

Serum Bilirubin Levels Can Predict Pancreatic and Biliary Malignancies in Patients with Obstructive Jaundice and Non-conclusive Cytology

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Abstract Background: Elevated serum bilirubin has been shown to be a reliable predictor of pancreatic and biliary malignancy but the relationship between serum bilirubin and inadequate (C1), benign (C2) and indeterminate (C3) cellular samples has not been explored. The aim of this study is to determine the relationship between serum bilirubin and pancreatic, biliary or ampullary malignancy in the context of non-confirmatory cytology. **Methods:** This is a retrospective analysis of patients with obstructive jaundice undergoing investigation for possible pancreatic, peri-ampullary or biliary malignancy between 2009 and 2013. **Results:** 135 patients were included; 84 had a malignant diagnosis and 51 benign. All patients with C4 or C5 cytology (n=49) had confirmed malignancy. 35 out of 86 C1 – C3 samples were falsely negative. ROC curve analysis demonstrated a strong association (AUC 0.912) between elevated serum bilirubin and malignancy; serum bilirubin ≥ 100 $\mu\text{mol/L}$ had a sensitivity of 86% and a specificity of 88%. In the C1-C3 subgroup, this association was maintained (AUC 0.905). Serum bilirubin ≥ 100 $\mu\text{mol/L}$ had a sensitivity of 80% and specificity of 88%. Using this cut-off highlighted 28 out of 35 of the malignancies missed by cytology ($p = 0.003$). **Conclusion:** Our study demonstrates that a serum bilirubin ≥ 100 $\mu\text{mol/L}$ is associated with malignancy and this relationship is maintained in C1-C3 cytology. When faced with non-confirmatory cytology in the absence of a benign aetiology and an elevated serum bilirubin ≥ 100 $\mu\text{mol/L}$, we advocate more aggressive investigation to avoid missing an occult malignancy.

Keywords: bilirubin, cytology, pancreatic cancer, adenocarcinoma

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1. Introduction

In the absence of choledocholithiasis or a radiologically visible mass, the underlying pathology in obstructive jaundice can be a diagnostic challenge. Inflammatory or neoplastic changes involving the biliary tree, ampulla, pancreas or duodenum are often responsible. The diagnosis of malignancy can be difficult because of subtle differences in appearance between benign and neoplastic aetiologies and the focus of investigation is ensuring that an occult malignancy is not missed.

When there is high radiological suspicion for malignancy and the lesion is potentially resectable with curative intent, it is accepted that surgery can be undertaken without pre-operative histological confirmation [1]. However, where there is diagnostic uncertainty about the aetiology of disease or the presence of an incurable malignancy,

histological or cytological confirmation of the diagnosis is imperative prior to embarking on treatment [1].

Tissue or cellular samples can be obtained via endoscopic retrograde cholangiopancreatography (ERCP), endoscopic ultrasound and fine needle aspiration cytology (EUS-FNAC) or percutaneous biopsy. These techniques can have variable sensitivity for malignancy, with EUS tending to demonstrate a greater diagnostic ability over ERCP [2]. More recently, the advent of peroral cholangioscopy has allowed for direct visualisation of the biliary tree and greater accuracy with tissue sampling.

However ERCP is more commonly used in patients in whom drainage of the biliary tree is deemed necessary prior to surgery and it allows for opportunistic cytological sampling at the same time as biliary stenting [3].

Because biliary brush cytology (BBC) has a low sensitivity for neoplasia, the potential of missed malignancies exists in patients whose cytology returns as either inadequate, benign or indeterminate [4]. Garcea et al demonstrated

that an elevated serum bilirubin $> 100 \mu\text{mol/L}$ has been shown to be a reliable predictor of pancreatic/biliary malignancy, with a high sensitivity and specificity [5]. This link between elevated bilirubin and pancreatic/biliary malignancy has been replicated in other studies [6-11]. However there is no published data on the sensitivity and specificity of serum bilirubin in inadequate (C1), benign (C2) and indeterminate (C3) cytology. The aim of the study was to assess if serum bilirubin is a reliable predictor of malignancy within the context of C1-C3 cytology.

2. Materials & Methods

This is a retrospective single institution study performed at a tertiary referral centre for hepatobiliary and pancreatic disease. Case records of patients presenting with obstructive jaundice between January 2009 to December 2013 were analysed. Biochemical blood tests including liver function tests with the serum bilirubin 24 hours prior to ERCP were recorded; computed tomography (CT) images were reviewed by a specialist hepatobiliary radiologist; ERCP reports were reviewed; BBC samples were obtained, analysed and graded based on the Royal College of Pathologists guidelines; endoscopic ultrasound (EUS) reports were analysed where available, as were histological samples (targeted biopsy or surgical specimen where available). Confirmation of malignancy was done directly (through positive tissue diagnosis) or indirectly (using surrogate markers like death certification or post-mortem reports). Patients were included in the study only if they had undergone all of the above investigations as part of their diagnostic workup, with the exception of EUS. All patients were discussed at the specialist HPB multidisciplinary team (MDT) meeting. The study was registered and ethical approval was granted locally (Reference 17883) Cases were excluded if they were undergoing surveillance of a suspected lesion or if the pancreaticobiliary lesion was metastatic in origin.

Statistics were analysed using SPSS version 20 (IBM Corp 2012). Mann Whitney-U tests were used to compare the distribution of continuous variables between groups. Chi-square analysis was used to compare dichotomised data. The level of serum bilirubin that demonstrated significant sensitivity and specificity for malignancy in C1-C3 cytology was ascertained using ROC curve analysis. Statistical significance was set at $p \leq 0.05$

3. Results

The case notes of 145 patients were scrutinised for the study. No cases were excluded because of incomplete data. Ten patients were excluded because their disease was metastatic disease to the site of interest leaving 135 patients in the study. The male to female ratio was 1:1 with a median age of 67 (interquartile range 59-76). 84 (62%) patients had a malignant diagnosis and 51 benign (38%) (Table 1). The most common malignant diagnosis was pancreatic ductal adenocarcinoma (51%) and the most common benign diagnosis was a benign biliary stricture (59%). Nine (7%) patients had C1 cytology. C2 and C3 accounted for the majority of cytology reports from ERCP

(57%). All patients with C4 or C5 cytology (36%) had a confirmed final diagnosis of malignancy. Twenty-two patients had EUS-FNAC and 38 had a percutaneous biopsy as part of their investigative work-up.

Table 1. Patient demographics, indications for investigations and final diagnoses

Demographic	n (%)
Median age	67 (IQR 59 – 76)
Sex (M:F ratio)	1:1
Indication	
Biliary tree pathology	64 (46)
Ampullary/Periampullary lesions	13 (10)
Pancreatic pathology	58 (44)
Methods of investigation	
ERCP + BBC	135 (100)
Bilirubin	135 (100)
EUS-FNAC	22 (16)
Percutaneous biopsy	38 (28)
Surgical specimen	21 (16)
CT abdomen/pelvis	135 (100)
Malignant diagnosis (n=84)	
Pancreatic adenocarcinoma	43 (51)
Cholangiocarcinoma	32 (38)
Ampullary adenocarcinoma	9 (11)
Benign diagnosis (n=51)	
Biliary stricture	30 (59)
Chronic pancreatitis	12 (23)
Duodenal ulcer	5 (10)
Gallstone disease	4 (8)
Cytological grading	
C1 (inadequate)	9 (7)
C2 (benign)	40 (30)
C3 (atypical, likely reactive)	37 (27)
C4 (suspicious for malignancy)	26 (19)
C5 (malignant)	23 (17)

IQR – inter-quartile range, ERCP – endoscopic retrograde cholangiopancreatography, BBC – biliary brush cytology, EUS – endoscopic ultrasound, FNA – fine needle aspiration cytology, CT – computed tomography.

The mean serum bilirubin for benign and malignant disease was $48 \mu\text{mol/L}$ and $240 \mu\text{mol/L}$ ($p < 0.001$) respectively in the total cohort. ROC curve analysis illustrated a close association between serum bilirubin levels and malignancy with an area under the curve (AUC) of 0.912 ($p < 0.001$). A serum bilirubin level $\geq 100 \mu\text{mol/L}$ demonstrated a sensitivity and specificity for malignancy of 86% and 88% respectively. A serum bilirubin of $< 100 \mu\text{mol/L}$ was also associated with a high sensitivity and specificity for benign disease (Figure 1)

35 out of 86 C1 – C3 samples were falsely negative. In the C1-C3 subgroup, the mean serum bilirubin was $48 \mu\text{mol/L}$ and $239 \mu\text{mol/L}$ between the benign and malignant causes respectively ($p < 0.001$).

ROC curve analysis for this subgroup demonstrated that serum bilirubin maintained a strong association with malignancy (AUC 0.905). A serum bilirubin of $\geq 100 \mu\text{mol/L}$ demonstrated a sensitivity and specificity of 80% and 88% (Figure 2). Univariate analysis using a serum bilirubin cut-off of $100 \mu\text{mol/L}$ was statistically significant for predicting malignancy in this group ($p < 0.001$ 95% CI 0.838 – 0.973) (Table 2) and would have identified 28 of the 35 malignancies.

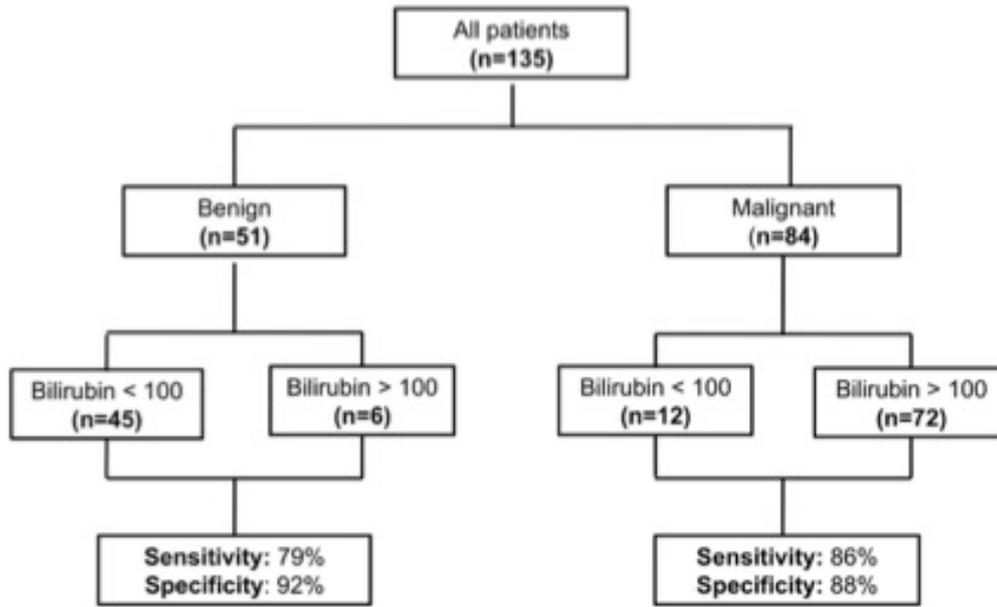


Figure 1. Flow chart of the sensitivity and specificity of serum bilirubin in both benign and malignant disease states

Table 2. Serum bilirubin > 100 µmol/L can help distinguish between benign and malignant aetiologies in those with C1-C3 cytology

Serum Bilirubin vs. Final Diagnosis				
C1/2/3 cytology samples (n = 86)				
		Final Diagnosis		Total
		Benign	Malignant	
Bilirubin	< 100 µmol/L	45	7	52
	> 100 µmol/L	6	28	34
Total		51	35	86

p = 0.003.

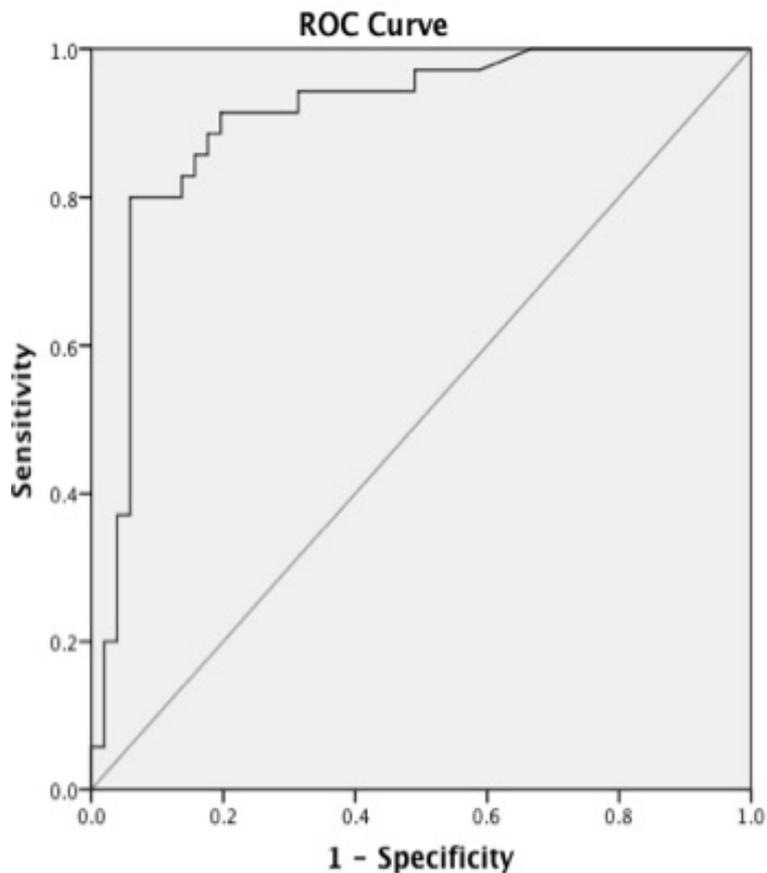


Figure 2. ROC curve analysis looking at the correlation between serum bilirubin > 100 µmol/L and malignant disease in patients with C1-C3 cytology

4. Discussion

Serum bilirubin has previously been demonstrated to be a good marker of pancreaticobiliary malignancy. Our study is, to our knowledge, the first that specifically explores the relationship between serum bilirubin and C1-C3 cytology.

Garcea et al have demonstrated that serum bilirubin $>100 \mu\text{mol/L}$ is closely correlated with a diagnosis of malignancy irrespective of the initial reason for investigation and independently of any other investigation [5]. Patients were significantly less likely to harbour a malignancy if the serum bilirubin was $< 100 \mu\text{mol/L}$, a finding replicated in many other studies [6,7,8,9]. This is possibly due to progressive and irreversible extra-luminal compression of the biliary tree that is associated with a malignancy.

Patients with obstructive jaundice in whom pancreatic or biliary malignancy is suspected and tissue biopsies prove elusive present a management challenge to clinicians¹¹. The poor sensitivity associated with BBC is well documented, and can make the confident exclusion of malignancy difficult. Furthermore, although CT has a high sensitivity and specificity for malignancy it can sometimes be radiologically difficult to distinguish between benign and malignant aetiologies, particularly smaller lesions and on a background of pancreatitis [12-17]. In our study, the sensitivity and specificity of CT at diagnosing benign lesions was 40% and 73% respectively. This contrasts with a 97% sensitivity and 88% specificity for malignancy. Just as certain radiological features may raise suspicion of a possible malignancy [15], we propose that an elevated serum bilirubin $>100 \mu\text{mol/L}$ may help identify patients who would also benefit from closer scrutiny in order to exclude malignancy, especially in those patients where radiology is indeterminate.

Despite the mean serum bilirubin for all malignant disease being $> 200 \mu\text{mol/L}$, we chose a cut-off of $100 \mu\text{mol/L}$. This is because the ROC Curve at $200 \mu\text{mol/L}$ demonstrates a specificity of 95% but a sensitivity of 61%. Whilst this is comparable to the sensitivity and specificity of BBC in some published studies [18], the sensitivity is still too low, as over a third of malignancies would be missed at this level. Indeed, the specificity of BBC is 100% in our series. Therefore, applying a cut-off at $> 200 \mu\text{mol/L}$ with a specificity lower than BBC would fail to add anything useful to the diagnostic work-up.

We acknowledge that there are limitations to the study. The small sample size may introduce a type 2 error and limits the ability to generalise to all patients with obstructive jaundice and a potential malignancy. In addition we did not investigate the ability of serum bilirubin to determine the difference in sensitivities between biliary, ampullary and pancreatic pathology. However, the statistical analyses conducted still demonstrate a strong, significant correlation. Also, we acknowledge that EUS-FNAC is more sensitive at detecting pancreatic and biliary malignancy in comparison to ERCP and BBC [4,19,20]. However, there is evidence to suggest that patients with obstructive jaundice that need stenting and without a clear tissue diagnosis should have ERCP

performed as it allows for cytological sampling at the same time as stenting [1,12,21,22,23]. Peroral cholangioscopy has been shown to demonstrate a high visual and tissue diagnostic rate for biliary tract lesions, but there is less evidence for its superiority over other modalities in the diagnosis of pancreatic pathologies [24], which comprise a substantial number of the patients in this series.

We conclude that in the face of non-confirmatory investigations and an elevated serum bilirubin; we advocate more aggressive investigation to avoid missing a malignancy.

Fund

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Conflicts of Interest

We have no conflicts of interest to declare.

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