| Survival by polysomy status (de novo) | 0.0265d | 0.5942e |
| Disomy | NE (15.2–NE) | Reference | — | Reference | — |
| Nonpolysomy | 28.6 (18.2–NE) | 1.15 (0.54–2.46) | 0.7168a | 0.70 (0.30–1.62) | 0.4069 |
| Polysomy | 12.7 (10.6–NE) | 2.09 (1.14–3.83) | 0.0174a | 1.00 (0.45–2.25) | 0.9911 |

CI, confidence interval; FISH, fluorescence in situ hybridization; HR, hazard ratio; PSC, primary sclerosing cholangitis.

aKaplan-Meier method.

bCox model.

cCox model with adjustment of age, sex, cancer antigen, 19-9, cytology results, brushing location, and whether patients received surgery or radiation.

dLog-rank test.

eWald χ² test.
(16.3%) had intrahepatic CCA, and 37 (14.0%) had extrahepatic CCA. When comparing patient characteristics by PB-FISH result type (disomy, nonpolysomy, and polysomy), the groups were fairly similar. When comparing the characteristics of the biliary brushing specimens, patients with polysomy were statistically more likely to have a suspicious or positive cytology result when compared with disomy and nonpolysomy PB-FISH results (Table 2). There was a significant difference in several clinical characteristics between patients with PSC CCA and de novo CCA, including the presence of a mass on imaging (31.9% vs 66.4%, \( P < 0.0001 \)) and CA 19-9 level (1,240.5 ± 4,878 vs 7,005.9 ± 44,712.03, \( P = 0.0024 \)). Follow-up duration for all patients ranged from 1.6 to 2.2 years, with a median follow-up of 1.84 years.

**Associations of PB-FISH polysomy and OS**

Within the entire cohort, 75 patients (28%) had no abnormalities by PB-FISH (i.e., disomy), 41 patients (15.5%) had a nonpolysomy abnormality, and 148 patients (56.1%) had polysomy. The median OS was 22.7 months (95% CI = 16.8–NE), 21.7 months (95% CI = 14.1–NE), and 20.3 months (95% CI = 13.1–42.8) for patients with disomy, nonpolysomy, and polysomy PB-FISH results, respectively (logrank \( P = 0.286 \)) (Table 3 and Figure 1). Results were consistent when censoring the OS during transplant for those who underwent transplant. However, among those who did not undergo transplant (\( N = 222 \)), patients with polysomy had significantly shorter OS compared with those with disomy, with a 59% higher risk of death (HR = 1.59, 95% CI = 1.02–2.50, \( P = 0.042 \)).

**Subgroup analysis by PSC**

In patients with de novo CCA (\( N = 144 \)), 43 patients (29.9%) had disomy by PB-FISH, 28 (19.4%) had a nonpolysomy abnormality, and 73 patients (50.7%) had polysomy. Significant differences in OS were observed (logrank \( P = 0.027 \)); the median OS was not reached for disomy (95% CI = 1.3–NE), and the median OS was 28.6 months (95% CI = 1.5–NE) and 12.7 months (95% CI = 0.9–4.9) for nonpolysomy and polysomy, respectively (Table 3 and Figure 2). The OS was significantly shorter in patients with de novo CCA with a polysomy or nonpolysomy abnormality on PB-FISH when compared with disomy (polysomy: HR = 2.09, 95% CI = 1.14–3.83; nonpolysomy: HR = 2.4, 95% CI = 0.54–2.46; \( P = 0.027 \)); however, the significant association diminished after adjusting for known prognostic factors probably due to the small sample sizes. In patients with PSC CCA (\( N = 120 \)), 32 patients (26.7%) had no abnormalities (i.e., disomy) by PB-FISH, 13

![Figure 1. Kaplan-Meier curve of overall survival in all patients with CCA stratified by polysomy status. CCA, cholangiocarcinoma; CI, confidence interval; HR, hazard ratio.](image-url)
patients (10.8%) had a nonpolysomy abnormality, and 75 patients (62.5%) had polysomy. No significant differences were observed in OS: the median OS was 20.6 months (95% CI 51.3–NE), 11.4 months (95% CI 50.9–2.6), and 30.0 months (95% CI 51.1–3.6), respectively (logrank P = 0.27) (Table 3 and Figure 3).

Associations of PB-FISH subgroups and OS
Because 1 patient can have multiple FISH aberrations, we classified patient FISH patterns by identifying their worst FISH type, assuming the hierarchy as polysomy > single locus gain with concurrent 9p21 loss > homozygous 9p21 loss > tetrasomy > single locus gain. We analyzed each PB-FISH abnormality separately and found the median OS for disomy, homozygous 9p21 loss, polysomy, single locus gain and single locus gain with concurrent 9p21 loss were 22.8 months (95% CI 51.4–NE), 18 months (95% CI 50.9–NE), 20.4 (95% CI 51.1–3.6), 37.2 months (95% CI 51.2–NE), and 28.8 (95% CI 51.5–NE), respectively (logrank P = 0.47). There were no statistically significant associations within these PB-FISH subgroups.

DISCUSSION
The aim of our study was to investigate whether PB-FISH assay abnormalities in biliary stricture brushings were associated with mortality in patients with CCA. Although there were no significant associations across all patients based on PB-FISH abnormalities, we found that the OS was significantly shorter in de novo CCA patients with a polysomy PB-FISH pattern. Notably, this statistical significance diminished in our multivariate analysis possibly due to small sample size.

UV-FISH increases the sensitivity of diagnosing malignancy in suspicious biliary strictures when compared with routine cytology. Only 1 previous study investigated whether abnormalities in UV-FISH in transpapillary forceps biopsy specimens could predict outcomes in patients with CCA (7). The study included a total of 49 patients with CCA and used the UV-FISH probe set that was originally developed to diagnose bladder cancer. The investigators did not differentiate between patients with PSC and those with de novo CCA in the study. The study found that increased chromosome 7 copy number was predictive of poor prognosis in patients with CCA, potentially related to overexpression of the oncogenes EGFR and MET (7). The PB-FISH assay was developed to improve the sensitivity for detecting pancreatobiliary malignancy and uses probes to chromosomal regions commonly aberrant in these cancers, including 1q21 (MCL1), 7p12 (EGFR), 8q24 (MYC), and 9p21 (CDKN2A). PB-FISH was found to improve the
sensitivity of cancer detection when compared with UV-FISH and routine cytology alone (8). To our knowledge, our study is the first to establish that polysomy PB-FISH from biliary brushings in patients with de novo CCA is associated with higher mortality rates.

Aneuploidy is a quantitative abnormality of cellular DNA content, and polysomy is considered a subset of aneuploidy. Polysomy by the PB-FISH assay is defined as 3 or more copies of at least 2 probes, which include MCL1, EGFR, MYC, and CDKN2A. EGFR mutations have been shown to be an independent prognostic factor for CCA (9). Previous studies have shown that aneuploidy in gastrointestinal lesions could be used to predict the development of gastrointestinal cancers and has been associated with histologic tumor grade, stage, and prognosis (10,11). Aneuploidy has also been associated with worse prognosis and higher recurrence in several gastrointestinal cancers including colorectal cancer, esophageal cancer, and hepatocellular carcinoma (10,11). Our study supports that polysomy determined by PB-FISH is a prognostic parameter for patients with CCA, though not necessarily an independent factor.

In our study, polysomy PB-FISH was associated with higher mortality rates in patients with de novo CCA, but not in those with PSC CCA. Patients with PSC have a higher lifetime risk of CCA than those without PSC. Therefore, surveillance for CCA through some form of noninvasive imaging is recommended for this population, especially in the first year of PSC diagnosis (12,13). ERCP is recommended to further evaluate abnormalities in imaging or biomarkers in patients with PSC undergoing surveillance (13,14). Of interest, our study also showed that patients with de novo CCA had a significantly increased presence of mass on imaging and higher CA 19-9 level during biliary brushing when compared with patients with PSC CCA. This likely explains why the statistical significance of polysomy in OS is diminished in multivariate analysis. The limited effect of polysomy PB-FISH on PSC CCA survival could be explained by the lower threshold to obtain biliary brushings in patients with PSC, causing sampling to be obtained before biliary dysplasia and decreasing the yield of a PB-FISH abnormality in the first brushing. In addition, PSC CCA is more likely to be sampled earlier than de novo CCA tumors, so the tumors tend to be smaller and more difficult to sample.

In our subgroup analysis, we did not see a significant difference in OS among patients with disomy, homozygous 9p21 loss, polysomy, single locus gain, and single locus gain with...
concurrent 9p21 loss. Although previous studies found increased detection of malignancy with the inclusion of 9p21 deletion in FISH studies, we did not see any statistical significance for prognostication when compared against other FISH aberrancies (9,15). However, this interpretation may be limited due to the size of each cohort and the fact that we are only including patients with CCA.

This study had several limitations. First, it was a retrospective, single-center study. In addition, many patients underwent multiple ERCPs before the diagnosis of CCA; however, this study used only the first PB-FISH result that the patient had received. This led to some differences in the PSC and de novo patient groups because the reason for evaluation differed between the 2 groups. The PB-FISH assay used in this study was performed on biliary brushings, which requires successful brushing of malignant cells at ERCP. Previous studies on biliary stricture sampling have shown that false negatives can occur in the context of inadequate tumor cell representation within a brushing sample.

Our study suggests that PB-FISH aberrations from biliary brushing specimens can potentially be used for the prognostication of CCA. A prospective trial including multiple and consistent time points for PB-FISH assays that each patient receives with a longer follow-up and larger cohort would further elucidate the prognostic utility of PB-FISH in both patients with PSC and those with de novo CCA.

CONFLICTS OF INTEREST
Guarantor of the article: Lewis R. Roberts, MB, ChB, PhD.
Specific author contributions: H.J.: contributed to the conception and design of the work, drafted the manuscript, interpreted the statistical analysis, critically revised the manuscript for important intellectual content, and gave final approval of the article. E.G.B.F.: contributed to the design of the work, critically revised the manuscript for important intellectual content, and gave final approval of the article. J.Y.: provided statistical analysis, drafted the manuscript, and critically revised the manuscript for important intellectual content. T.M.B.: provided statistical analysis, critically revised the manuscript for important intellectual content, and gave final approval of the article. T.J.Z.: provided statistical analysis, critically revised the manuscript for important intellectual content, and gave final approval of the article. G.J.G.: contributed to the conception and design of the work, critical revision of the manuscript, and gave final approval of the article. K.C.H.: contributed to the conception of the work, critical revision of the manuscript, and gave final approval of the article.

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Study Highlights

WHAT IS KNOWN

- Cholangiocarcinoma is difficult to diagnose and has a poor prognosis.
- We can use FISH on ERCP brushing specimens to help us diagnose cholangiocarcinoma.

WHAT IS NEW HERE

- Polysomy on Pancreatobiliary FISH on brushings from biliary strictures is associated with poorer survival in patients with de novo cholangiocarcinoma in univariate analysis.

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